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Magic-Angle Spinning NMR of Cold Samples

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CONSPECTUS

Magic-angle-spinning solid-state NMR provides site-resolved structural and chemical information about molecules that complements many other physical techniques. Recent technical advances have made it possible to perform magic-angle-spinning NMR experiments at low temperatures, allowing researchers to trap reaction intermediates and to perform site-resolved studies of low-temperature physical phenomena such as quantum rotations, quantum tunneling, *ortho-para* conversion between spin isomers, and superconductivity. In examining biological molecules, the improved sensitivity provided by cryogenic NMR facilitates the study of protein assembly or membrane proteins. The combination of low-temperatures with dynamic nuclear polarization has the potential to boost sensitivity even further. Many research groups, including ours, have addressed the technical challenges and developed hardware for magic-angle-spinning of samples cooled down to a few tens of degrees Kelvin.

In this Account, we briefly describe these hardware developments and review several recent activities of our group which involve low-temperature magic-angle-spinning NMR. Low-temperature operation allows us to trap intermediates that cannot be studied under ambient conditions by NMR because of their short lifetime. We have used low-temperature NMR to study the electronic structure of bathorhodopsin, the primary photoproduct of the light-sensitive membrane protein, rhodopsin. This project used a custom-built NMR probe that allows low-temperature NMR in the presence of illumination (the image shows the illuminated spinner module).

We have also used this technique to study the behavior of molecules within a restricted environment. Small-molecule endofullerenes are interesting molecular systems in which molecular rotors are confined to a well-insulated, well-defined, and highly symmetric environment. We discuss how cryogenic solid state NMR can give information on the dynamics of *ortho*-water confined in a fullerene cage.

Molecular motions are often connected with fundamental chemical properties; therefore, an understanding of molecular dynamics can be important in fields ranging from material science to biochemistry. We present the case of ibuprofen sodium salt which exhibits different degrees of conformational freedom in different parts of the same molecule, leading to a range of line broadening and line narrowing phenomena as a function of temperature.

Introduction

Magic-angle spinning (MAS) is a technique in nuclear magnetic resonance (NMR), in which a solid sample is rapidly spun around an axis tilted by ~54.74° (the "magic angle") with respect to the static magnetic field of a NMR magnet. MAS greatly improves the spectral resolution and sensitivity in solid samples, by averaging out a range of anisotropic nuclear spin interactions.[1,2] In most cases, magic-angle-spinning NMR is performed at temperatures above ~150 K. However, in some cases, it is advantageous, or necessary, to perform magic-angle spinning NMR on colder samples. The main motivations are as follows:

1. *Improved Signal-to-Noise Ratio*. Low temperatures offer a significant increase in signal strength, since the thermal nuclear magnetization depends on temperature as T^{-1} (the Curie law). Furthermore, if the receiver/transmitter coil is cooled at the same time as the sample, the net signal-to-noise ratio acquires a

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$T^{-3/2}$ temperature dependence, since the Johnson–Nyquist noise (which is caused by thermal motion of the conduction electrons in the resonant circuits and coil material) is proportional to $T^{-1/2}$. For example, performing MAS NMR experiments at 10 K, rather than 300 K, potentially enhances the signal-to-noise ratio by a factor of ~160. In practice, such gains can be hard to realize, since relaxation times and line widths also tend to increase at low temperature. Low-temperature relaxation rates may be increased by paramagnetic doping.[3]

2. *Access to Low-Temperature Phenomena*. A wide range of physical phenomena, including superconductivity,[4] quantum tunnelling, spin isomer conversion, magnetic phenomena, and so forth, occurs at low temperatures. The study of the molecular basis of these phenomena by high-resolution NMR requires the MAS of cold samples.
3. *Trapping of Kinetic Intermediates and Unstable Molecules*. Many molecules or reaction intermediates are unstable at high temperature. Their study by high-resolution NMR requires the MAS of cold samples.
4. *Dynamic Nuclear Polarization (DNP)*. In DNP-enhanced NMR,[5−11] a sample containing unpaired electrons is illuminated with microwaves whose frequency is slightly displaced with respect to the electron resonance frequency. Saturation of the electron spins gives rise to a large nuclear polarization, which may then be exploited to provide greatly enhanced NMR signals. The largest signal enhancements are achieved at low sample temperatures, where both the electron and the nuclear spin–lattice relaxation time (T_1) are relatively long.

