NMR INSTRUMENTS

The MAS CryoProbe

Exploring New Frontiers in Solid-State NMR

Innovation with Integrity

Introduction

The CryoProbe technology has profoundly influenced the world of solution-state NMR. And after more than 25 years of continuous developments and improvements, the CryoProbe has become a widely adopted product in NMR laboratories worldwide (Kovacs & Moskau, 2013). Sensitivity enhancements of up to 4 compared with equivalent room temperature (RT) probes have been reported on various types of samples, leading to experimental time savings of up to an order of magnitude. Such dramatic increase in productivity has led many research groups, both academic and industrial, to equip their laboratories with a CryoProbe.

The success of the technology spurred its expansion into Magnetic Resonance Imaging (MRI) and later, into solid-state NMR. In 2019 Bruker pioneered the introduction of a commercial triple-resonance 600 MHz MAS CryoProbe specifically designed for structural biology applications, marking a first in the industry. Recently, our MAS CryoProbe portfolio has been extended by introducing a double resonance probe equipped with a broadband channel, especially designed to provide innovative solutions in the field of material science. The double resonance MAS CryoProbe is the newest member of the CryoProbe family, and a few such devices exist worldwide, located in industrial and academic NMR laboratories. Similarly to the liquids CryoProbes, the MAS CryoProbe offers a typical mass sensitivity enhancement factor of 3-4 compared with equivalent room temperature probes, providing a game changing boost in sensitivity.

Increased sensitivity does not only permit faster spectral recording but offers other experimental advantages like recording an additional dimension, for enhanced resolution. Another advantage of such a boost in sensitivity is the ability to study dilute samples, with concentrations typically under the limit of detection of typical RT probes. The sensitivity enhancement provided by the MAS CryoProbe can be further applied to the study of native samples, or samples with lower amounts of labelling, effectively reducing the cost of producing samples.

The construction of a cryogenically cooled probe for solid-state NMR presents unique challenges. In amongst them, the highly demanding RF field performance, the requirement for an effective thermal isolation between the sample and the cryogenic components of the probe circuitry, the sample accessibility, the magic angle adjustment, and the spinning stability present significant technical demands. Bruker is proud to have achieved a successful balance between performance requirements and practical constraints. The MAS CryoProbe is a true game changer in the field of solid-state NMR.

The present document summarizes our collaborative efforts with customers, using the MAS CryoProbe to study challenging samples. The work is extensive, ranging from biological systems to material science, even reaching the

field of pharmaceuticals. Some samples are studied at natural abundance level for the first time. Other samples contain various amounts of isotopically enriched spin systems, on which multiple dimension high quality experiments were acquired for the first time. Employing the 800 MHz double resonance low gamma MAS CryoProbe, insensitive nuclei like ^{47}Ti, ^{49}Ti, ^{25}Mg and ^{67}Zn were analyzed.

The results are presented in a concise manner to showcase the various ways the MAS CryoProbe has produced unprecedented data. For more detailed information on each topic, the reader is referred to the literature articles cited at the end of this document. We aim to offer the reader a valuable review suitable for grant proposals or justification purposes.

Description of the Equipment

The design of the Bruker cryogenically cooled MAS probe meets different technological challenges among which the demanding RF requirements for solid-state NMR spectroscopy as well as allowing magic angle spinning (MAS) and easy change of the rotor while keeping probe components at cryogenic temperatures. While the probe is constructed to fit in standard bore (SB) magnets it can also be mounted in wide bore (WB) magnets with a special adaptor insert.

Figure 1a shows a MAS CryoProbe (MAS CRP). It consists of two parts connected with a flexible line: (i) a body containing the cooled preamplifiers for optimal signal enhancement of the observe nuclei, (ii) a unit where the sample rotor is loaded, which is inserted into the magnet and houses the RF coil and circuitry, the MAS related components, and an integrated automatic tuning and matching unit for all RF channels. Figure 1b shows where the rotor is inserted.

The MAS CryoProbe delivery includes accessories that enable convenient and safe operation. The additional equipment includes:

- An automated lift for safe probe insertion and easy rotor exchange. The motorized, software-controlled lift moves the probe out and into the magnet in cold mode for rotor change and guarantees reproducible MAS positioning of the probe's MAS module. The lift is mounted under the magnet as shown in figure 2 (a and b) and does not need to be removed when other probe types are used. Its design ensures compatibility with standard room temperature, solids and liquids probes. It can also be used to mount the liquids CryoProbe or the CryoProbe Prodigy into the magnet before cooling down.
- A lift motion control unit and a mounting hardware for the probe body outside the magnet and the flexible interconnecting line.
- A motorized and automated magic angle setting mechanism with control electronics.
- A spinning test station to ensure that the rotor has been properly packed and rotates well before inserting it into the MAS CRP.
- A T-type BOSS shim system that is compatible with all other probes.
- A pump station to ensure a good quality vacuum in both parts of the MAS CRP before cooling down and while in cold mode.
- 3.2 mm special MAS CryoProbe rotor.
- A dedicated set of packing tools for different types of samples.

Figure 1a The MAS CryoProbe with its special two parts design: the body with the cold preamplifiers on the right side connected through a flexible line to the unit housing the RF and MAS components.

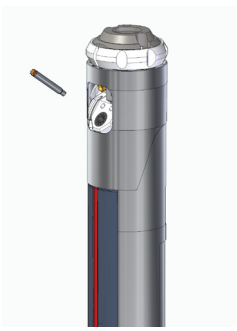

Figure 1b Top part of the MAS CryoProbe where the sample is inserted.

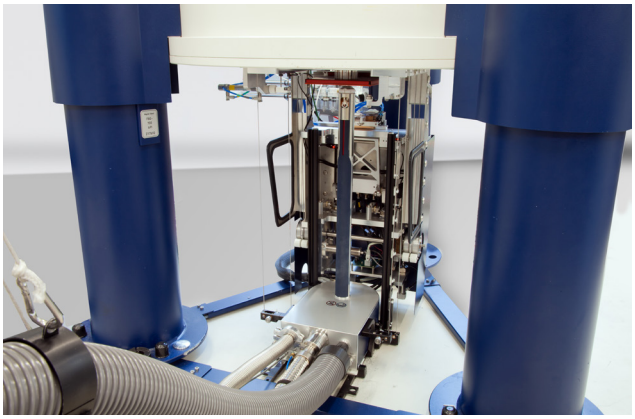

Figure 2a The automated lift used to move the probe into and out of the magnet for measurement or rotor exchange.

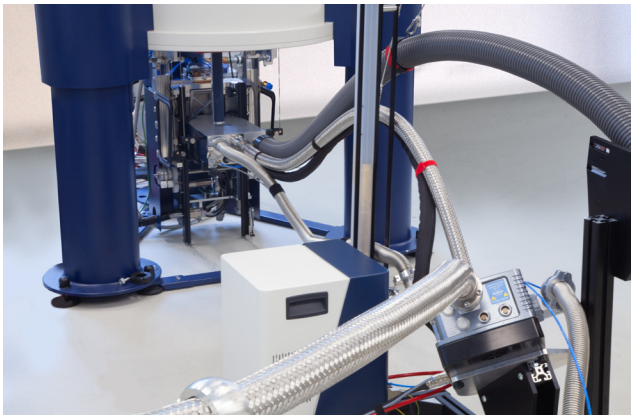

Figure 2b Probe midway through being inserted into the magnet.

A Bruker CryoPlatform (CU/4 and higher) is needed to cryogenically cool the probe with cold helium gas. Cooling is accomplished with a closed-looped gas flow via a flexible transfer line. This design ensures minimal helium gas consumption. The option of a BSNL (Bruker Smart Nitrogen Liquefier) add-on makes the operation of the entire spectrometer more convenient. The BSNL uses the extra cooling capacity of the CryoPlatform reducing drastically the need of LN_2 refills of the magnet.

For independent sample temperature regulation and operation below room temperature, the use of a BCU unit is required. Specifically, a BCU-II is required to work below 10 °C. Figure 3 illustrates an overview of a spectrometer equipped with a MAS CryoProbe and its required accessories.

The special 3.2 mm rotors for the MAS CRP are designed to be longer for a larger filling volume of up to 87 microliters. Smaller sample volumes can be center-packed using bottom and top spacers of different dimensions. With an outer diameter of 3.2 mm and available sample volume similar to a conventional 4 mm MAS probe, the MAS CryoProbe is capable of high-speed rotation up to 20 kHz using a MAS3 controller.

Figure 3 Site layout of a spectrometer equipped with the MAS CRP and its accessories.

The MAS CryoProbe brings typically a 3 to 4-fold S/N improvement relative to a conventional probe for the same sample amount. Figure 4 shows the mass sensitivity enhancement measured on an unlabeled and unaltered glycine sample, at room temperature.

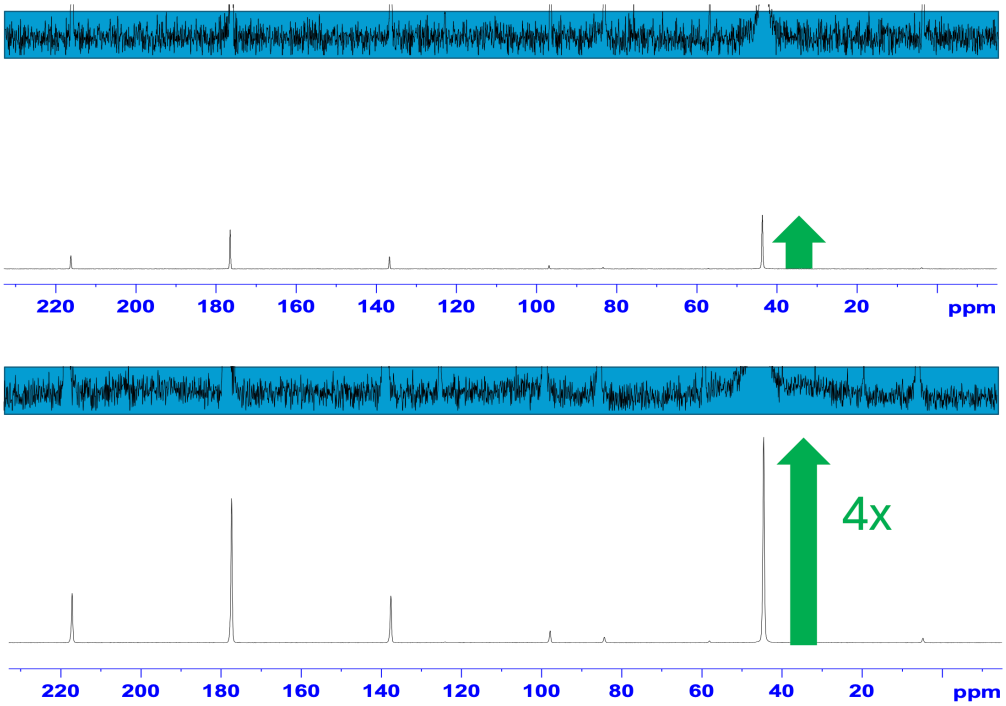

Figure 4 Glycine is Bruker's NMR standard for the evaluation of signal to noise performance. The standard ^{13}C signal-to-noise ratio is calculated from the intensity of the α-carbon signal in a cross-polarization experiment performed under ^{1}H decoupling. A ^{13}C CPMAS signal-to-noise test is performed on a 4 mm RT HX CPMAS probe (top) and a HCN MAS CryoProbe (bottom). The arrow illustrates the improvement of the signal intensity under the same experimental conditions and equivalent noise level.

By using glycine as a longstanding standard sample, we established a reference point for the ^{13}C mass sensitivity enhancement brought about by the MAS CryoProbe. Since the standard workflow for many studies, especially in the field of structural biology, relies on ^{13}C observe experiments, this reference is important. However, Bruker acknowledges that the research sample is what truly matters to our customers. In that respect, we have collaborated with a large group of scientists from all over the world, to explore further the sensitivity enhancement of our MAS CryoProbe. The following sections summarize our findings in the field of small molecules, pharma, material science and structural biology.

We trust that the application examples will effectively demonstrate that the MAS CryoProbe is offering a game changing sensitivity enhancement for solid-state NMR.

Structural Studies of Large Biomolecular Assemblies

In structural biology, the analytical quest aims to unravel the intricate function of proteins through structure and dynamics. Structure is obtained through various amino acid sequence specific ^{13}C-^{15}N correlation experiments which are complemented by spin diffusion based ^{13}C-^{13}C correlation experiments for distance constraints between the various protein building blocks. While such experiments are part of a standard toolset, the acquisition time can often be quite lengthy. The MAS CryoProbe adds value to such experiments by substantially reducing the required experimental time. Larger biological assemblies are optimally studied at higher magnetic fields to improve sensitivity and resolution. Selective labelling strategies can be used to alleviate resonance overlap. Such sparse isotopic labelling reduces sensitivity and experiments become impractical through excessively long data acquisition times. The MAS CryoProbe helps to overcome sensitivity challenges, rendering resonance assignments possible.

Microtubules (Hassan et al.)

Microtubules are dynamic polymers made up of tubulin subunits. They are closely involved in various cellular processes, as they provide structural support to the cell. Their dynamic nature allows them to constantly assemble and disassemble, which is essential to their function within the cell. The motor protein kinesin binds to microtubules and plays a key role in intracellular transport.

To gain a better understanding of the assemblies, a comprehensive set of experiments was performed by the group of T. Polenova on a sample of U(^{13}C-,^{15}N-) labelled Kif5b kinesin bound to microtubules. Although only about 9 % of the sample was isotopically labelled, excellent quality 1D ^{13}C and ^{15}N CPMAS spectra were immediately obtained, using the MAS CryoProbe. Figure 5 shows a 2D ^{13}C-^{13}C CORD experiment recorded in about 1 hour with a single scan. Such an experiment leads to a fast sample quality assessment and estimation of the secondary structure of the protein.

