High-Frequency Dynamic Nuclear Polarization NMR for Solids: Part 1 - An Introduction

Michelle Ha and Vladimir K. Michaelis*

Department of Chemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2G2

*Correspondence: vladimir.michaelis@ualberta.ca

Abstract

Dynamic nuclear polarization (DNP) NMR spectroscopy, a high-polarization method, is rapidly changing the landscape of what is possible in solid-state nuclear magnetic resonance (NMR) spectroscopy. To date, there have been over 200 publications discussing high-frequency DNP NMR of solids with more than half being released within the past few years. Below we provide for researchers that may be interested in this high-sensitivity technique an introduction to high-frequency DNP NMR spectroscopy, including instrumentation, mechanisms, polarizing agents and sample preparation. While there are many applications utilizing high-frequency DNP NMR, Part II will deal with recent advances in method development and applications to biomolecular solids and materials science.

Introduction

Solid-state nuclear magnetic resonance (NMR) spectroscopy is a mature field and arguably one of the most robust analytical techniques for characterizing atomic- and molecular-level structure in solids. It can be found in nearly every scientific discipline such as biomolecular, chemical, materials and earth science due to the unique ability to probe sub-nanometer short- and medium-range structure of ordered and disordered solids. A particular strength of NMR spectroscopy is its ability to elucidate various isotropic and anisotropic interactions that are rich in atomic- or molecular-level structural and dynamic information. For example, dipolar coupling (a through space interaction) is readily used to address medium-range order within solids.[1-5] The isotropic chemical shift is vital in identifying functional groups within organic molecules, polymorphs in pharmaceutical compounds, or coordination environments and bonding arrangements important in materials science and geoscience.[6, 7] The quadrupolar interaction, affecting over 70% of the NMR-active nuclei on the periodic table, is highly sensitive to the

overall molecular and atomic environment. The magnitude and shape of the interaction has aided in studying many chemical systems.[8, 9]

The large versatility of NMR spectroscopy can be overshadowed by the small nuclear Zeeman polarization resulting in poor overall sensitivity. Solids are particularly disadvantaged as many suffer from broad NMR resonances ranging between 10's of kHz (dipolar coupling, magnetic shielding) to MHz (quadrupolar coupling) and a range in relaxation properties (*e.g.,* T_1 and T_2). Over the past 50 years, several innovations have advanced the field, providing practical gains in sensitivity. The first breakthrough was made by Andrew and Lowe[10, 11] who introduced magic-angle spinning; another important advance was the introduction of cross-polarization[12] (CP). In the early 1990's progress in high-field NMR magnets surged, with the introduction of ultrahigh-field magnets from a host of commercial and home-built systems; today commercial systems exist as high as 1 GHz, with plans for 1.2 and 1.3 GHz in the very near future.[13, 14] At each stage these developments have left a lasting impact in the field of NMR spectroscopy.

With the recent commercialization of dynamic nuclear polarization (DNP), a high-polarization technique in NMR spectroscopy, the field is undergoing a rapid change due to the technique's unprecedented sensitivity.[15, 16] DNP NMR involves transferring the large electron polarization of unpaired electron spins to nearby insensitive nuclei via microwave irradiation of the electron-nuclear transitions, resulting in sensitivity gains in varying orders of magnitude (*i.e.,* 10 to 10^3) over traditional NMR spectroscopy (*e.g.,* up to 658-fold (γ_e/γ_n) increase in the case of ^{1}H). This makes otherwise impossibly long experiments practical to complete. For example, an enhancement factor (ε) of 20 reduces the required experimental time by a factor of 400 (20^2), reducing experimental times from months to hours and opening new frontiers for scientific exploration.

To gain a better understanding of the various components necessary for high-frequency DNP NMR, this article contains two parts, below (Part I) will provide an overview of the instrumentation, mechanisms, polarizing agents, and sample preparation that are commonly used within the field; Part II highlights the new areas DNP NMR is making available to the research community including DNP method development, biomolecular solids and materials science. Many excellent reviews have emerged over the years and the following articles are recommended for interested readers.[17-28]

High-Frequency DNP NMR Instrumentation

Robust and reliable instrumentation is essential to performing experimental high-frequency DNP research and is still a key barrier in implementing DNP NMR within international research groups. A DNP NMR spectrometer (Figure 1) is comprised of four major components: (i) a solid-state NMR spectrometer, (ii) a microwave device and accessories, (iii) cryogenics and (iv) a DNP NMR probe.