The experimental implementation of cold-sample MAS requires that several technical obstacles are overcome. A stream of cold gas must be generated and exposed to the rotating sample while minimizing heat losses. Ideally, the sample temperature and spinning frequency should be controlled independently. In the case of helium cooling, arcing in the radio frequency field must be avoided. In addition, a reliable method of measuring the temperature of the rotating sample is required.

The group of Yannoni pioneered cold-sample magic-angle-spinning NMR, achieving MAS NMR on samples as cold as 7 K.[12−15] However, this early work had a limited impact, probably because of the difficulty in achieving stable and reproducible operation, and severe limitations in the achievable spinning frequency. Attempts at commercializing cryogenic MAS probes by the NMR instrument company Doty were also not very successful. The "modern era" of cold-sample MAS NMR was initiated by the Tallinn group of Samoson, who achieved stable MAS NMR of samples in the cryogenic temperature regime, at spinning frequencies exceeding 15 kHz.[16] As discussed below, several other equipment designs are now in operation, with particular capabilities and limitations.

For several years, our group has been involved in both the technological development of cold-sample MAS and in some of its applications. Here, we briefly review some of the hardware developments as well as the research activity of our group on this topic. In order to limit the discussion, we do not discuss the hardware used in DNP-enhanced MAS experiments, and we do not present experimental results obtained by other research groups.

Equipment

Most modern magic-angle-spinning NMR systems use three gas streams: A *bearing* gas stream for supporting the sample rotor on cylindrical journal bearings; a *drive* gas stream for propelling the rotational motion; and a *sample* gas stream which is blown onto the sample region at the center of the rotor for controlling the sample temperature. The current designs for cold-MAS systems differ in (i) the gas used for the three streams, helium, nitrogen, dry air, or a mix of gases in the same system; (ii) the systems used for generating cold gas and regulating its temperature; and (iii) the dimensions of the MAS rotor. Important system parameters are (i) the minimum achievable sample temperature; (ii) the generation of temperature gradients within the sample; (iii) the accessible range of spinning frequencies; (iv) the stability and controllability of spinning and cooling; (v) the degree of coupling between the sample temperature and the spinning frequency; (vi) the operational costs; and (vii) the ease of setup and of operation.

Modest Cooling (down to about 90 K). Standard Commercial Systems. At the time of writing, most NMR instrument manufacturers offer standard solid-state NMR systems (probe + cooling equipment) that can achieve sample temperatures down to about 150 K. These commercial MAS systems typically use a heat-exchange dewar and heaters to control the temperature of the sample gas stream, while the bearing and drive gas streams are kept warm. The lowest achievable sample temperature is limited, and the sample often experiences strong temperature gradients.

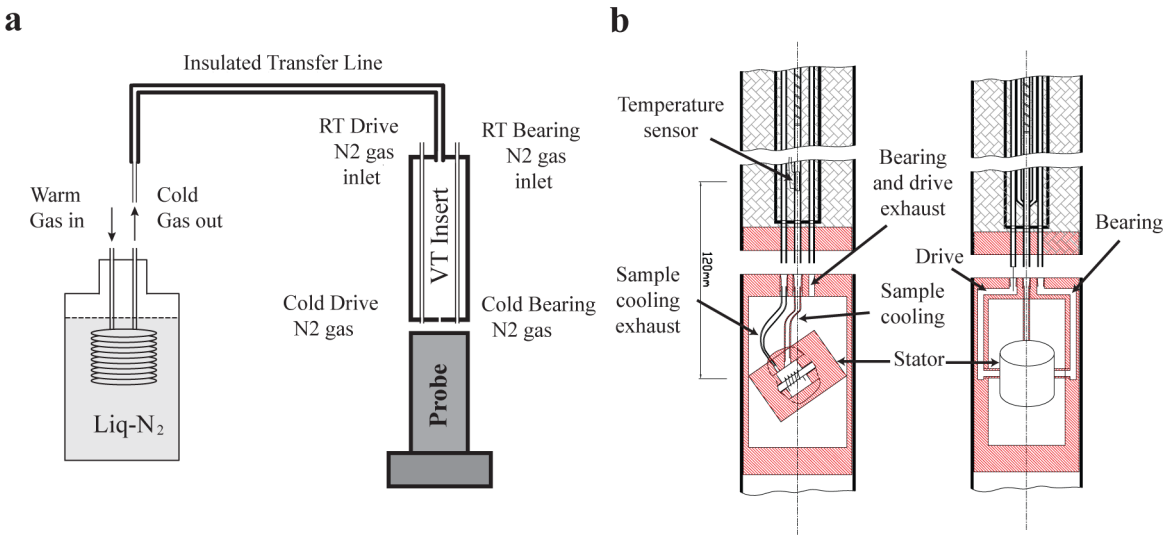

FIGURE 1. (a) Equipment for performing MAS NMR down to about 120 K. (b) Two views of the customized VT insert and the top of the NMR probe with the spinning module.