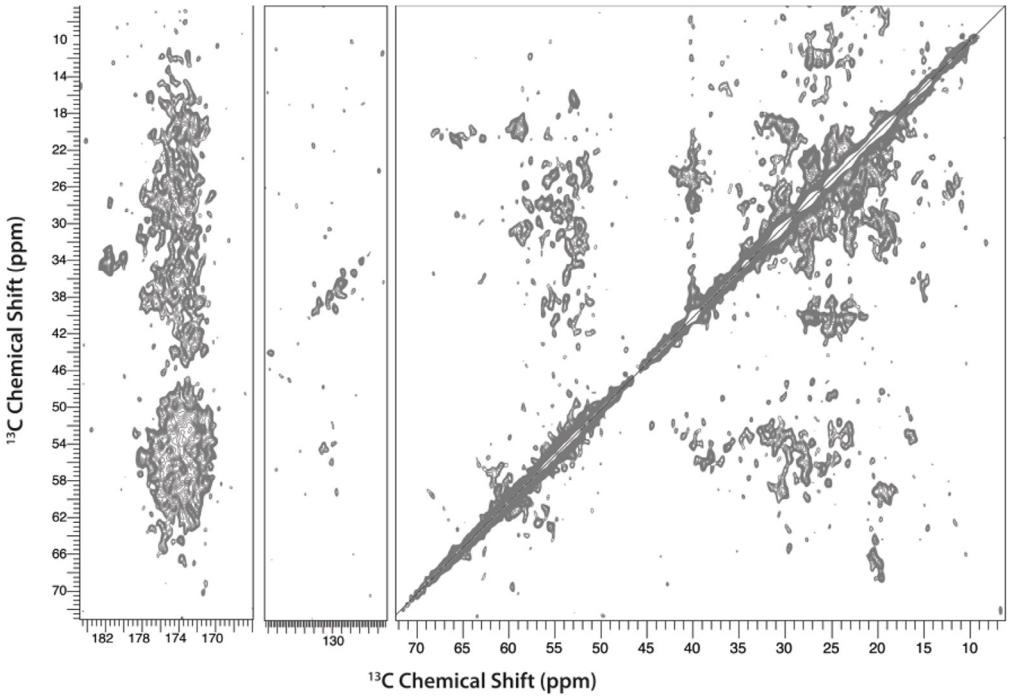

Figure 5 2D ^{13}C-^{13}C CORD experiment acquired on a sample of U(^{13}C-,^{15}N-) labelled Kif5b kinesin bound to microtubules. The total amount of isotopically labelled material is 9 %. The experiment time was 1 hour and 15 minutes.

The sensitivity enhancement of the MAS CryoProbe allows the acquisition of 3D NCACX/NCOCX data sets in a fraction of the time required with a conventional probe.[2] The significant reduction in acquisition time provided by the MAS CryoProbe results in superior data quality with respect to both signal to noise and resolution.

Assemblies of HIV-1 U^{15}N Capsid Protein Without ^{13}C Labelling (Hassan et al. 2020)

The MAS CryoProbe has proven instrumental in providing informative NMR spectra from samples with carbon at natural isotopic abundance. The study reported that a combination of four 2D experiments, based on ^{1}H-^{15}N, ^{15}N-^{15}N, NCA, and NCO correlations, provides a viable strategy for obtaining resonance assignments of proteins and protein assemblies, even in the absence of ^{13}C labels.

While there are only a few examples of ^{15}N-^{15}N protein spectra due to their low sensitivity and long experimental time, the MAS CryoProbe facilitated the acquisition of a ^{15}N-^{15}N PDSD spectrum of exceptional quality. The spectrum contained numerous well-resolved cross peaks that could be readily assigned. Moreover, the data allowed sequential correlations to preceding and subsequent residues to be observed, enabling the unequivocal identification of all proline residues, which are typically elusive in 2D and 3D NCACX/NCOCX datasets.

Although ^{15}N double cross polarization transfers to natural abundance carbons are generally considered unfeasible in proteins, the superior signal-to-noise ratio provided by the MAS CryoProbe made it possible to obtain high-quality 2D NCA and NCO spectra with many resolved peaks in reasonable time frames.

Human Blood Protein Vitronectin (Vn) Bound to Hydroxyapatite (HAP) (Gopinath et al. 2024)

Vitronectin is a multifunctional glycoprotein which is synthesized and excreted by the liver and found in various bodily fluids including serum. Its association with hydroxyapatite (HAP) leads to the formation of large complexes involved in ectopic deposition of lipids and protein, which is related to various pathological disorders like macular degeneration

and Alzheimer's disease. Probing these assemblies at the atomic level is crucial for understanding their formation mechanisms and the underlying molecular interactions.

Preliminary results obtained on a conventional probe underscored severe limitations in sensitivity: recording a set of multidimensional experiments for resonance assignments would require an impractical amount of time. With the MAS CryoProbe, the essential 3D experiments (3D NCACX and 3D NCOCX) were collected in approximately 10 days.

Furthermore, dynamic residues and water-protein interactions were investigated by utilizing the INEPT-based and water-edited NCA experiments. Fast dynamic processes cannot be detected using a dipolar-based cross-polarization approach. However, J coupling transfer schemes such as INEPT transfer can be applied to gain information on the mobile sites. The large number of scans required for such an experiment demonstrates the impracticality for acquiring similar data with a conventional probe. Finally, the WATER-edited NCA was recorded with short $^{1}H^{1}H$-mixing times (10 milliseconds), preserving the specificity at the expense of signal intensity. An approach that can still be afforded with the sensitivity enhancement of a cryogenic probe.

Study of Fibrils (Hassan et al., 2020)

Fibrils consist of linear biopolymers arranged in a rod-shape structure, characterized by a high length-to-diameter ratio. Common examples are collagen, elastin, and even spider silk. Given the ubiquitous presence of fibrils in biological systems, their characterization holds paramount importance in the field of biomaterials and biomechanics. Fibrils are also present in amyloids, which are associated with the onset of various diseases in the human body, with Alzheimer's disease being one of the most devastating.

Our first application example deals with the HET-s (218–289) protein, which forms amyloid fibrils with a rigid amyloid core and limited structural polymorphism. The solid-state NMR spectral quality of HET-s is considered a benchmark for insoluble and non-crystalline protein assemblies. Our results with the MAS CryoProbe show that for a uniformly labeled concentrated sample, high quality ^{13}C NMR spectra can be obtained with 1 scan, as shown in figure 6.

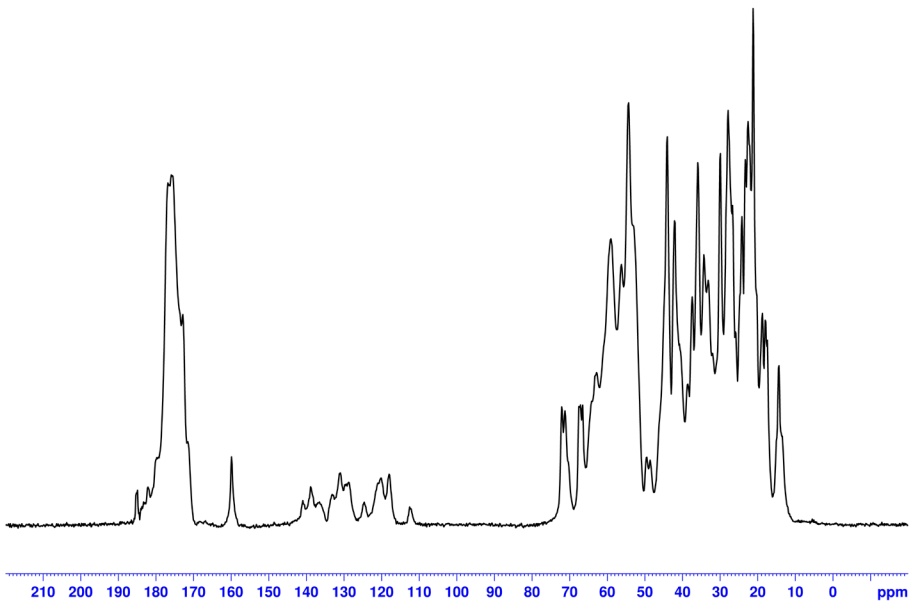

Figure 6 1D ^{13}C CPMAS spectrum of amyloid fibrils formed by the HET-s (218-289) prion domain, uniformly ^{13}C and ^{15}N labelled. The spectrum is obtained with 1 scan. The amount of sample is approximately 50 mg.

Because of the sensitivity enhancement brought about by the MAS CryoProbe, subsequent multidimensional experiments can be optimized on the fly and in a timely fashion on the sample of interest. In the field of solid-state NMR, the ability to perform real-time optimization on such complex systems is a rare occurrence.

Our second example of amyloid fibrils pertains to fibrils of Human Y145Stop (huPrP23-144). Figure 7 shows a ^{13}C-^{13}C correlation spectrum obtained with the MAS CryoProbe. The extra peaks obtained because of the sensitivity enhancement of the CryoProbe are highlighted in yellow, compared to a conventional probe (Helmus et al., 2008).

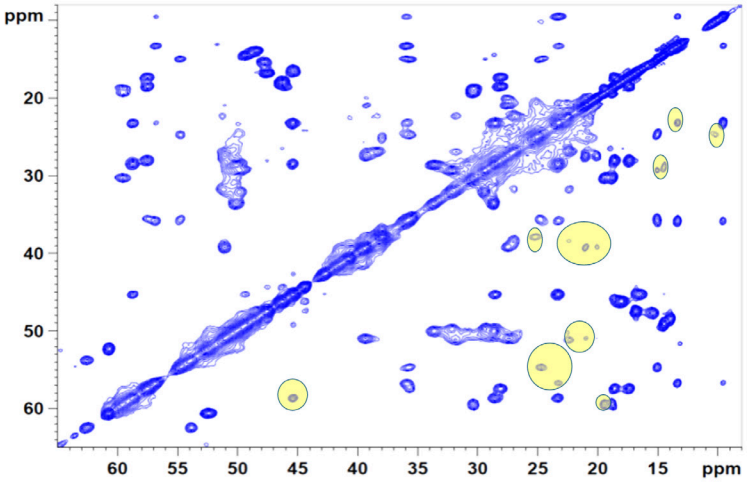

Figure 7 2D ^{13}C-^{13}C DARR spectrum of ~20 mg of (U-^{13}C,^{15}N-) labelled human Y145Stop (huPrP23–144). Experiment time is 5 hours.

The data represents a striking example of the enhanced sensitivity of the MAS CryoProbe: more correlations can be recorded in a similar amount of time, as evidenced by the peaks in the yellow circles. Subsequently, the MAS CryoProbe was used to obtain high-resolution and high sensitivity 2D NCACX and NCOCX spectra, with experiment times ranging from only ~1 – 4 h.

Native Collagen Inside Bone Matrix (Tiwari et al., 2021)

Most structural studies of collagen protein focus on model peptides or extracted collagen, using analytical techniques which offer only static structural details. In bone, the native environment of collagen is shaped by its interactions with minerals, lipids, non-collagenous proteins, citrate, and water. The ability to capture dynamics, as well as orientation-dependent molecular interactions is therefore essential, especially since these interactions result in unique structural arrangements compared to extracted collagen. Understanding collagen's behavior in its native state, both healthy and diseased, can provide insights for bone degenerative disorders, tissue engineering, and bone implants.

However, structural studies of native collagen using solid-state NMR remain challenging due to the low concentration within the bone. The natural abundance spectrum of collagen suffers from very weak sensitivity, therefore only one-dimensional data can be obtained. Increased resolution using 2-dimensional spectroscopy is therefore not possible for these complex materials.

N. Sihna and colleagues have compared the sensitivity performances of their conventional solid-state NMR equipment with the MAS CryoProbe. In a ^{13}C CPMAS experiment recorded on a sample of cortical femora bone of goat (Capra hircus), a sensitivity enhancement of a factor of four was obtained, corresponding to a factor of 16 in experimental time saving. Additionally, signals arising from natural abundance ^{15}N resonances of collagen protein were observed with reasonable signal averaging.

The boost in sensitivity by using the MAS CryoProbe is key for the study of the noncovalent interactions such as hydrogen bonds, electrostatic interaction, as well as hydrophobic interactions responsible for the folding stability of the collagen protein.

Ant Metabolomics Using Solid-State NMR Spectroscopy (Duplais et al., 2021)

In a collaborative effort, researchers from various laboratories joined forces and employed NMR spectroscopy to explore the metabolism of ants. (Duplais et al., 2021). The study primarily aimed to understand the evolutionary process that prompted the *Cephalotes* turtle ants, originally carnivorous, to adopt a vegetarian diet. The research emphasized the significant role of gut bacteria in the cuticle development of these herbivorous ants. The developed methodology holds potential for extension to agricultural and environmental sectors, particularly in studying metabolic alterations in small animals exposed to pesticides.

To identify metabolites using the ^{15}N signals as markers, the ants were fed with ^{15}N-enriched urea. Despite the enrichment and pre-existing knowledge of the chemical shift of several cuticle components, the researchers faced substantial challenges in metabolite assignment. A double-cross polarization (DCP) experiment, which transferred the signals from the ^{15}N (from the processed ^{15}N-enriched urea) to the nearby C atoms at natural abundance was instrumental for further assignments. The experiment was successfully conducted in a few days of acquisition time using a cryogenically cooled MAS probe. From the DCP results it was demonstrated that ant-associated bacteria not only contribute to the production of tyrosine and phenylalanine, but to the formation of cuticular components, such as chitin, cuticular proteins and catecholamine cross-linkers. Hyperpolarization techniques were attempted with limited success, due to the non-wettable nature of the sample.

The challenges posed by the limited sample amount and the sparse concentration of the ^{13}C spins present at natural abundance for the DCP experiment can only be overcome with a cryogenically cooled probe.

Bacterial Cell Wall and Entire Bacteria (Vallet et al, submitted)

Bacterial cell walls have a distinct structural architecture that set bacteria apart from other types of cells. Due to its critical importance for mechanical stability and impermeability, the bacterial cell wall represents a significant target for antimicrobial drugs. Because of the intrinsic dynamic disorder and fluidity of bacterial cells, studies by crystallography or electron microscopy are essentially impossible. Atomic level characterization of such intact systems is amenable with solid-state NMR, and largely benefits from the sensitivity boost provided by the MAS CryoProbe.