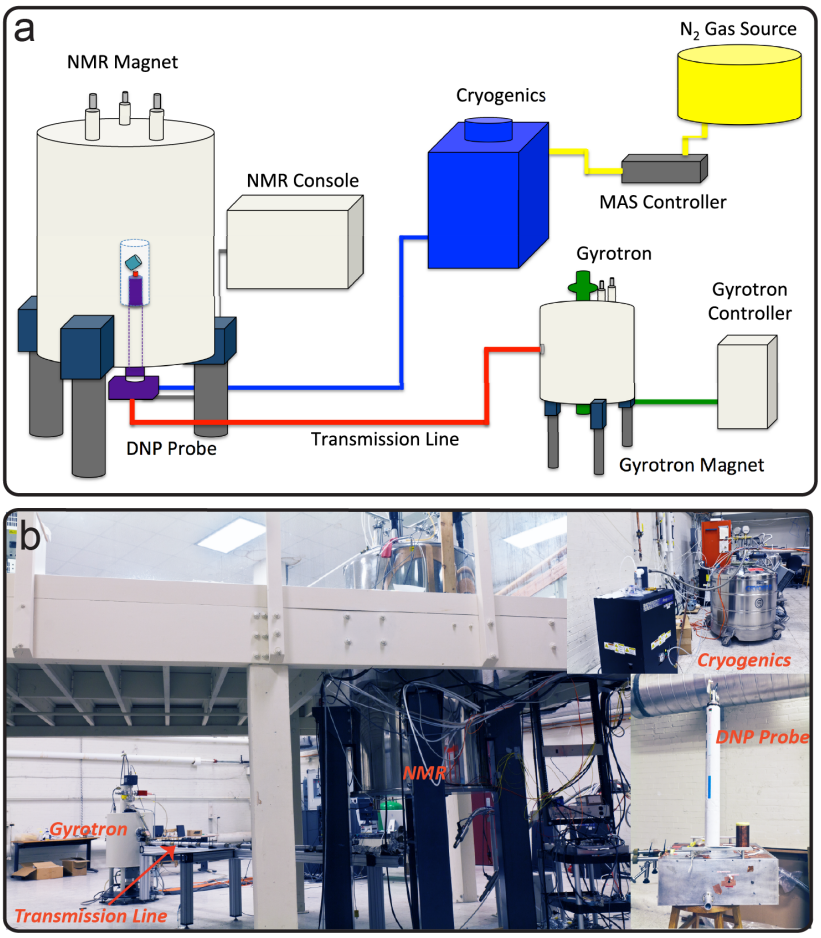


Figure 1: (a) Schematic of a high-frequency DNP NMR Spectrometer and (b) photo of a 699 MHz / 460 GHz DNP NMR Spectrometer (Francis Bitter Magnetic Laboratory, Massachusetts Institute of Technology, Cambridge, MA, USA) including (i) solid-state NMR magnet, (ii) gyrotron and transmission line, (iii) cryogenics and (iv) DNP NMR probe.

i. Solid-state NMR Spectrometer

A conventional NMR spectrometer (*i.e.,* superconducting magnet, spectrometer, pneumatic control system, user interface, *etc*.) is required to perform NMR experiments. For DNP NMR, the magnet bore must be 89 mm (*i.e*., wide-bore) rather than a standard bore of 51 mm to accommodate the probe electronics, dewar, microwave waveguide, sample eject and insulation requirements for performing low-temperature experiments. Another consideration is whether the main magnetic field is coupled to an outer coil that can be adjusted to varying degrees (*i.e.,* a sweep coil) to increase or decrease the field strength in fine steps. This is

particularly important for radical development and for other targeted applications. The sweep coil enables one to optimize the strength of the main magnetic field for advanced applications.

ii. Microwave Devices

One of the most challenging pieces of instrumentation to develop is a source that generates high-frequency microwaves. With high-frequency DNP NMR now ranging from 5 to 19 T (200 to 800 MHz), microwave devices must generate microwaves in the 140 to 527 GHz frequency range. The most commonly applied device is known as a gyrotron. This is in part due to the significant development in gyrotron technology for the application in DNP NMR carried out by Temkin and Griffin at MIT,[29-33] by Osaka University and the University of Fukui[34-36] and commercially by Bruker Biospin, Communications and Power Industries (CPI) and by Bridge12.