The Southampton Rhodopsin Probe. For our project on the solid-state MAS NMR of the photoreceptor protein rhodopsin (see below) we required a true sample temperature lower than 120 K. We developed MAS NMR hardware for reaching reliable sample temperatures down to 90 K, in the presence of light illumination.[17] A simplified sketch of the apparatus is shown in Figure 1. For a description of the illumination hardware, consult ref 18.

A stream of nitrogen gas is passed through a heat-exchange dewar filled with liquid nitrogen and sent to the NMR probe via a well-insulated transfer line and a custom-built variable-temperature bore insert (VT). To reduce thermal losses the bearing and drive nitrogen gas streams are cooled by bringing them into thermal contact with the cold VT gas (Figure 1b). The magnet bore is kept warm by a flow of nitrogen purge gas. A true sample temperature of 90 K was reached while spinning the sample at a frequency of 7 kHz with a stability of $\pm$10 Hz.

DNP-MAS Probes. Dynamic nuclear polarization experiments require low sample temperatures to achieve large enhancements in the nuclear spin magnetization. At the time of writing, most equipment of this type is limited to temperatures above 90 K, which is compatible with the use of nitrogen gas. Nevertheless trial experiments at lower sample temperatures are under way.[11,19] Bruker have commercialized a MAS-DNP integrated solution that comprises a low temperature MAS probe with built-in waveguide and cold gas supply that reaches sample temperatures of $\sim$100 K.

Cryogenic Magic-Angle Spinning NMR. MAS at temperatures below $\sim$90 K is more difficult. There are currently three main designs in operation, which have different advantages and disadvantages; these are briefly described in the following subsections.

At the time of writing, and to the knowledge of the authors, none of the designs incorporate cryogenic cooling of the receiver electronics to take advantage of the reduced Johnson noise in the cold receiver coil. Future designs are expected to incorporate this feature.

The Tallinn Design. The custom-built cryogenic NMR equipment available in Tallinn (Estonia) performs MAS solid-state experiments at temperatures down to 7 K.[16] All three gas streams are helium, with sample cooling achieved by controlled boiling of a liquid He reservoir in the base of the NMR probe, which is inserted into the vertical-bore NMR magnet from the top. The drive and bearing gas lines normally use room-temperature He gas, although when the lowest temperatures are required the He drive gas is precooled by passing it through a liquid-nitrogen Dewar. Sample temperatures of 7 K and spinning frequencies of 20 kHz have been reached (although not at the same time). All the helium is recovered and recycled.

The Tallinn design is relatively simple and compact. Its major disadvantage is its limited stability and a high degree of coupling between sample temperature and spinning frequency.

The Southampton Design. In the cryogenic MAS NMR apparatus designed and built in Southampton (U.K.), all three gas streams are generated from a pressurized vessel of supercritical He, and transported to the probe through a custom-built triple-core transfer line. The use of supercritical