Experiments were performed on samples composed of isolated cell walls from *C. glutamicum* and *M. smegmatis* intact bacteria, which were grown in ^{13}C-enriched media. The data in figure 8 shows a 2D ^{13}C-^{13}C correlation spectrum, obtained using J-transfer (SQJ). In black is the spectrum recorded with a 600 MHz 3.2 mm HCN CryoProbe, and in red is the same spectrum acquired with a 3.2 mm conventional probe at the same field strength. The acquisition of the spectrum with the MAS CryoProbe required significantly less time (6.5 hours vs 16 hours) and resulted in better quality data.

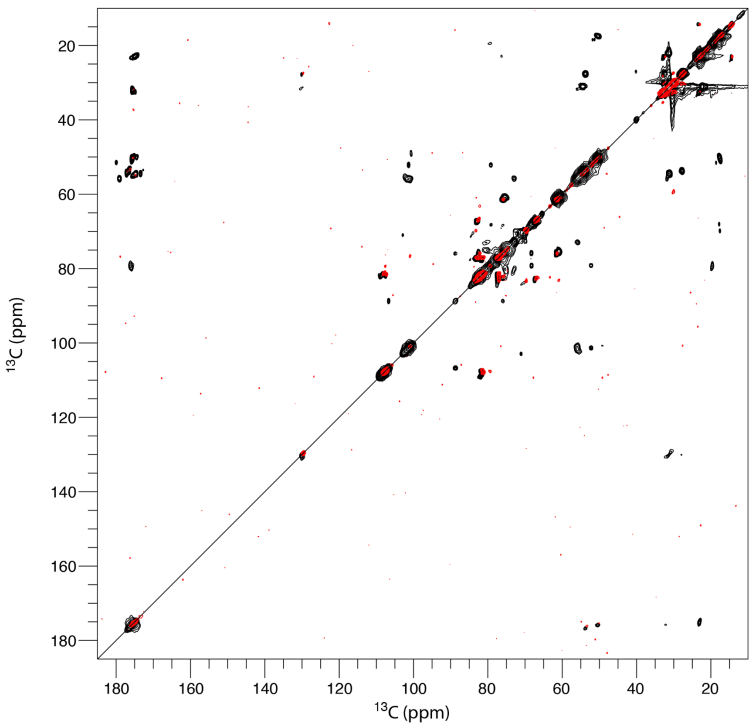

Figure 8 ^{13}C- ^{13}C single quantum J-transfer correlation spectra conducted on *M. smegmatis* entire bacteria and *C. glutamicum* cell wall. In black, the spectrum obtained on a sample of *C. glutamicum* cell wall with the 3.2 mm HCN MAS CryoProbe on a 600 MHz instrument (total experimental time 6.5 hours) and in red the same experiment recorded on a sample of *M. smegmatis* cell wall with a 3.2 mm HCN conventional probe at the same field strength (total experimental time, 16 hours).

Considering the ratio between the rotor volumes, the enhancement corresponds to a sensitivity increase per mg of sample of about 4. However, the absolute sensitivity improvement between both probes is a factor of 8, leading to an experimental time saving of 64.

Plant Cell Wall (Temple et al., 2022)

The leaves of Arabidopsis thaliana, a widely used model organism in plant biology and genetics, have been the subject of extensive study using solid-state NMR. The primary aim of these studies is to enhance the understanding of the molecular mechanisms underlying plant growth and to explore potential ways to influence this process. *A. thaliana* plants, from both the wild type and mutant, were grown under ^{13}C-enriched CO_2, and the leaves collected and subsequently used for solid-state NMR experiments without further purification steps.

PDSD experiments recorded on wildtype uniformly ^{13}C labeled *A. thaliana* leaves using a 600 MHz HCN MAS CryoProbe are shown on figure 9: (black, 44 hours) and a 3.2 mm MHz conventional probe (red, 87 hours) on the same sample and sample amount. At the bottom of figure 9, two representative traces (89 and 105 ppm cross-sections) of the 2D experiments are compared, scaling them according to the ratio of the number of scans. The averaged improvement factor of the CryoProbe calculated along four (only two shows here) 1D sections is about 3. The sample temperature was set to ~ -20° Celsius for both experiments. The superior data quality obtained with the MAS CryoProbe is a testimony to its higher B_1 homogeneity and shorter experimental time.

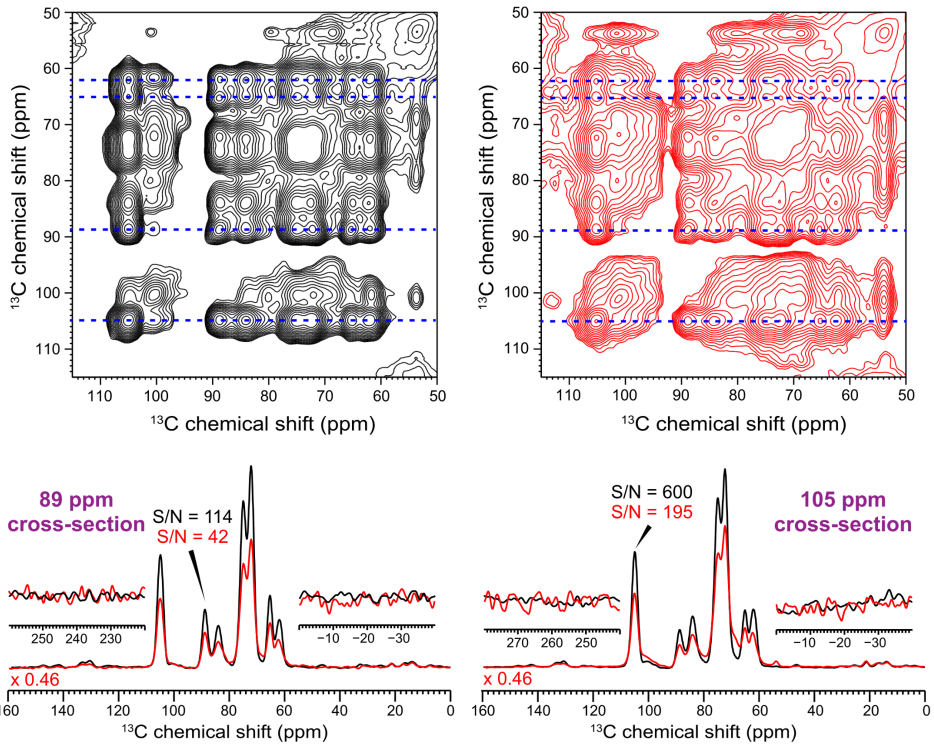

Figure 9 Top: PDSD experiments recorded on wildtype *A. thaliana* leaves (grown under ^{13}C-enriched CO_2) using a 600 MHz HCN MAS CryoProbe (black, 44 hours) and a 3.2 mm MHz conventional probe (red, 87 hours) on the same sample and sample amount. Bottom: Two representative traces (89 and 105 ppm cross-sections) of the 2D experiments are compared, scaling them according to the ration of the number of scans. The averaged improvement factor of the CryoProbe calculated along four (only two shows here) 1D sections is about 3.

Other Biological Systems

The combination of solution- and solid-state NMR with the MAS CryoProbe has proven to be a valid method to achieve atomic-level descriptions of many other biological systems, like intrinsically disordered proteins (IDP), as well as folded domains, within phase-separated droplet.

R. Kriwacki and co-workers used solution-state and solid-state cryogenically cooled probes to investigate how Alternative Reading Frame (ARF) promotes phase separation when mixed with Nucleophosmin (NPM) (E. B. Gibbs et al., 2020), (E. B. Gibbs et al., 2022) and (E. Gibbs et al., 2023).

P. Schanda and F. Napoli (Institute of Science and Technology Austria, Austria) conducted a comparative study of two strategies for the backbone assignments of *Ignicoccus islandicus malate dehydrogenase*, a tetrameric protein composed of four units, each weighing 33.4 kDa and consisting of 310 amino acids (unpublished results).

Another striking demonstration of the sensitivity enhancement of the MAS CryoProbe was evidenced in the study of a catalytic process. More specifically, an enriched kinase is prepared and allowed to react with an isotopically enriched

substrate. The reaction is monitored through the chemical resonance signature of the participating amino acids. Such a study requires very high sensitivity to acquire good signal to noise ^{15}N-^{13}C correlation spectra from the active site. Holmes and coworkers (Holmes et al. 2022) monitored the enzymatic activity of tyrosine kinase, a slow but dynamic process where the MAS CryoProbe was instrumental for acquiring the necessary ^{15}N-^{13}C correlations, showing the detailed chemistry of the catalytic process.

To summarize, the MAS CryoProbe offers significant benefits for the atomic-resolution characterization of various biological systems. We anticipate that these advantages will continue to expand the capabilities of MAS NMR spectroscopy in the field of structural biology.

Materials Science

In material science, NMR is a powerful technique to investigate and characterize the detailed structure and properties of materials. However, samples often suffer from long experiment times and challenging setup requirements. Quadrupolar and low gamma nuclei are especially demanding of hardware performance, while requiring long spectrometer sessions to achieve adequate signal-to-noise ratios. Two-dimensional spectroscopy, such as MQMAS experiments or 2D homo/hetero-nuclear correlation, proves even more difficult.

Material science applications which were previously inaccessible by solid-state NMR for reasons of poor sensitivity are now made possible in these demanding situations with the help of the game-changing enhancement of the MAS CryoProbe, particularly those involving low-gamma and low-natural-abundance nuclei. The following section provides examples showcasing the MAS CryoProbe performance and its impact on results.

Metal-Organic Framework: ^{67}Zn at natural abundance (Zhang et al., 2023)

Metal-Organic Frameworks (MOFs) are an important class of materials with applications in for example separation, gas storage, sensing, catalysis, CO_2 sequestration, and even in biomedicine. These materials present attractive characteristics such as high surface areas, adjustable chemical functionality, and structural diversity. Characterization is crucial to improving the performance of MOFs and designing new MOFs for targeted applications.

Metal centres play key roles in MOF applications, influencing framework topology, stability, and applications. They can be studied by solid-state NMR if the metal ion is an NMR-active nucleus, examples are ^{67}Zn, ^{25}Mg, $^{47/49}Ti$ and ^{43}Ca. Due to their unfavorable NMR properties (large quadrupolar interactions, low natural abundance and small gyromagnetic ratios) these materials exhibit low sensitivity, which hinders the acquisition of useful NMR spectra for characterization. The sensitivity problems are further compounded by the fact that MOFs have very low density, further diluting the spins of interest. In most cases, isotopic enrichment is practically impossible.

Yining Huang and coworkers managed to characterize the Zn ion in a MOF with the ^{67}Zn isotope at the natural abundance level of 4.1 % and a frequency ratio relative to ^{1}H resonance of 6.256803 %. For successful spectroscopy, it is necessary to employ all available sensitivity improvement tools available to NMR (Cavanagh & Rance, 1993). The NMR spectra were obtained employing Double Frequency Sweep (DFS) (Kentgens & Verhagen, 1999) using apodization weighted sampling (Simon & Koestler, 2019) and ultimately, the MAS CRP. Figure 10 shows natural abundance ^{67}Zn 3QMAS spectra of a ZIF-4 MOF with multiple Zn sites in their unit cells. These spectra were collected on an Avance NEO NMR spectrometer at a B_0 field of 18.8 T equipped with a MAS CryoProbe.

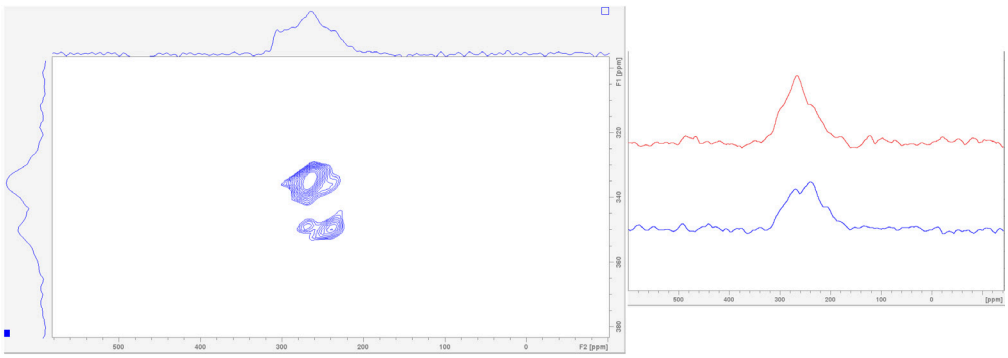

Figure 10 ^{67}Zn DFS-triple quantum MQMAS spectrum of the ZIF-4 sample. With an acquisition time of 3 days, the two sites can be resolved, and the data could be successfully analyzed.

Battery Materials & Catalysis

Solid-state nuclear magnetic resonance has shown tremendous potential in the study of battery materials, particularly in revealing structural details and structural changes (in the charging/de-charging cycle, for example) which can influence their performance. In addition to ^{6}Li, ^{7}Li, and ^{23}Na, a variety of other nuclei are frequently studied in battery materials using solid-state NMR, including quadrupolar nuclei like ^{17}O, ^{25}Mg, ^{27}Al, ^{33}S, ^{39}K, ^{43}Ca, ^{51}V, and ^{67}Zn. As mentioned earlier, NMR measurements on some of these isotopes can pose challenges due to large quadrupolar coupling, low natural abundance and/or low frequency (gyromagnetic ratio), which affect the NMR sensitivity and detectability.

Titanium is a key player in the development of electrodes for various energy storage systems. Specifically, batteries have recently emerged with $Li_4Ti_5O_{12}$ (LTO) as an alternative anode material for rechargeable lithium-ion (Li+) batteries. Their distinguishing features include exceptional cycling stability, long cycle life, superior safety against the formation of lithium dendrites and whiskers which can cause short circuits, failure, and hazardous accidents. They also exhibit better low-temperature performance and higher power density. However, these anodes are highly active catalysts: their propensity to adverse reactions with electrolytes can lead to problems like the development of gas associated with the swelling of the battery pack.