The basis of a gyrotron is its use of stimulated cyclotron radiation generated when present in a superconducting magnet. A gyrotron is capable of generating high-power microwaves at high frequency (> 100 GHz). The fast-wave device is often operated in a higher mode which improves the robustness and cooling abilities providing a microwave device that is extremely stable, with high output power and continuous operation for days to months. The design of these devices is also attractive for their longevity with lifetimes expected to be 10+ years. These features make them ideal for NMR studies where experiments often require days to weeks of continuous acquisition. As discussed above, the NMR spectrometer may be equipped with a sweep coil to allow fine adjustments of the field strength. If this is not possible, an alternative is having a tunable microwave device such as a tunable gyrotron, as one can fluctuate the output of the microwave frequency while keeping the main magnetic field of the NMR instrument constant.[33, 37-40] However, an issue to be aware of is maintaining a stable and constant microwave output over a wide tuning range.

Alternative microwave sources, optimal for applications below 5 T, including extended interaction Klystrons (EIK), oscillators (EIO) and amplifiers (EIA), have also been successfully applied to higher field strengths; for example the Tycko group has an operational system at 9.4 T (400 MHz, ^{1}H and 264 GHz, e^{-}).[41] Unfortunately, these alternatives suffer from limited output power at high frequencies, ~ 5 W at 265 GHz, which affects enhancements and strains the devices, limiting their longevity (lifetimes of ~ 10,000 hrs, ~1.2 yrs at 265 GHz). Furthermore, commercial units are unavailable for applications above 265 GHz. As advancements and demand of microwave technology continue, these sources may be attractive for certain DNP applications as they become available at higher magnetic fields and/or microwave output powers.

iii. Cryogenics

Conventional DNP NMR relies on the ability to cool the sample to cryogenic temperatures to improve the electron and nuclear relaxation behaviour, aiding the effective transfer of bulk polarization within the sample. This can be accomplished by using a cryogenic heat exchanger whereby a sealed can within a larger container of liquid nitrogen is pressurized to provide a stream of gas at cryogenic temperatures.[42] By adjusting the pressure within the heat exchanger one can control the output temperature. Finer control can easily be achieved using a cryogenic temperature control (*e.g.,* Lakeshore unit) that can be equipped with a heater to regulate the output gas to within 1 K or better. Similar types of devices have been successfully implemented using liquid He although the increasing cost of He is a prominent concern. Breakthroughs in DNP have occurred with recirculated He; in particular, the groups of De Paëpe[43] and Matsuki[35] have successfully designed a closed-loop system for their DNP NMR instrument that can operate at He temperatures. Tycko *et al.*[44, 45] and Levitt *et al.*[46] have also recently contributed to the He-cooled MAS NMR area. The major expense of DNP beyond the

initial investment in infrastructure is its thirst for cryogens (both N_2 and He). Hence, the authors believe a worthwhile goal of this field as it matures should be to strive to reduce the operating costs associated with the cryogens. For example, cryogenic pre-chillers and nitrogen gas generators have been successfully used in Osaka University[36] and MIT.[47]

iv. DNP NMR Probe

The NMR probe is responsible for sending and detecting the signal during an NMR experiment, and if necessary, cooling the sample, rotating the sample and providing the conduit for microwave irradiation prompting the transfer of polarization between electron and nuclear spins. A cryogenic DNP NMR probe is based on conventional home-built or commercial MAS NMR probes, but a few key modifications are required. The lines that provide a stream of compressed gas (*i.e.,* drive and bearing) to levitate and rotate the rotor must be vacuum jacketed. The insulation enables the transfer of chilled gas for the drive and bearing to cool the sample and probe. Typical spinning frequencies are between 4 and 20 kHz, but newly designed commercial probes are available capable of spinning samples up to 40 kHz (depending on rotor size & experimental temperature). In the foreseeable future, frequencies >40 kHz will surely be attained as room temperature probes are capable of spinning frequencies beyond 100 kHz.[48] The stator housing requires a modification near the coil to accommodate the microwave transmission line, responsible for guiding and projecting the microwave beam onto the rotor. The probe is often encased within a vacuum jacketed dewar to aid in localized cooling of the probe, and more importantly to protect the bore of the NMR magnet (T = ~290 K) from the cryogenic temperatures (< 120 K) located within the probe. Samples can be placed into zirconia or sapphire NMR rotors, the latter appear to provide larger enhancements, although thin-wall zirconia is proving quite successful commercially and is slightly more robust. The drive caps can be Torlon®

160 or Vespel® that are glued using cryo-epoxy or machined zirconia in combination with a small
161 polymer plug.