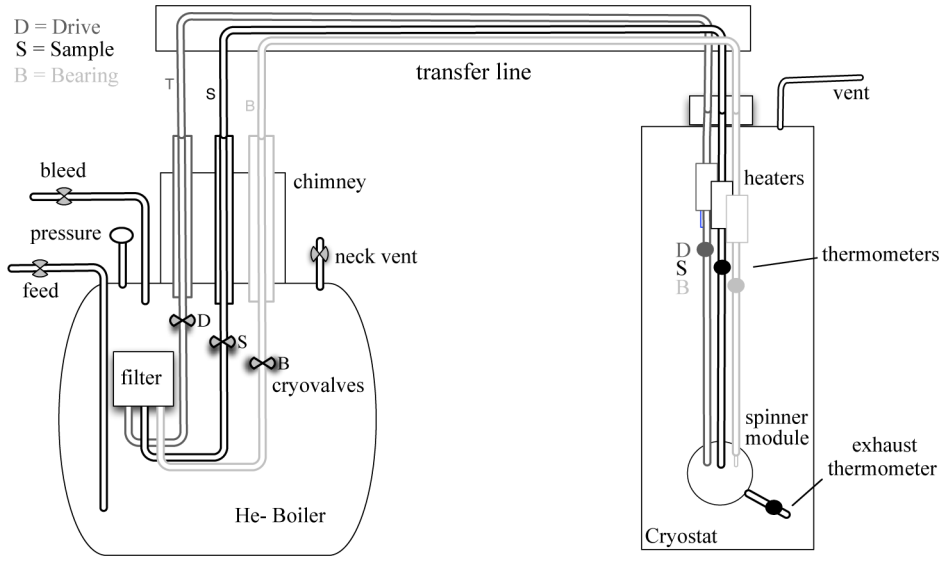

FIGURE 2. Southampton cryogenic MAS NMR equipment, showing the He pressure vessel, the triple-core transfer line, the cryostat, and the NMR probe, which is inserted into the magnet from the top.

He, rather than liquid He, avoids the inherent instability of two-phase flow. The equipment is shown in a simplified form in Figure 2.

The supercritical helium generator is charged with up to 5000 L of liquid He and pressurized to between 2.5 and 4.5 bar to obtain supercritical He. The cold supercritical He is filtered and divided into three separate streams, which are individually controlled by fine cryogenic needle valves to control the sample, bearing and drive gas flows independently.

The three gas streams are transported to the NMR probe, using a custom-made superinsulated transfer line with three cores. The outer lines (bearing and drive) act as radiation shields for the inner line (sample), allowing a low temperature of ~8 K to be maintained for the sample gas.

A set of electrical heaters allows the temperature of the three streams to be adjusted independently. In a typical run, the temperature of bearing and drive are kept around 90 K whereas the sample gas is kept at 8 K. In the current mode of operation, warm bearing and drive gas was found to be necessary to maintain the hydrodynamic properties of the bearings, and to avoid problems caused by thermal shrinkage of the drive tips. We are, however, experimenting with colder bearing and drive gases. This allows a minimum sample temperature of 9.6 K.

At the time of writing, the maximum spinning frequency is 15 kHz. This speed is limited by the capacity of the heater needed to stabilize the temperature of the bearing gas stream. In the future, the installation of a more powerful heater should allow spinning frequencies exceeding 20 kHz.

The probe is housed in a brass cryostat that fits inside the room-temperature shim tube of a 14.1 T 89 mm bore magnet. The probe is inserted from above. The probe contains a modified spinner module for 2 mm outer-diameter rotors, incorporating axial thrust bearings and asymmetric journal bearings in order to stabilize the rotor, damp vibrations, and reduce friction at low rotation frequencies. The rotor drive tips were modified in order to hold tight at cryogenic temperatures.

The filling of the He boiler requires about 150 L of liquid helium to cool down the boiler from ambient temperature before charging with liquid He, and takes up to 10 h. The sample temperature can be lowered from 300 to 13 K in about 30 min in a safe regime. Autonomy of about 20 h is available when working at the lowest temperature and highest spinning speed.

The Southampton system is relatively complex and expensive to run. Great care is needed to avoid the sucking of air or nitrogen into the system during the cooling phase (cryopumping). Minor contamination of the He with N_2 or other gases readily leads to the development of ice blockages and spinning failure. Cooling protocols have been developed which avoid this problem with high reliability. Although the system has a very high He consumption, we are now installing a He recycling system with sufficient capacity to handle the large output of cold He gas. The main advantage of the Southampton design is its very high operational stability, low minimum temperature, and good independent control of spinning and temperature.

The Bethesda (NIH) Design. The design of the Tycko group[3] uses nitrogen bearing and drive streams and cold helium for sample cooling. A 4 mm outer-diameter rotor is used to accommodate relatively large sample volumes, which are necessary to study proteins at low concentration in frozen solutions or in biological membranes. The rotor (4 mm outer diameter) is longer than standard MAS rotors, which permits the engineering of a clean separation between the helium-cooled region of the sample and the warmer nitrogen bearing and drive gas flows used for MAS. This physical separation avoids the build-up of nitrogen ice and minimizes temperature gradients across the sample. Cooling is provided by liquid helium from a pressurized helium dewar which is sent to the probe through a vacuum insulated transfer line. A needle valve is used to control the helium flow. The advantage of the Bethesda design is its relatively low He consumption and running cost. However, the minimum sample temperature (~20 K) is higher than in the other two designs.