Titanium remains until now one of the most challenging nuclei to study by solid-state NMR, as it possesses two NMR active nuclei often referred to as unreceptive, due to their low natural abundance and low gyromagnetic ratios. In addition, their similar resonance frequencies lead to overlapping of their respective NMR signals, even at high magnetic fields. Larger sample volumes and sensitivity enhancement techniques like quadrupolar CPMG experiments have been used to boost the sensitivity, but with limited efficiency.

A research group at EMPA (Duebendorf, Switzerland) successfully conducted ^{49}Ti/^{47}Ti solid-state NMR experiments on $LiTi_5O_{12}$ battery material, using the MAS CryoProbe. The spectra were recorded under static conditions as well as under MAS (15 kHz) using pulse sequences that involve quadrupolar CPMG schemes for sensitivity enhancement (figure 11). Because of resonance overlap between the two isotopes, modelling and line-shape fitting analysis was performed to derive values of the isotropic chemical shift (δ_{iso}=–800 ppm), quadrupolar coupling constant (CQ = 8.3 MHz) and asymmetry factor (η=0.5), which are indicative of Ti nuclei in a distorted octahedral surrounding. As shown in figure 11, the high magnetic field combined with the MAS CryoProbe enabled the acquisition a ^{47}Ti/^{49}Ti 1D-spectrum in just a few hours. With such a short experiment time, 2D-experiments could be envisioned to increase the resolution and highlight distinct species.

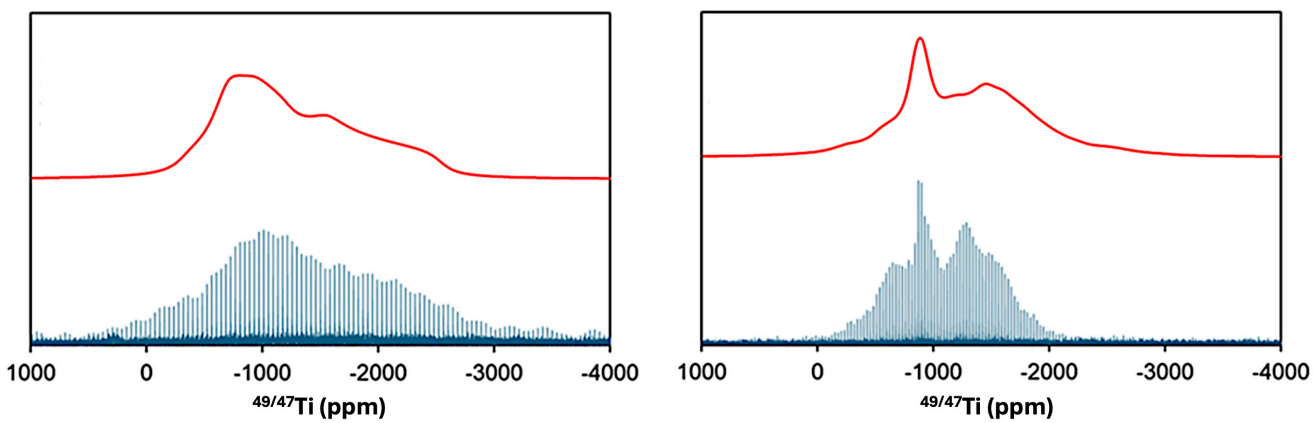

Figure 11 ^{49}Ti/^{47}Ti QCPMG NMR spectra of $LiTi_5O_{12}$ (battery active material) recorded a) under static conditions and b) at 15 kHz MAS rate on an 800 MHz NMR instrument using a MAS CryoProbe (3 hours of measuring time). The spikelets were generated by the CPMG echo train acquisition are shown in blue and the simulated lines in red.

Due to its unique properties, titanium has also emerged as a powerful catalyst in chemical reactions. Titanosilicates are a key group of heterogeneous catalysts where Ti is commonly incorporated into the crystalline lattice of a zeolite-type framework. The efficiency of Ti as a catalyst is closely related to its coordination environment and can be significantly influenced by the preparation method and the state of the catalysts. In this respect, solid-state NMR spectroscopy represents the characterization method of choice, enabling detailed studies of the local coordination geometry of Ti. This contrasts with other spectroscopy methods that only provide averaged structural information.

Sensitivity enhancement techniques like double frequency sweep have been combined with quadrupolar CPMG experiments for more efficient acquisition of 1D titanium NMR spectra. Combining high field with the MAS CryoProbe has allowed the study of Ti in zeolites samples, with industrially relevant Ti loadings of typically 1.5 % by weight. The combination of high field NMR and the enhanced sensitivity provided by the MAS CryoProbe provides the ultimate solution for the investigation of such a challenging sample.

The exceptional performance of the MAS CryoProbe, estimated to provide a 10 to 14-fold improvement in signal-to-noise ratio compared with a 3.2 mm LTMAS probe, has allowed Christophe Copéret and his team (Lätsch et al., 2023) to perform the first-ever ^{47}Ti/^{49}Ti solid-state NMR characterization of such a dilute zeolite sample. The data obtained with the MAS CryoProbe has provided substantial insight on the local structure of the active site of this catalyst, a topic of debate for over four decades.

This study emphasizes the effectiveness of solid-state NMR characterization, by obtaining directly structural information on industrially relevant materials, despite their extremely low loading. It also underscores the superior performance delivered by high-field NMR instruments equipped with a MAS CryoProbe.

Cementitious Materials

Concrete is the second-most-used substance in the world after water, and is the most widely used building material, with a worldwide production exceeding 10 billion tons per annum. Portland cement, the most common cement used to produce concrete, is associated with significant CO_2 emissions, accounting for approximately 8 % of current global anthropogenic CO_2 emissions. Consequently, efforts are currently underway to develop low-CO_2 cementitious materials as sustainable alternatives.

The complex chemical nature of cement systems often involves disordered phases that continue to evolve for many years after initial mixing. This complexity has posed significant challenges in understanding their atomic structure.

High-field solid-state NMR is a powerful analytical tool used in the study of cements and cementitious materials, providing valuable insights into the hydration processes, the formation of calcium silicate hydrate (CSH) phases, and the interaction of additives with the cement matrix. This information is vital for the development of high-performance concrete materials with improved durability and strength. Furthermore, solid-state NMR can be used to study the effects of aging and degradation of concrete materials. Such an initiative can help in the development of strategies for the repair and maintenance of concrete structures, extending their lifespan and overall reducing costs.

An extensive collection of NMR experiments on cement material has been published, probing challenging nuclei such as ^{17}O, ^{25}Mg, ^{29}Si, ^{33}S, ^{35}Cl, ^{39}K and ^{43}Ca. Because of several limitations including low gamma, quadrupolar interactions, long relaxation times and overall low sensitivity, the general strategy is to employ a probe with the largest sample volume available.

The example below on figure 12 shows a comparison of one dimensional ^{29}Si NMR spectra between a 7 mm conventional probe and a 3.2 mm MAS CryoProbe. Taking into consideration experimental parameters and different magnetic fields, the MAS CryoProbe delivers twice the signal to noise, despite using about 1/3 of the sample amount, which is a factor of 4 in experimental time. Quantitative ^{29}Si spectroscopy has been used to determine the C3S/C2S ratio in cements, a parameter which is related to their settling properties. Given the shorter relaxation times at higher fields and the presence of paramagnetic impurities, further optimization would have allowed for shorter acquisition times. In addition, the higher spinning speed capability allowed by the smaller rotor diameter of the MAS CryoProbe would lead to an improvement of the sensitivity and resolution.

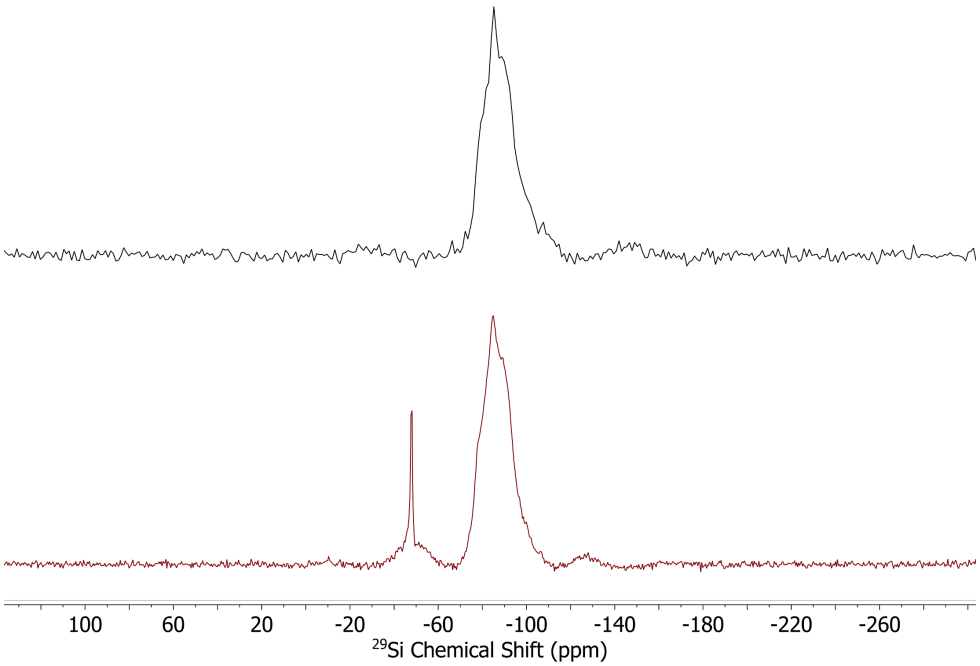

Figure 12 ^{29}Si spectra recorded on Magnesia Silicate Cement (at natural abundance). Top: ^{29}Si direct polarization (hpdec) experiment recorded on a 400 MHz 7 mm broad-band two-channel MAS probe (7168 scans, 30 s of repetition delay, 60 hours of acquisition time). Bottom: ^{29}Si triple-pulse experiment recorded on an 800 MHz 3.2 mm MAS CryoProbe (1024 scans, 30 seconds of repetition delay, 8 hours and 30 minutes of acquisition time). The narrow peak is arising from the ^{29}Si signal coming from the SiN rotor.

The next example presents results obtained on ^{25}Mg at natural abundance. Due to the disordered structure of many cementitious phases, significant line broadening is observed, even at higher spinning speed. Frequently, the ^{25}Mg 1D NMR spectrum does not have sufficient resolution to differentiate sites. A multidimensional experiment can improve the resolution and isolate different species, but unfortunately requires a much longer experimental time. The acquisition of such data is too often out of reach for low gamma nuclei at natural abundance level on a conventional RT probe.

In figure 13 we report an example of a ^{25}Mg 1D and 2D-dimensional spectra recorded at natural abundance of a cementitious sample, using the MAS CryoProbe. The percentage of Mg is estimated to be of 15 %. The overlapping of resonances makes the 1D spectrum of little use. However, the 2D 3QMAS experiment shows at least 3 different sites which can be attributed to different chemical environment around the ^{25}Mg atom.

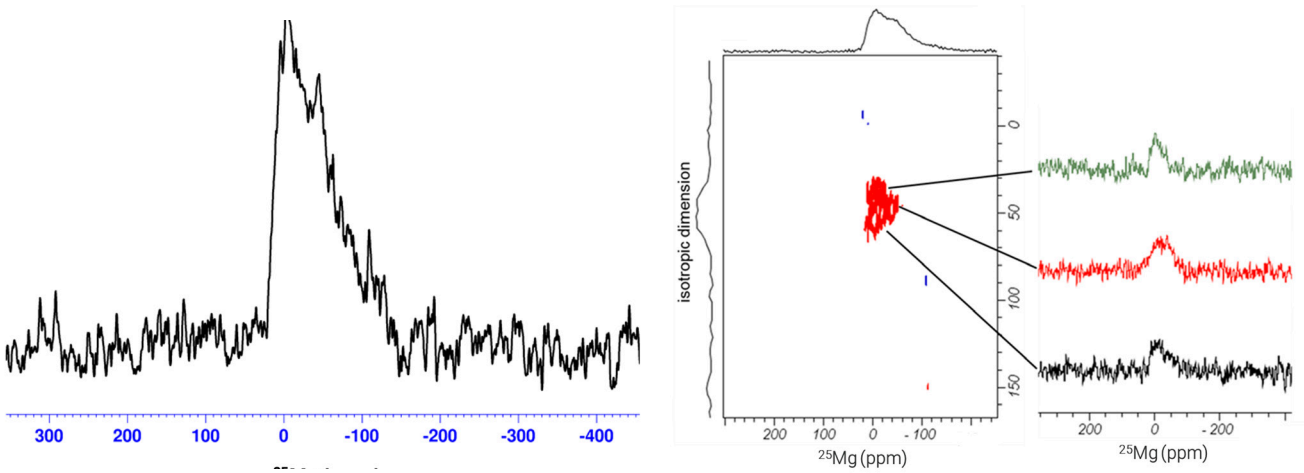

Figure 13 ^{25}Mg spectra recorded on Magnesia Silicate Cement (at natural abundance). Left: Hahnecho spectrum (full-echo acquisition) recorded in about 3 minutes (400 scans, processed with 100 Hz of line broadening). Right: 2D DFS-3QMAS recorded in 1 day and 14 hours (recycle delay 1 second, 34 indirect increments, 3840 scans). At least 3 different sites (black, red, green lines) can be distinguished and assigned to different ^{25}Mg environments.

In summary, the MAS CryoProbe offers a superior solution based on higher sensitivity, higher spinning speed, better B_1 homogeneity and higher RF fields, compared to a larger diameter conventional probe.

Environmental Protection, Food Safety and Sustainability

Solid-state NMR is a versatile analytical technique well suited to address the complexity of environmental systems, and the processes affecting them. Also, solid-state NMR can provide atomic-level information on contemporary topics such as environmental protection, food security and sustainability.

In agriculture, understanding the structure and behavior of cellulose, lignin and other carbohydrates of plant origin can improve crop management practices and contribute to the development of more sustainable and productive farming systems. For instance, NMR has been used to study the structure of plant cell walls (Temple et al., 2022), aiding in the selection of crop varieties with desirable traits, and has contributed to a better understanding of the metabolism of insects (Duplais et al., 2021). Please refer to the section on Biomolecules Assemblies for detailed examples and references.