Temperature Estimation

There are many influences on the sample temperature in MAS NMR experiments, apart from the temperature of the VT gas stream. These are as follows:

Thermal Conduction. It is usually necessary to keep the bearing and drive gas streams relatively warm, in order to maintain the hydrodynamic properties of the gas within acceptable limits. In addition, there can be local frictional heating in the bearings. The temperature of the sample is therefore influenced by thermal conduction through the rotor material from the relatively warm bearings to the cold sample region. Thermal conduction effects depend strongly on the material of the rotor, the cooling power of the VT gas, and the distance between the bearings and the sample region.

Radiofrequency Heating. The application of radiofrequency fields during the NMR experiments may warm the sample, either by direct radiofrequency heating of the sample, or by heating of the nearby electrical components. Direct radiofrequency heating of the sample tends to be insignificant at temperatures below ~140 K, since frozen samples lack mobile ions that respond to electric fields.

The sample temperature may be estimated as follows:

Gas Temperature Measurements. The temperature of the gas streams entering and exiting the sample region may be used as a rough guide for the sample temperature. The gas temperatures may be measured by temperature-sensitive electrical components such as thermistors, or by nonmagnetic optical temperature sensors which may be placed very close to the spinning rotor.

NMR Thermometers. A complementary method is to observe the NMR signals of substances with temperature-sensitive NMR parameters, and which are placed inside the spinning rotor. Ideally, these substances are loaded into the rotor at the same time as the substance of interest, often separated by an inert physical barrier such as Teflon. Alternatively, experiments on the NMR thermometer are used to determine the sample temperature at a range of physical spinning conditions, such as gas temperature, spinning speed, gas pressure, and so on. This provides a set of calibration curves which may be used to estimate the sample temperature during independent measurements on the substance of interest. This indirect approach must be used cautiously since the spinning conditions depend on the mass and the mass density of the sample, and the sample itself may change the temperature distribution through its thermal conductivity or radiofrequency heating characteristics.

In some materials, phase transitions cause sharp changes in the chemical shifts or other NMR parameters at well-defined temperatures.[20,21] This allows the calibration of a small number of specific transition temperatures. Continuous temperature-dependent NMR parameters such as dipolar couplings,[22] spin−lattice relaxation times[23−25] and chemical shifts[17,23,26−30] have all been used for temperature calibration. The temperature-dependent ^{207}Pb chemical shift in $Pb(NO_3)_2$ is widely used.[17,26,27] The ^{79}Br chemical shift in KBr can be used to calibrate the temperature in the range 100−400 K.[23−25] The ^{15}N chemical shift in the organic dye TTAA may be used as a thermometer in the temperature range of 130 to 400 K.[28] The ^{119}Sn chemical shift in $Sm_2Sn_2O_7$ can be used down to 85 K.[29,30]

The use of chemical shifts to calibrate the sample temperature may be confounded by susceptibility shifts in the cold probe and the purge gas. We have used the narrow low-temperature proton line of the endofullerene $H_2@C_{60}$ to take this into account[17,31]

Most chemical shift thermometers are unsuitable in the cryogenic regime. Thurber and Tycko[23] showed that the T_1 relaxation time constant of ^{79}Br in KBr may be used as a thermometer in the temperature range of 20−320 K.[23] The field-dependence of the ^{79}Br T_1 is weak.[25] The ^{127}I T_1 of CsI may be used as a convenient thermometer down to ~10 K.[24]

Applications

In this section, we review some of the NMR experiments performed in our own laboratory involving the MAS NMR of cold samples.

Trapping of Bathorhodopsin. Rhodopsin is an integral membrane protein that is sensitive to light and is found in the retina of the eye. Light-induced structural changes in rhodopsin are the first step of the visual response.[32] We have been interested in the primary photointermediate of rhodopsin, called *bathorhodopsin*. Bathorhodopsin has a short lifetime at room temperature, but may be trapped indefinitely if the sample is kept below 120 K. Solid-state NMR permits study of the local electronic structure and dynamics of the chromophore in this energy-rich photointermediate.