The pulp industry stands to benefit greatly from the insights provided by solid-state NMR. Understanding the detailed structure and behavior of wood-derived materials can provide guidance for more efficient processes, leading to cost savings and improved product performance. Furthermore, this understanding can aid in the development of more sustainable practices, contributing to the industry's environmental stewardship. For example, NMR studies have facilitated the development of high-performance wood adhesives and coatings and of biofuels from lignocellulosic biomass.

Recently, solid-state NMR has emerged as a powerful tool for studying soil, cereals, flour, meat, and a wide range of other dietary products. For instance, in soil studies, solid-state NMR can reveal the composition and behavior of organic matter, contributing to the understanding of nutrient cycles and soil fertility.

Solid-state NMR is commonly employed also in the research fields related with the thematic of energy saving and CO_2-capture, and we invite the reader to refer to section Material Science for examples of how the MAS CryoProbe can be instrumental in the development of better batteries and cements with reduced environmental impact.

Cellulose and Wood Materials

Cellulose is a complex carbohydrate found in plant cell walls. Despite many years of research on the subject, the supramolecular structure of cellulose is still debated. Understanding the intricate structure of cellulose is crucial for various applications in industries such as textiles, paper, and biofuel production. Some technical celluloses, like the bleached wood pulp, contain extra carbonyl and carboxy groups. Understanding these structures can lead to advancements in the processing and utilization of cellulose-based products.

Solid-state NMR provides a means of investigating this structure in detail, offering insights into how the arrangement of these units affects the material's properties. Cellulose is composed of several repeating units of glucose, as illustrated in figure 14. It has been shown that the chemical shift of the C4 carbon is a sensitive probe to the supramolecular structure in which the glucose unit is present. A model of cellulose widely spread in the literature states that the C4 chemical shift region of the cellulose (80-92 ppm) can be attributed to C4-carbons in glucose units in different environments (figure 14). Among other things, solid-state NMR spectroscopy allows the determination of some critical parameters of cellulose and its derivatives with minimal sample treatment. In particular, the crystallinity index of cellulose and the stoichiometric surface-to-volume ratio, which are two important parameters used to determine the material properties, are readily accessible using ^{13}C solid-state NMR.

The analysis of the CPMAS spectrum requires deconvolution of the C4 region into its subcomponents, as shown in figure 15. The operation of deconvolution is more robust and more accurate when the spectrum presents an optimal signal-to-noise ratio. However, many commercial materials such as bleached wood pulp, contain a high amount of water and typically require extended experimental times. The use of a MAS CryoProbe, with its large sample volume and larger than three-fold increase in sensitivity, is an ideal tool to enhance the throughput of these measurements.

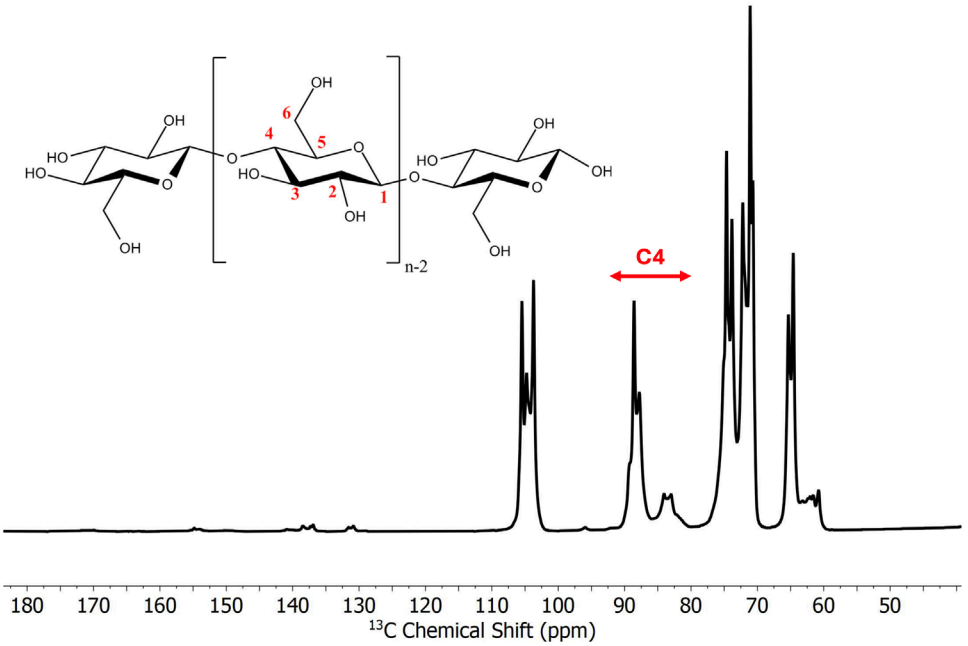

Figure 14 Glucose units forming the cellulose backbone and ^{13}C CPMAS spectrum obtained from a sample of commercial eucalyptus pulp.

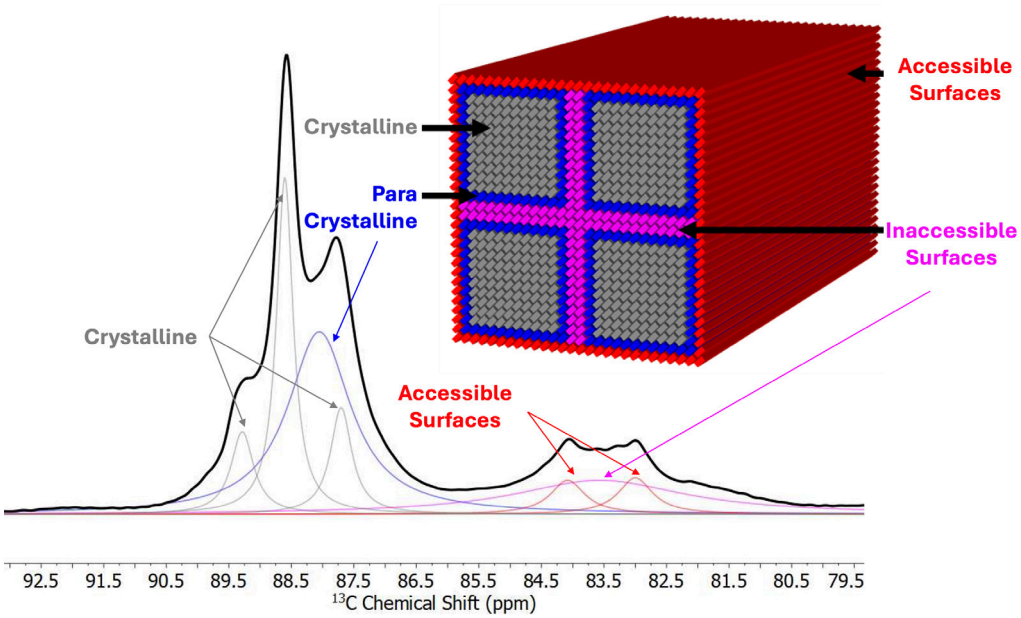

Figure 15 ^{13}C CPMAS experiment recorded on a sample of eucalyptus pulp with a 600 MHz 3.2 mm HCN MAS CryoProbe (11 minutes). The signal region between 80 and 92 ppm is used to derive the crystallinity index and the accessible and inaccessible surfaces according to the deconvolution model drafted at the bottom (picture courtesy of T. Larsson).

The group of T. Larsson from RISE, Stockholm University, Sweden, compared the performance of the MAS CryoProbe with a conventional MAS probe on wet samples of commercial eucalyptus pulp and HCl-hydrolyzed cotton linters. The gain in sensitivity, scaled according to the different number of scans and field strengths, resulted in an enhancement factor of 5.6 for the wet pulp sample and 3.7 for the hydrolyzed cotton linters sample (comparison not shown). In the specific case, the measurement time of 11 hours required on their setup (400 MHz instrument with a 4 mm CPMAS HX probe) was reduced to 11 minutes on a instrument equipped with a MAS CryoProbe (600 MHz HCN MAS CryoProbe).

This gain in sensitivity allows to pursue more advanced experiments. In particular, the 2D refocused INADEQUATE highlights details which might be missed in the lower resolved monodimensional spectra. In figure 16, we report the 2D refocused inadequate on unlabelled wet samples of commercial eucalyptus pulp, recorded in just 8 hours. The assignment to the different carbons is readily accessible.

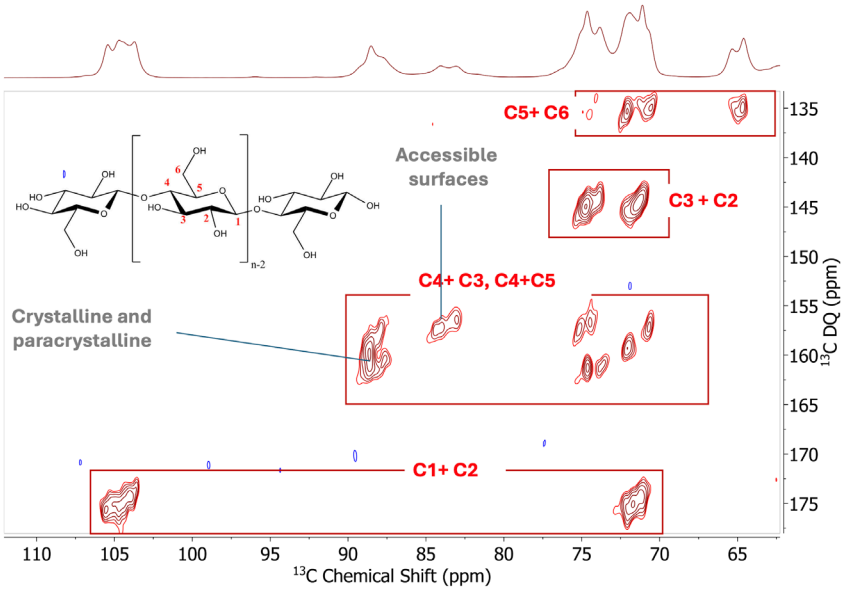

Figure 16 2D ^{13}C-^{13}C refocused INADEQUATE recorded with a 600 MHz HCN MAS CryoProbe on wet samples of commercial eucalyptus pulp with ^{13}C at isotopical natural abundance. The spectrum shows the assignment to the different carbon moieties, and was recorded in just 8 hours employing an apodization weighted sampling scheme (see the Material Science section for the reference).

Because the time required to record such an information-rich spectrum is short with the MAS CryoProbe, the 2D refocused INADEQUATE can be added to the standard tools to characterize wood pulp. As an example in figure 17, we report the overlap between two samples of commercial eucalyptus pulp treated under different conditions. This comparison highlights down to molecular-level precision how the different treatments affect the material, giving fundamental insights for developing new processes and formulations.

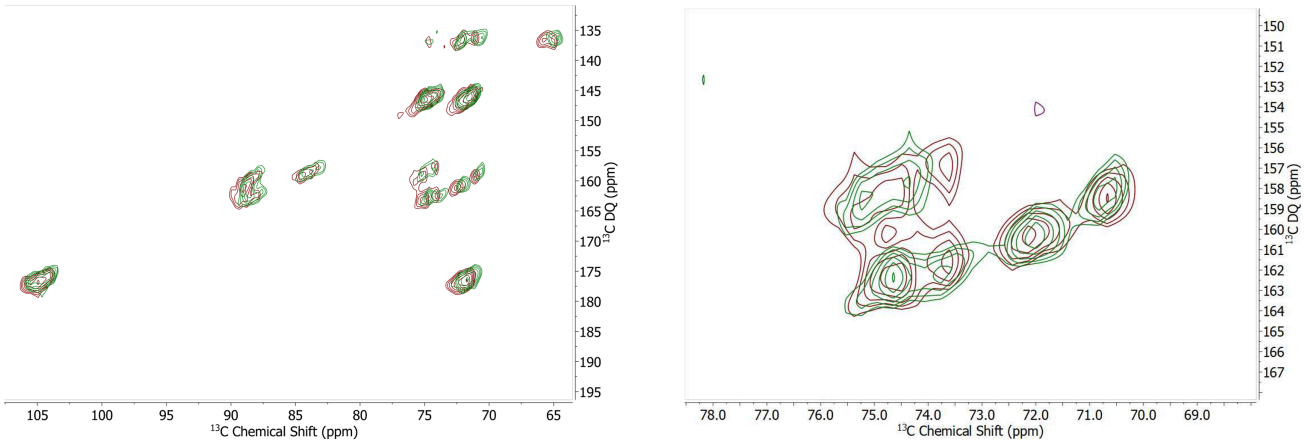

Figure 17 Left: Overlap of 2D ^{13}C-^{13}C refocused INADEQUATE experiments conducted on two samples of commercial eucalyptus wet pulp. One sample (green) was undergoing to a process of hydrolyses by HCl treatment, while the other was not treated (red). Right: The zoom on the accessible and inaccessible regions associated with the C4 position of the glucose units in the cellulose: the broad component is absent in the hydrolysed spectrum, suggesting a high reduction of the inaccessible surface.

In figure 18, we report a spectrum from wood of grapevines collected in the wine-producing region of Bordeaux, France. In this region, there is an economic and ecological concern about an emerging fungal infection of the grapevines. The scientific community is making efforts to study the degradation effects of these microorganisms on the wood. Solid-state NMR is a well-suited analytical technique to provide valuable insights into the mechanisms of degradation, as it can monitor changes in wood composition and structure. Extracting data from a ^{13}C CPMAS spectrum of wood, for subsequent analysis, requires line-fitting and deconvolution, which can be implemented once a model has been developed. To build a robust model for deconvolution, it is very helpful to consult a 2D ^{13}C-^{13}C correlation spectrum, like the rINADEQUATE shown in figure 18. The sensitivity improvement provided by the cryogenically cooled probe is critical to record such a spectrum in a reasonable amount of time.