The Southampton "rhodopsin probe", described above, allows MAS NMR at temperatures down to ~100 K, with in situ light illumination, which is required to generate the bathorhodopsin photointermediate from the rhodopsin starting material. The details of the illumination set up are given in ref 17.

The original intention was to use a sapphire rotor in order to maximize light transport into the sample. However, sapphire has a high thermal conductivity at low temperature. However, rapid heat transport from the warm rotor bearings made it difficult to achieve the required low temperature when using a sapphire rotor. For this reason, a thin-wall zirconia rotor was used instead.[17]

Using this custom-built MAS equipment, we were able to lower the sample temperature below 120 K, trigger the rhodopsin photocycle by illumination, and observe the NMR peaks of bathorhodopsin.[18] We used a set of rhodopsin isotopomers, each labeled with pairs of ^{13}C nuclei at different positions along the retinylidene chromophore (Figure 3a). Double-quantum filtered dipolar recoupling[33] was used to suppress the natural abundance ^{13}C background signals. Figure 3b shows the double-quantum-filtered ^{13}C NMR spectra of [9,10-$^{13}C_2$] retinylidene-rhodopsin before (above) and after (below) 10 h of continuous illumination at a temperature of 120 K.

The spectrum obtained after illumination shows a clear splitting of one of the ^{13}C peaks, indicating the generation of bathorhodopsin. The ~35% bathorhodopsin yield is limited by the partial penetration of light into the optically dense particles and by secondary photoisomerization of bathorhodopsin.[18] The analysis of the chemical shifts of bathorhodopsin together with the estimation of the changes (upon isomerization) of the torsional angles of the H−C11−C12−H retinal's fragments[34] made it possible to characterize the electrostatic and steric contributions to the storage of photon energy in this photointermediate.

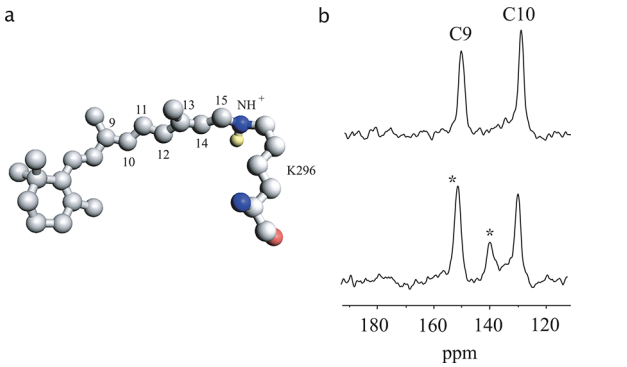

FIGURE 3. (a) Structure of the chromophore in bathorhodopsin, showing the numbering of the carbon sites. (b) Double-quantum-filtered ^{13}C NMR spectra of [9,10-$^{13}C_2$] rhodopsin before (above) and after (below) 10 h of illumination with a 420 nm light, at a sample temperature of 120 K and a spinning frequency of 7.00 kHz. Asterisks indicate the chemical shifts of the bathorhodopsin signals. Adapted with permission from ref 17. Copyright 1990 American Chemical Society.

Rotation of *Ortho*-Water. Recently, our laboratory become interested in the study of endohedral light-molecule fullerenes. These compounds consist of small, light, molecules (such as H_2 or H_2O) encapsulated in fullerene cages (C_{60}, C_{70}, and derivatives thereof). Macroscopic quantities of these remarkable systems are accessible through "molecular surgery".[35−37] The encapsulated guest molecule behaves as a quantum rotor whose rotational and translational levels are mixed together by the confinement provided by the cage. The rotor energy levels may be studied by infrared spectroscopy[38−40] (FIR) and inelastic neutron scattering[41,42] (INS) as well as NMR.[22,31,43,44]

One example is the water-endofullerene $H_2O@C_{60}$.[45] Water displays two spin-isomers, called *para*-water and *ortho*-water, determined by the relative orientation of the proton spins (opposite for *para*, and parallel for *ortho*). Since only *ortho*-water has a nuclear spin ($I = 1$), the NMR signals from water derive exclusively from the *ortho* spin isomer. Although *ortho*-water is metastable at temperatures below ~30 K, it may be observed by NMR since the *ortho*-to-*para* conversion is sufficiently slow.[45]

Ortho-water molecules rotate freely and isotropically inside the fullerene cage. This averages out the dipole−dipole couplings between the water protons, so that the ^1H MAS spectra are narrow and do not display strong spinning sidebands at temperatures above 20 K (see Figure 4). However, MAS NMR at the lowest achievable temperature of 9.6 K resolves a pattern of strong spinning sidebands. This is due to residual dipole−dipole coupling between the water protons, which indicates that the degeneracy in the *ortho*-water ground state is broken.[45] The origin of this broken

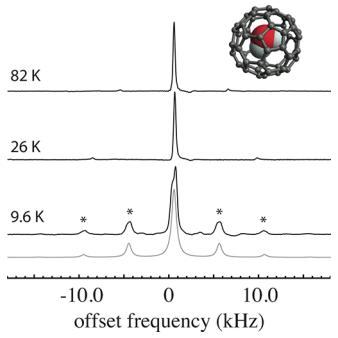

FIGURE 4. Magic-angle-spinning proton NMR spectra of $H_2O@C_{60}$ at a field of 14.1T acquired using the Southampton low-temperature MAS equipment. The spinning frequencies are 5.82, 8.79, and 5.04 kHz from top to bottom. Spinning sidebands are indicated by asterisks. The gray line is a simulation for randomly oriented pairs of protons with a magnetic dipole−dipole interaction of −5.5 kHz. Reprinted with permission from ref 45. Copyright 2010 National Academy of Sciences.

degeneracy is unknown, but it may be linked to electrical interactions between neighboring water dipoles.[46]

Frozen Molecular Motion in Ibuprofen. Molecular motions are often connected with fundamental properties such as stability of solid phases, solid−solid phase transitions, intra- and intermolecular interactions, chemical reactivity, and so on. Although some molecular motions may be slowed down at moderately low temperatures, a full characterization of molecular motional properties by NMR requires equipment for accessing site-resolved NMR spectra down to cryogenic temperatures.

Recently, we have investigated molecular motions in a solid sample of sodium ibuprofen dihydrate (IBU-S) as a function of temperature from ∼350 down to 20 K (manuscript in preparation). The different fragments of this pharmaceutical molecule are known to undergo motions on the nanoseconds time scale, at room temperature.[47] Cryogenic solid-state NMR equipment offers the possibility to study these motions over a wide range of temperatures and to characterize their physical parameters.

Following previous high-temperature observations,[47] we acquired a set of ^{13}C−CP-MAS spectra in the temperature range of 239−20 K, in order to follow the changes in the aromatic ring, methyl and isobutyl NMR peaks (Figure 5). The Southampton "rhodopsin probe" (Figure 1) was used to collect the spectra taken at temperatures in the range from 250 K down to about 140 K, while the cryogenic MAS probe (Figure 2) was used for experiments below 150 K.

Our results show a clear selective broadening of NMR transitions at many, but not all, sites in the molecule. The most polar part of the molecule (the isopropionic fragment) freezes into a well-defined single conformation, giving rise

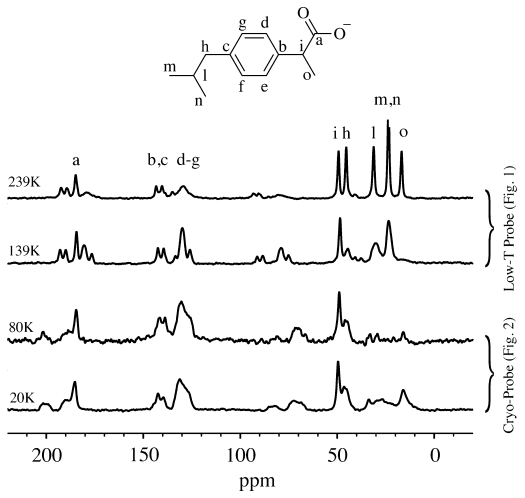

FIGURE 5. ^{13}C spectra of ibuprofen sodium salt dihydrate at different temperatures. The upper two spectra were acquired with the Southampton "rhodopsin probe", using a 4 mm rotor, spinning the sample at 5.0 kHz and summing over 80 transients. The lower two spectra were acquired with the Southampton cryogenic MAS probe, using a 2 mm rotor, spinning the sample at 9.0 kHz and summing over 16 and 8 transients, respectively.

to sharp peaks even at the lowest temperature. Conversely, the apolar part (the isobutylic fragment) freezes into a set of conformations giving rise to broad chemical shift distribution resulting in very broad peaks and unresolved resonances. A detailed explanation of the experiment together with an interpretation of the results is in preparation.