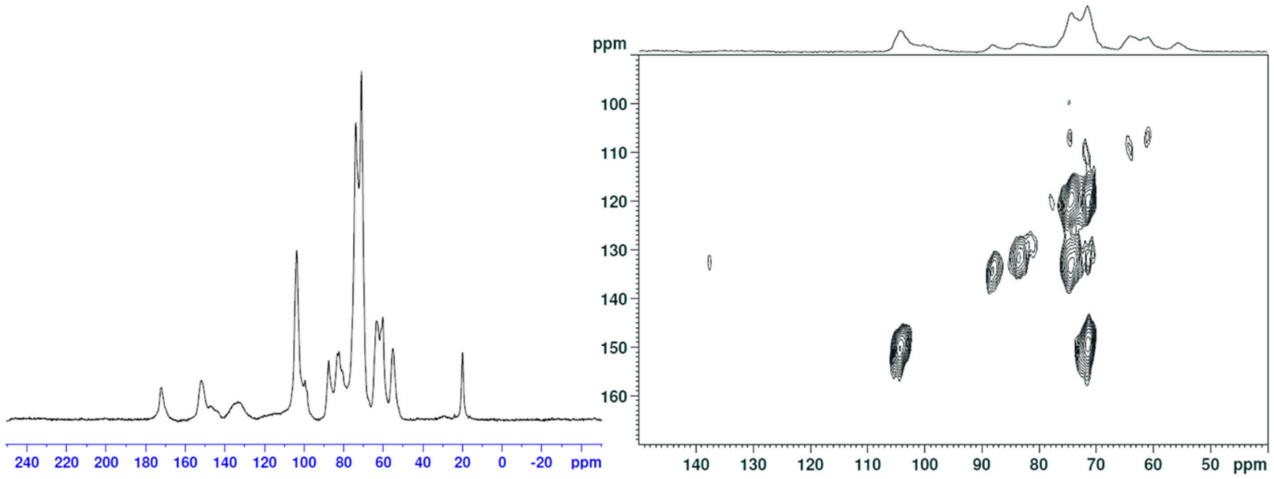

Figure 18 Left: ^{13}C CPMAS spectrum of grapevine wood, recorded on a 600 MHz 3.2mm HCN MAS CryoProbe with 512 scans in 20 minutes. Right: 2D ^{13}C-^{13}C rINADEQUATE recorded on the same sample in 36 hours. Sample courtesy of A. Loquet.

Food Dietaries

In the context of cereals and flour, NMR can elucidate starch crystallinity and protein structures, which are crucial for determining their nutritional and functional properties. When applied to meat, solid-state NMR can provide insights into the water-protein-fat interactions that influence texture and taste. To showcase the expected sensitivity and resolution that could be achieved on these classes of samples, we report in figure 19 a few examples of spectra recorded on samples of bean wheat and chicken sliced meat.

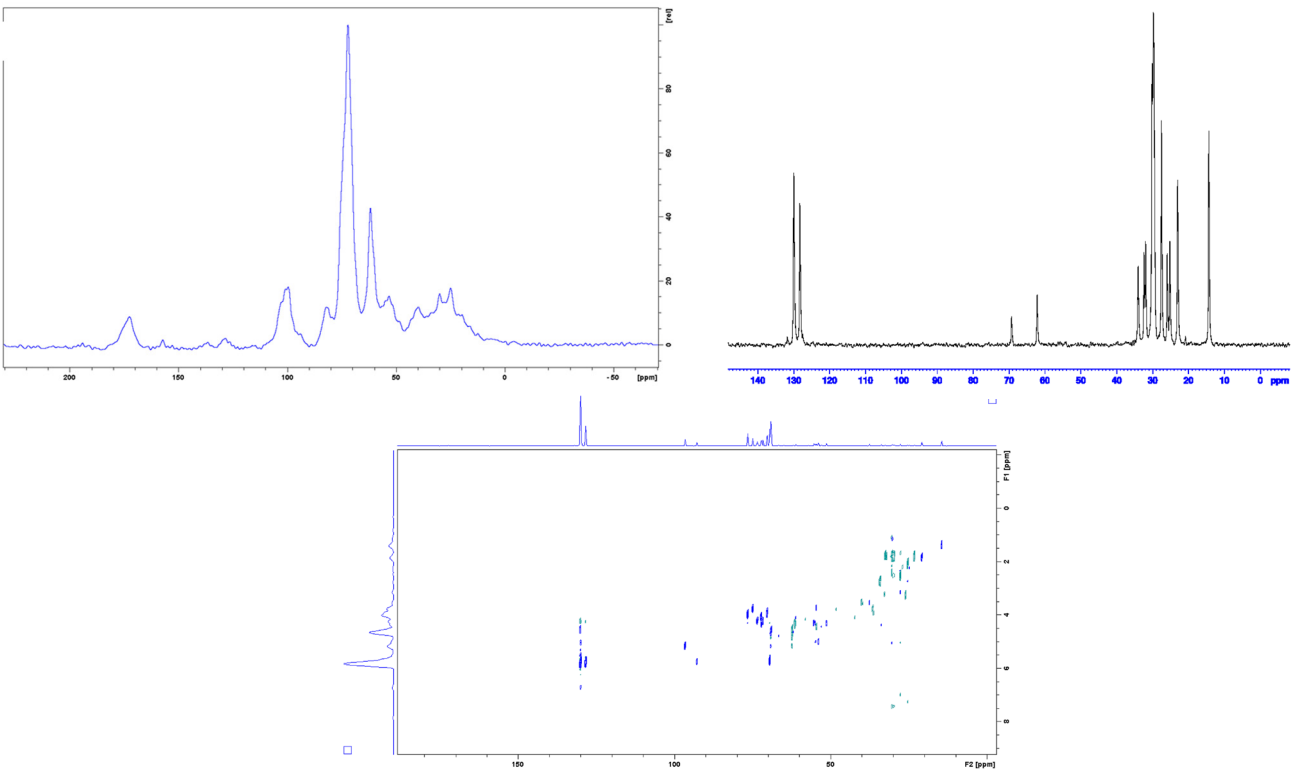

Figure 19 Top: ^{13}C CPMAS and ^{13}C INEPT experiments recorded on a rotor filled with a powder of broad bean. Bottom: 2D Edited ^{1}H-^{13}C INEPT experiment recorded on a sample of minced chicken meat.

Although the application of the MAS CryoProbe in these fields is still not well established, we can envisage that the gain in sensitivity offers the possibility to obtain a more detailed characterization of these complex samples, contributing significantly to advancements in food science, nutrition, and emerging topics in sustainable agriculture and environmental protection.

Small Synthetic Organic Molecules

Cyanide Dye

The 2D refocused INADEQUATE is an invaluable tool for organic chemists, as it enables the connection of neighboring ^{13}C atoms via their J-coupling. However, this experiment lacks sensitivity because the correlations depend on the occurrence of two ^{13}C nuclei adjacent to each other. Given the low natural abundance of ^{13}C, the experiment is unfortunately impractical and rarely used.

The introduction of the MAS CryoProbe has essentially eliminated this sensitivity limitation for small organic solids, allowing for high-quality recordings within a span of 1.5 to 3 days, contingent on the amount of sample and complexity.

Figure 20 presents a 2D ^{13}C-^{13}C correlation spectrum recorded on a highly symmetrical cyanine dye molecule. The assignment of the peaks becomes a very challenging task and necessitates a relatively large number of increments in the indirect dimension to achieve the required resolution. The spectrum was recorded using an apodization weighted sampling scheme (Simon & Koestler, 2019) and a restricted indirect spectral width, both of which contributed to reducing the total experimental time to 3 days. It should be noted that only half of the rotor was filled, thus the experimental time could be significantly shortened if more material is available.

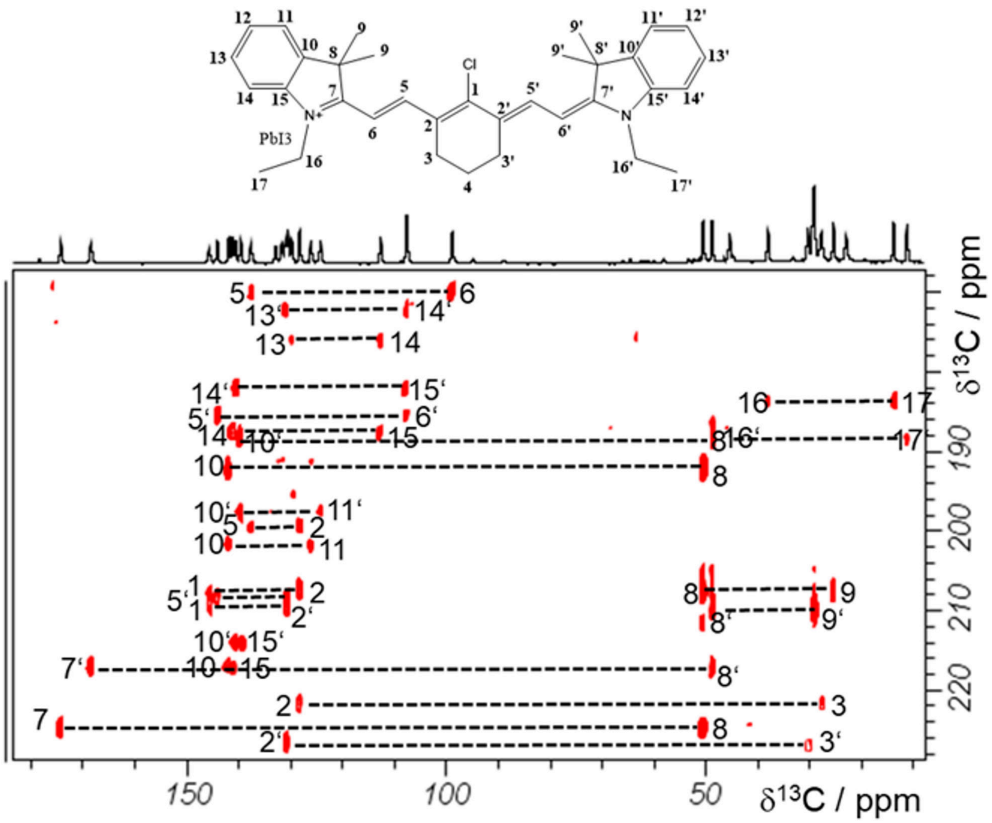

Figure 20 2D refocused INADEQUATE on a medium/small-size organic molecule. The experiment was recorded in about 3 days on a half-filled 3.2 mm MAS CryoProbe rotor (about 55 mg of compound) with a 600 MHz HCN CryoProbe.

For this sample, the enhancement factor measured with the MAS CryoProbe using a 1D ^{13}C CPMAS spectrum was 5.6, compared to a RT probe (data not shown). We can therefore conclude that the INADEQUATE experiment would not be feasible on a conventional probe.

^{17}O NMR in Small Molecules (Shen et al, 2022) (Ha et al., 2021)

Oxygen is a central constituent in biological molecules. Organic functional groups containing oxygen are directly involved in a multitude of reactions, including enzymatic reactions. Despite its central function, oxygen is rarely used as a probe in structural biology for its elusive nature. The NMR active isotope ^{17}O has a natural abundance 0.037 %, a nuclear spin of 5/2, and a relatively low gyromagnetic ratio. The MAS CryoProbe seems the ideal tool for studying biological systems using ^{17}O for its high sensitivity.

Vladimir Michaelis and coworkers (Ha et al., 2021) have developed a very efficient labelling procedure for ^{17}O enrichment of the three amino acids of N-formylmethionine-leucyl-phenylalanine (f-MLF). This approach permits inexpensive ($0.25 USD/mg) insertion of ^{17}O labels, an important experimental technology for biomolecular studies based on ^{17}O detection.

The MAS CryoProbe enabled the ^{17}O NMR study of the tripeptide, employing all spin manipulation and data acquisition techniques available: double-frequency sweep and apodization weighted sampling (Simon & Koestler 2019, Kentgens & Verhagen 1999). The results demonstrated the analytical potential of ^{17}O based solid-state NMR using the CryoProbe technology by revealing an intermolecular hydrogen bond between amino acids using a 2D ^{1}H-^{17}O heteronuclear correlation experiment (data not shown).

Gang Wu and coworkers (Shen et al., 2022) showed how functional groups in α-D-glucose can be characterized using a full toolbox, including ^{17}O enrichment, paramagnetic doping for faster data acquisition, polarization enhancement techniques through spin manipulation, and finally, the sensitivity enhancement of the MAS CryoProbe. The results shown on figure 21 represent a high-quality ^{17}O multiple-quantum (MQ) MAS 2D spectra of a doped and ^{17}O labeled glucose sample. The unprecedented spectral resolution permitted the detection of a key structural difference for a single hydrogen bond between two types of crystallographically distinct α -D-glucose molecules.

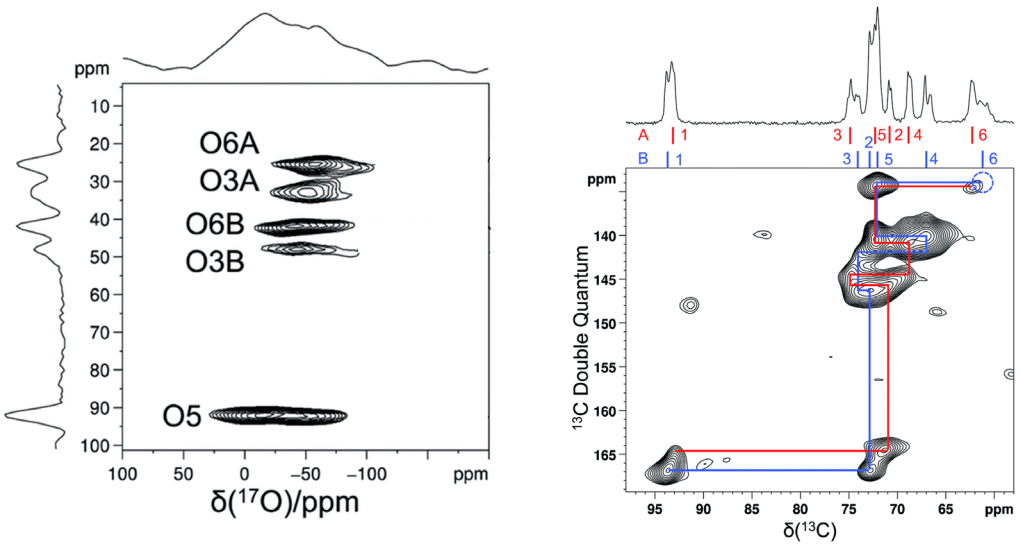

Figure 21 2D refocused INADEQUATE on a medium/small-size organic molecule. The experiment was recorded in about 3 days on a half-filled 3.2 mm MAS CryoProbe rotor (about 55 mg of compound) with a 600 MHz HCN CryoProbe.

These results represent the first case where all oxygen-containing functional groups in a carbohydrate molecule are site-specifically ^{17}O-labeled and fully characterized by solid-state ^{17}O NMR. They also constitute the first set of ^{17}O 3QMAS spectra ever reported for a carbohydrate compound. Employing high field instruments and the MAS CryoProbe to study unreceptive nuclei will be of fundamental importance for recording multidimensional experiments on even more complex systems in the future.

Pharmaceutical Applications: NMR Crystallography, Polymorphism and API Characterization

Most Active Pharmaceutical Ingredients (APIs) are manufactured, distributed, and consumed as solids. Many APIs have multiple polymorphic forms, each of which can have distinct physicochemical properties influencing their bioavailability and stability.

Therefore, it is essential for the pharmaceutical industry to have reliable techniques for the characterization of APIs at every stage of their development, including the bulk forms and the final dosage formulations (i.e., tablets and capsules). ^{13}C solid-state NMR represents a common method for such a characterization, but often suffers from low sensitivity and interference with excipients. In the next section, we will outline how the sensitivity enhancement of the MAS CryoProbe not only allows a faster characterization of APIs, but also enables the probing of more challenging nuclei like ^{15}N and ^{35}Cl.

Vitamin D_3

Homonuclear ^{13}C-^{13}C correlation experiments are important for structural analysis (Olson et al., 2003). At the natural abundance ^{13}C level, such correlation experiments of molecular solids are challenged with low sensitivity requiring impractically long experiment times with conventional probes. Figure 22 shows an example of vitamin D_3 recorded with the MAS CryoProbe with 16-fold reduction of experiment time.

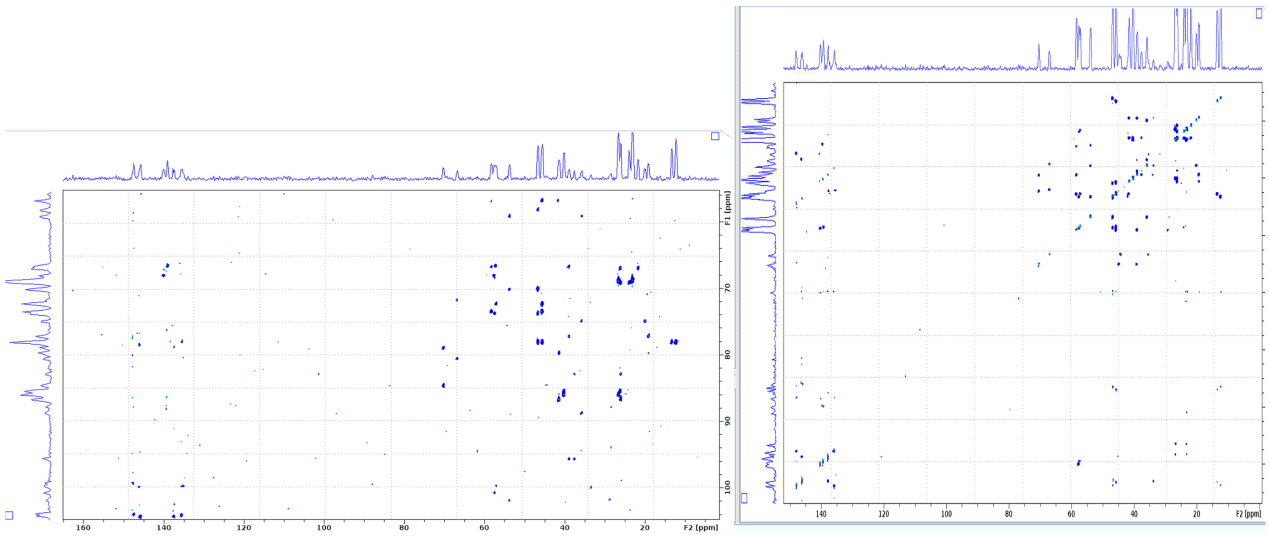

Figure 22 ^{13}C-^{13}C through bond correlation experiments of Vitamin D3 at the natural abundance level show acquired in 11 hours for the rINADEQUATE (left) and 18 hours for the SAR-COSY (right) (Lee et al., 2009).

Using the rINADEQUATE and a narrow spectral width in the indirect dimension requires careful analysis relying on proper identification of the sum frequencies and is less obvious to analyze, yet quicker. The SAR-COSY offers direct analysis of cross peaks but takes more time. Compared to a 14 day experiment for the UC2QF-COSY (Olson et al., 2003), the experiment with the CryoProbe is by a factor of 16 faster and makes these experiments accessible for structural analysis and NMR crystallography.

Posaconazole (Du et al. 2023)

The crystal structure of Posaconazole could be analyzed with a refocused INADEQUATE experiment (figure 23 left, 512 transients, 60 increments in t1, for a total acquisition time of 2 days), and a SAR-COSY experiment (figure 23 right, 192 transients, 368 increments in t1, for a total acquisition time of 4.3 days). Figure 24 shows the 1D ^{13}C and ^{15}N CPMAS spectra of Posaconazole, with experiment time of 17 minutes and 4 hours respectively. The signal enhancement for ^{13}C as well as for ^{15}N was found to be greater than a factor of 4, with a SINO per scans of 25:1 for the 3.2 mm MAS CryoProbe and 5.8 for the 4 mm conventional probe, scaled for the field difference.

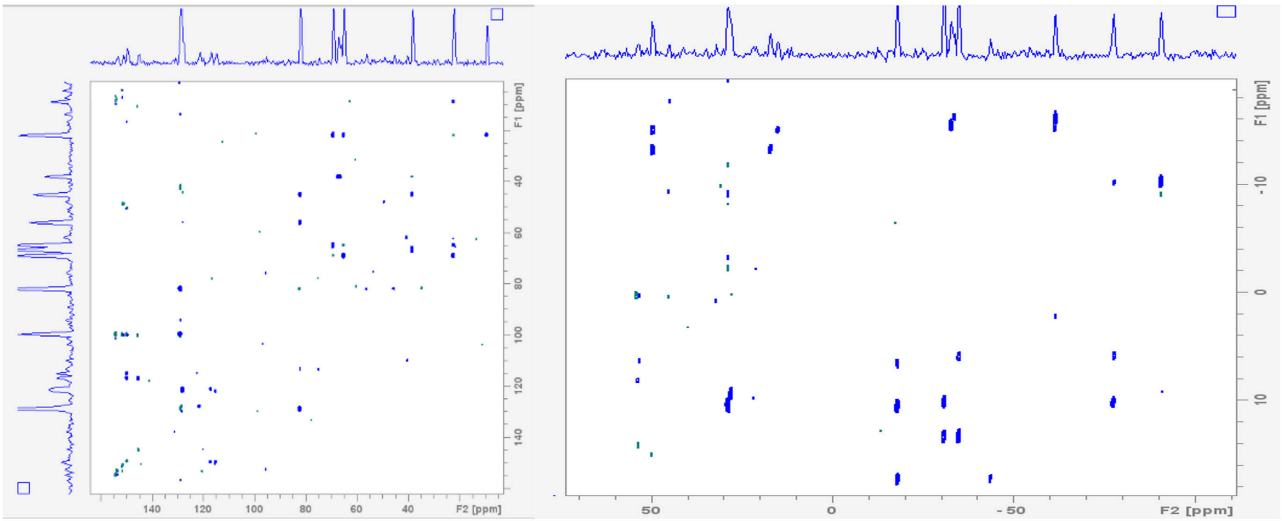

Figure 23 ^{13}C-^{13}C through bond correlation experiments of Posaconazole requires longer recycle delays for data acquisition, namely 5.8 s. The data acquisition time for the SAR-COSY experiment for posaconazole was 113 hours using 192 transients, 368 experiments in t1, while the INADEQUATE had taken 18 h 22 min using the same number of transients, 512 scans were used for the rINADEQUATE experiment requiring 49 hours data acquisition time with excellent signal-to-noise.

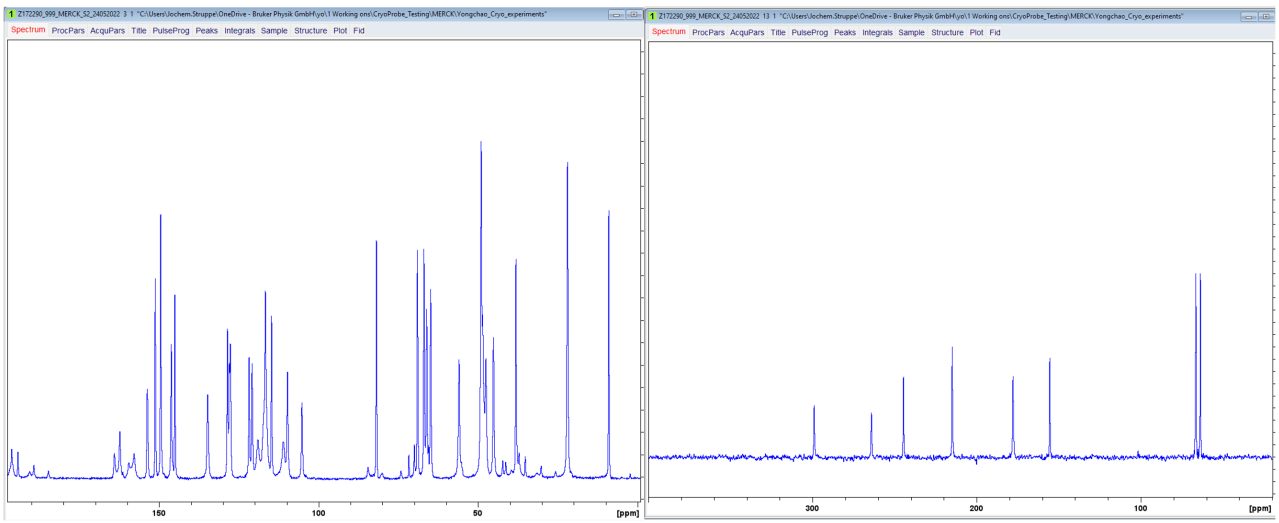

Figure 24 ^{13}C and ^{15}N CPMAS spectra of Posaconazole demonstrating that while the ^{13}C spectrum takes 17 minutes with the CryoProbe (left), the ^{15}N spectrum (right) was acquired in 4 hours with 2432 accumulated transients with an excellent signal to noise permitting reliable CS assignment.

Yong Du and collaborators also show an application to an amorphous preparation of posaconazole with the successful analysis of the molecular network through ^{1}H-^{13}C correlation spectra. One caveat for the rINADEQUATE or SAR-COSY experiments lies in the fact that these experiments need a long enough T_2 for the magnetization to survive the J-evolution periods of 4 milliseconds lasting in total 16 milliseconds for the 4 evolution periods. Nevertheless, based on structure and results of ^{13}C-^{1}H HETCOR experiments with the MAS CryoProbe, all necessary structural studies are available for the analysis of the amorphous preparation.

Lansoprazole (Li et al. 2020)

Enhancement factors of similar magnitude have been recorded for ^{15}N. Figure 25 shows the comparison between the ^{15}N CPMAS spectra of Lansoprazole, a proton pump inhibitor, recorded on a 400 MHz 4 mm room temperature probe and on a 3.2 mm 600 MHz HCN MAS CryoProbe. Even taking into consideration the different magnetic fields, the sensitivity is significantly improved with the MAS CryoProbe, allowing to acquire the data in just 44 minutes. This is in contrast with the experimental time of more than 3 days using a conventional RT probe.

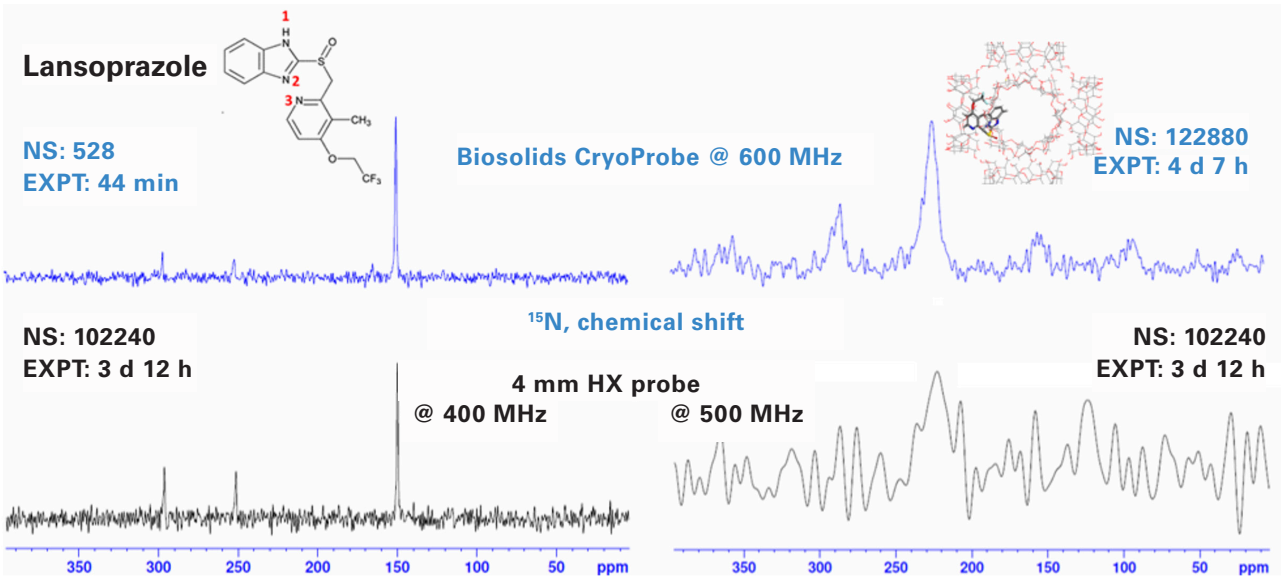

Figure 25 Top: ^{15}N spectra of Lansoprazole acquired with a 600 MHz 3.2 mm HCN MAS CryoProbe. Bottom: A 4 mm 400 MHz HX room temperature probe. The resonance at 150 ppm appears in both spectra with similar intensity, but the spectrum on the top shows significantly less noise, by approximatively a factor of 2. Considering the scaling factors due to the different magnetic field strengths and the square root of the ratio of the number of transients acquired for each spectrum, 0.072, the signal to noise improved by a factor of 7. The resonances at 300 and 250 ppm show a signal to noise value of 3.5. The reason for the different improvement factor may be found in properties of the CP experiment being not quantitative and depending on the position of the carrier frequency and its offset to the ^{15}N resonances in Hz.