Conclusions

Advances in nuclear magnetic resonance have consistently been coupled to advances in hardware. In this short Account, we have described some of the efforts made by several research teams to develop magic-angle-spinning NMR equipment capable of operation at low temperatures. One motivation of this is to enhance the signal-to-noise of NMR experiments by improving the thermal polarization of the sample, and to reduce the noise generated by the receiver coil. In addition, the ability to conduct high-resolution NMR at low temperatures brings a whole range of materials phenomena into the range of MAS NMR study. These include superconductivity, low-temperature phase transitions, magnetic phase transitions, quantum tunneling, quantum molecular rotation, *ortho*−*para* transitions, the study of trapped intermediates, and so on.

This Account summarized some of the studies which have been performed by the Southampton group: NMR of a trapped membrane protein photointermediate; study of the quantum rotation of *ortho*-water molecules trapped inside fullerene cages; and the inhomogeneous freezing-out

of internal molecular motions in a small-molecule solid (the sodium salt of ibuprofen).

The latter example is particularly relevant to the solid-state NMR of biosolids at low temperature; it is anticipated that most biopolymers will behave in a similar way to ibuprofen at low temperature, and will exhibit a high degree of inhomogeneous line broadening for hydrophobic regions of the molecule (due to the freezing-in of a large number of conformations with similar energy) at the same time as maintaining acceptable resolution for hydrophilic and well-structured molecular regions. In the case of microcrystalline solids[48,49] and nanocrystalline proteins,[50] there are experimental evidence of high resolution even at temperature as low as ~90−100 K. These observations are of high concern for those applying DNP-MAS to the NMR of biosolids, where experiments are currently often performed at around ~100 K, with potentially greater gains in signal strength available in the cryogenic temperature regime.

The hardware challenges of cryogenic magic-angle-spinning NMR are considerable, and so far the solutions developed by the community are of a preliminary and exploratory nature. Nevertheless, the progress made so far indicates that the magic-angle-spinning NMR of cold samples is a rich and worthwhile field and is likely to receive much increased attention in the future.

We thank Mike Webb, Carlo Beduz, and Yifeng Yang for design and construction of the Southampton cryogenic NMR equipment; Ivo Heinmaa for providing details of the Tallinn cryogenic equipment and for help with experiments; Salvatore Mamone, Mark Denning, and Marina Carravetta for help with the endofullerene experiments; and Axel Gansmüller for help with the rhodopsin experiments. We thank EPSRC (U.K.) and the Royal Society (U.K.) for support.

BIOGRAPHICAL INFORMATION

Maria Concistrè received her Ph.D. in 2010 from University of Southampton (U.K.) where she is currently a postdoctoral researcher. Her research interests focus on low and cryogenic temperature solid-state NMR of materials and biological samples.

Ole Gustav Johannessen received a Masters degree in Physical Electronics from the Norwegian Technical University of Trondheim, Norway. Since 1994, he has supported the Levitt group by designing and building NMR hardware, first in Stockholm, Sweden, and since 2001 as a Research Engineer at the University of Southampton, U.K.

Elisa Carignani received her degree from the Università di Pisa in 2009 and she is currently third-year Ph.D. student at the Università di Pisa. She has published 3 papers concerning new approaches for studying the dynamics of organic molecules by solid-state NMR. Her research interests include solid-state NMR of organic materials, pharmaceuticals, and liquid crystals.

Marco Geppi received his degree from the Università di Pisa in 1991 and Ph.D. from the Scuola Normale Superiore di Pisa in 1997. He has been a Researcher in physical chemistry at the Università di Pisa since 2001. He has published approximately 90 papers on NMR of solids and liquid crystals. His research interests include solid-state NMR of materials, pharmaceuticals, and biomacromolecules; nuclear relaxation and molecular dynamics; and NMR of liquid crystals.

Malcolm H. Levitt received his PhD from Oxford University (1981) and performed postdoctoral research at the Weizmann Institute (Israel), the ETH-Zürich (Switzerland), and MIT (U.S.A.). He joined the faculty of Stockholm University (Sweden) and the University of Southampton (U.K.) in 2001. His research involves a wide range of theoretical and methodological advances in nuclear magnetic resonance, both in solids and in solution.

FOOTNOTES

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The authors declare no competing financial interest.

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