The MAS CryoProbe is a true game changer for the NMR analysis of small molecule crystals. The increased sensitivity offers fast data acquisition for various experiments on unlabeled samples. As pointed out earlier, ^{15}N NMR at natural abundance is very insensitive, requiring significant number of transients. Combined with long ^{1}H relaxation properties, the typical experimental times often exceed acceptable limits.

Hydrochloride Salts of APIs

More than 50 % of APIs are manufactured as HCl salts, for the purposes of stabilizing their crystalline forms, while allowing for a high degree of bioavailability upon dissolution. The chloride ions in HCl APIs sit in unique environments with intricate hydrogen bonding arrangements. Interestingly, there is an excellent NMR handle capable of providing a unique spectral fingerprint for each polymorph containing ^{35}Cl. We have used the MAS CryoProbe on static samples of APIs in their dosage form. Figure 26 shows in blue the static ^{35}Cl WURST-CPMG spectrum of a crushed Zantac tablet (150 mg) acquired on a 500 MHz WB system using a 4 mm MAS probe. The acquisition time is approximately 1 hour, which significantly contrasts with the spectrum in red, acquired at 800 MHz with the MAS CryoProbe in less than 2 minutes (128 scans). On the right, a similar comparison is shown for a Benadryl (diphenhydramin HCl) crushed tablet (25 mg).

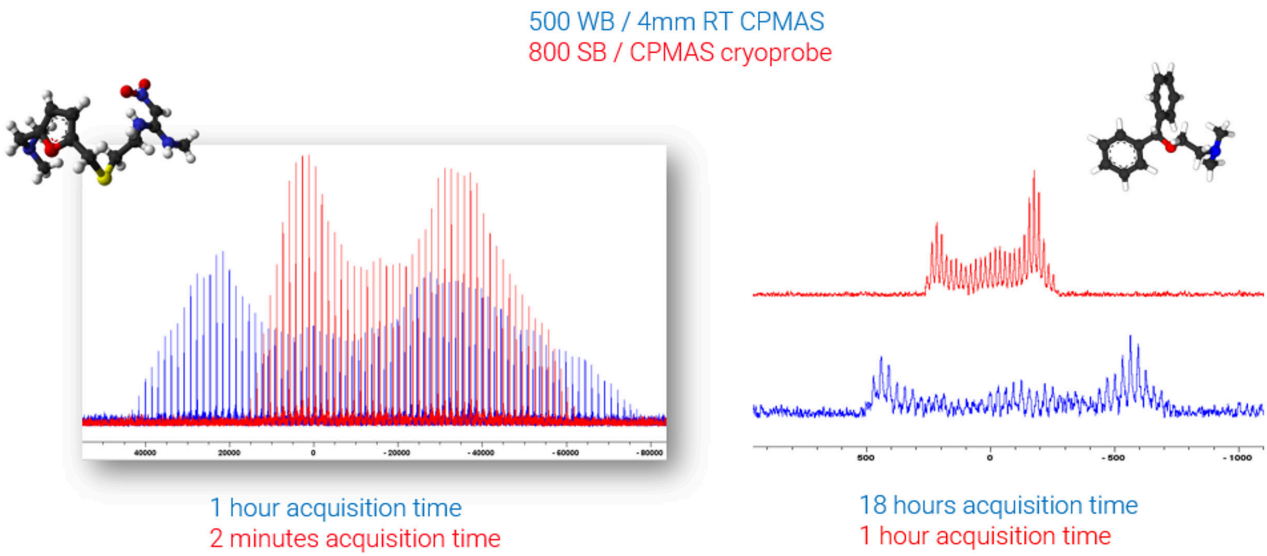

Figure 26 A comparison between the ^{35}Cl NMR results obtained with a 4mm 500 WB probe (blue) and the MAS CryoProbe (red). The enhancement for such an experiment is more than 10-fold.

Despite the different magnetic fields, the enhancement provided by the MAS CryoProbe is truly phenomenal.

Conclusion

The enhanced sensitivity of the MAS CryoProbe has been illustrated through numerous examples presented in this document. In multiple instances, the MAS CryoProbe yielded data that had never been obtained previously with conventional RT equipment. Several cases involving low sensitivity samples have demonstrated that additional dimensions could be recorded to achieve higher resolution. Other examples have shown a significant reduction of acquisition time, leading to an increase in productivity.

We believe the breakthrough in this technology will be applicable and relevant to the research community, allowing the exploration of new avenues in solid-state NMR. To conclude, we would like to share some testimonials from our collaborators:

Prof. Tobias Sparrman, Umea University, Sweden

With the astonishing sensitivity of our 3.2 mm HCN MAS CryoProbe for 600 MHz we enter a new world of possibilities for complex samples like membrane proteins and plant cell walls. I'm especially amazed by the DCP performance indicating not only the expected CryoProbe sensitivity gain but also a fantastic B1-homogeneity.

Prof. Len Mueller, University of California Riverside, USA

The sensitivity is game-changing: experiments that previously took us 5 days can now be done in less than 5 hours. This has allowed us to observe and characterize short-lived intermediates and minor species within the active site of a 72 kDa protein, all while the sample remains near room temperature and catalytically active.

Prof. Tatyana Polenova, University of Delaware, USA

The MAS CryoProbe is truly transformative technology. We tested the performances with assemblies of proteins that cannot be studied by other structural biology techniques due to their disordered nature. Gratifyingly, with the MAS CryoProbe we could acquire excellent sensitivity 3D spectra in a matter of a few days.

Dr. Miguel Mompean, IQF-CSIC, Spain

We conducted preliminary performance testing on the MAS CryoProbe: experiments that traditionally required overnight acquisition can now be completed within less than one hour and with an amazing signal-to-noise. The MAS CryoProbe exhibits exceptional capabilities and enables groundbreaking advancements in the emerging field of hybrid amyloids and their interactions and dynamics.

Dr. David Withall, Rothamsted Research, UK

The CPMAS BioSolids CryoProbe enables significant progress in crop protection and environmental research alongside other areas. It can help answer critical questions about pest biology, crop and plant science, and environmentally-friendly pest management solutions.

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Tobias Sparrman and Gerhard Gröbner, Department of Chemistry, Umeå University, Sweden.

Miguel Mompeán, Instituto de Química Física "Blas Cabrera", Spain.

David Withall, Rothamsted Research, England, United Kingdom.

Rachel Martin, Department of Chemistry, University of California, Irvine, California, USA.

Galia Debelouchina, Department of Chemistry and Biochemistry, University of California, San Diego, California, USA.

Greg Holland from Department of Chemistry & Biochemistry, San Diego State University, San Diego, California, USA.

Jacob B. Holmes, Viktoriia Liu, Bethany G. Caulkins, Eduardo Hilario, Rittik K. Ghosh, Adam D. Gill Jennifer A. Romero, Paul M. Bogie, Chia-en A. Chang, Richard J. Hooley, Yuliana K. Bosken ,Michael F. Dunn and Leonard J. Mueller, Department of Chemistry, University of California Riverside, USA.

Tata Gopinath, Kyungsoo Shin, Ye Tian, and Francesca M. Marassi, Department of Biophysics, Medical College of Wisconsin, Milwaukee, USA.

Marianna Porcino, Charlotte Martineau-Corcos, Ludovic Brutinot, Vincent.Sarou-Kanian, Franck Fayon and Pierre Florian, Université d'Orléans, France.

Eric G. Keeler, New York Structural Biology Center, New York, USA.

Kelsey McCoy and Ann E. McDermott, from the Department of Chemistry, Columbia University, NYC, USA.

Kong Xueqian, formerly Zhejiang University, Hangzhou, P.R. China.

Ivan V. Sergeyev, formerly Bristol Myers Squibb, Cambridge, MA, USA.

Cristina Coelho-Diogo, Cédric Lorthioir, Yannick Millot, Baptiste Rigaud and Christian Bonhomme, Sorbonne Université, Paris, France.

Caitlin M. Quinn, Chunting Zhang, Changmiao Guo, Brent Runge, Tatyana Polenova, Department of Chemistry and Biochemistry, University of Delaware, USA.

Angela M. Gronenborn, Pittsburgh Center for HIV Protein Interactions, University of Pittsburgh School of Medicine, USA.

Wonpil Im, Departments of Biological Sciences, Chemistry, and Bioengineering, Lehigh University, USA.

Theint Theint, Hanh H. Dao and Christopher P. Jaroniec, Department of Chemistry and Biochemistry, The Ohio State University, USA.

Mélanie Berbon, Alons Lends, Birgit Habenstein, Antoine Loquet, Université de Bordeaux, France.

RamaNand Rai, Department of Chemistry, Institute of Sciences. Banaras Hindu University, India.

Nidhi Tiwari, Navneet Dwivedi and Neeraj Sinha, Centre of Biomedical Research, SGPGIMS Campus, India.

Corrie S. Moreau from the Department of Entomology, Department of Ecology and Evolutionary Biology, Cornell University, USA.

Christophe Duplais and Yannick Estevez, CNRS UMR8172 EcoFoG, AgroParisTech, Cirad, INRAE, Université des Antilles, Université de Guyane, France,

John T. Wertz from the Department of Biology, Calvin University, USA.

Estelle Martineau, Jonathan Farjon and Patrick Giraudeau, Université de Nantes, France.

Alicia Vallet, Isabel Ayala, Jean-Pierre Simorre, Catherine Bougault, Univ. Grenoble Alpes, France.

Federico Napoli and Paul Schanda, Institute of Science and Technology Austria, Austria.

Henry Temple, Alberto Echevarría-Poza, Jan J. Lyczakowski, Igor Yakunin, Oliver M. Terrett, and Paul Dupree, Department of Biochemistry, University of Cambridge, UK.

Weibing Yang, Sainsbury Laboratory, University of Cambridge, UK.

Ray Dupree, Department of Physics, University of Warwick, UK.

Juan Pablo Parra-Rojas, Susana Saez-Aguayo, and Ariel Orellana, Centro de Biotecnología Vegetal, FONDAP Center for Genome Regulation, Facultad de Ciencias de la Vida, Universidad Andrés Bello, Chile.

Pyae Phyo and Mei Hong, Department of Chemistry, Massachusetts Institute of Technology, USA.

Eric B Gibbs, Qi Miao, and Richard Kriwacki, Department of Structural Biology, St. Jude Children's Research Hospital, USA.

Victoria N. Drago and Timothy C. Mueser, Department of Chemistry and Biochemistry, University of Toledo, USA.

Robert P. Young from the Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory, USA.

Joana Paulino, Xiaoling Wang and Frederic Mentink-Vigier, National High Magnetic Field Laboratory, Florida State University, USA.

Gwladys Riviere and Joanna R. Long, Department of Biochemistry and Molecular Biology, McKnight Brain Institute, National High Magnetic Field Laboratory, University of Florida, USA.

Ivan Hung and Zhehong Gan, National High Magnetic Field Laboratory, Tallahassee, USA.

Lukas Lätsch, Imke B. Müller, Alexander V. Yakimov, Sadig Aghazada, Zachariah J. Berkson, and Christophe Copéret, ETH Zurich, Department of Chemistry and Applied Biosciences, Switzerland.

Andreas Brinkmann and Victor Terskikh, National Research Council Canada, Canada.

Wanli Zhang, Vinicius Martins and Yining Huang, Department of Chemistry, University of Western Ontario, Canada.

Tomas Larsson, RI.SE and Royal Institute of Technology (KTH), Sweden.

Alexander Remhof, Frederik Nüesch, Ellina Bernard and Daniel Rentsch, EMPA, Switzerland.

Job Boekhoven, Department of Chemistry, Technical University of Munich, Germany.

Michelle Ha, Serge Nader, Sheref S. Mansy and Vladimir K. Michaelis, Department of Chemistry, University of Alberta, Canada.

Jiahui Shen and Gang Wu, Department of Chemistry, Queen's University, Canada.

Yong Du and Yongchao Su, Analytical Research & Development, Merck & Co., Inc., USA.

Xue Li, Anne Zehnacker-Rentien and Ruxandra Gref, Université Paris-Saclay, CNRS, Institut des Sciences Moléculaires d'Orsay, France.

Tao Guo and Jiwen Zhang, Center for Drug Delivery Systems, Shanghai Institute of Materia Medica, China.

Ting Xiong and Weifeng Zhu, Key Laboratory of Modern Preparation of TCM, Ministry of Education, Jiangxi University of Traditional Chinese Medicine, China.

Gilles Patriarche, Université Paris-Saclay, CNRS, Centre de Nanosciences et de Nanotechnologies, France.

Christine Péchoux, Université Paris-Saclay, INRAE, AgroParisTech, GABI, France.

Alexandre Michelet, PerkinElmer, France.

Alexandre Poulhazan, Alexandre Arnold and Dror E. Warschawski, Department of Chemistry, Pharmaqam/NanoQAM, Université du Québec à Montréal, Canada.

Ieva Goldberga, César Leroy and Danielle Laurencin, ICGM, CNRS, Université de Montpellier, ENSCM, France.

Diana Bernin, Department of Chemistry and Chemical Engineering, Chalmers University of Technology, Sweden.

Emma Sparr, Maria Gunnarsson and Daniel Topgaard, Department of Chemistry, Lund University, Sweden.

Kanehashi Kohji, Takahashi Takafumi and Keiko Okushita, Advanced Technology Research Laboratories, Nippon Steel Corporation, Shintomi, Japan.

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