162 It should be noted that several variations from this general design model do exist. A few
163 noteworthy differences include: (i) an added variable temperature line (3rd vacuum jacketed line)
164 that can accommodate liquid N_2 or He to assist in cooling;[44, 47] (ii) the microwave waveguide
165 may be directed at the top of the stator or pointed to the head of the NMR rotor;[36] (iii) sample
166 ejection[47, 49, 50] for ease of changing samples (above or below models exist); and (iv) the gyrotron
167 may be placed above the probe using the same superconducting magnet to produce microwaves
168 and record the NMR experiment. As the probe body is cooled this does affect the overall
169 behaviour of the electronics which can have positive benefits in generating high RF fields (*i.e.,*
170 $\gamma B_1/2\pi$) but it can also affect the tuning circuit. In regards to the RF design, the most effective
171 approach has been a topic of discussion for many years; issues include whether the probe design
172 should be transmission line *vs* locally-tuned and weighing the benefits of implementing a
173 balanced RF circuit design.[44, 45, 51-58]

174 As the field has rapidly advanced with the introduction of commercial units in 2010,
175 systems have been successfully implemented in several research groups worldwide; a few are
176 summarized in Table 1.
177

Table 1: Examples of homebuilt and commercial DNP NMR instruments

Type	Location/ Manufacturer	B_0 (MHz / GHz)	Microwave Source	Completed
Home-Built	MIT	211 / 140[30, 59]	Gyrotron	*ca.* 1991-1993
		380 / 250[29, 38, 60]	Gyrotron	*ca.* 2002-2003
		700 / 460[23, 33, 47]	Gyrotron	*ca.* 2011-2013
		500 / 330[32, 39]	Gyrotron	*ca.* 2014-2016
		800 / 527[31]	Gyrotron	*in-prep.*
Home-Built	NIH	400 / 263[41]	Diode	*ca.* 2009-2010
Home-Built	Osaka	600 / 395[36, 40]	Gyrotron	*ca.* 2010-2012
		700 / 460[34, 35]	Gyrotron	*ca.* 2015-2016
Home-Built	Warwick	284 / 187 - 600 / 395[61]	Gyrotron	*ca.* 2012
Home-Built	Washington U. St. Louis	300 / 198[62]	Gyrotron	*in-prep.*
Commercial	Bruker Biospin[48, 50]	400 / 263	Gyrotron	*ca.* 2009-2010
		600 / 395	Gyrotron	*ca.* 2011-2012
		800 / 527	Gyrotron	*ca.* 2012-2013

DNP Mechanisms

For solids using a continuous microwave source there are four DNP mechanisms that can be considered in order to achieve bulk polarization transfer between an unpaired electron source (polarizing agent) and a nucleus: (i) thermal mixing (TM), (ii) Overhauser (OE), (iii) solid effect (SE), and (iv) cross effect (CE). The latter mechanism is by far the most targeted area in high-frequency DNP NMR applications due to the wide array of wide-line nitroxide radicals that favour the polarization of high-gamma nuclei (*i.e.,* ^{1}H). SE has been effective at lower field strengths[63] although a range of developments and results have recently appeared in the literature[64-66] so that it is still an active field of study in high-frequency DNP development. Likewise, Overhauser effect has recently emerged as a contender for higher field DNP NMR.[67, 68] Further developments with radicals could be fruitful as the enhancements scale linearly with

magnetic field strength. Below is a brief overview of the CE and SE DNP mechanisms; the following references are provided for a more comprehensive review.[15, 16, 21, 24, 27, 65, 67-83]

The dominant DNP mechanism depends on the targeted NMR-active nucleus and on the EPR characteristics of the selected polarizing agent. For example, the most common approach in DNP NMR applications of solids is through indirect polarization transfer using a CP step ($e^- \rightarrow {}^1H \rightarrow X$, where X is a lower gamma nucleus). Using this approach one can select for SE by using a narrow-line radical such as Trityl or for CE with TOTAPOL, a wide-line biradical. In other words, it is the relative magnitudes of the electron homogeneous (δ) and inhomogeneous (Δ) linewidths, and the nuclear Larmor frequency (ω_{0I}) that guide the DNP mechanism.

i. Cross Effect DNP mechanism

The CE mechanism can be described as a three-spin flip-flop-flip process between two electrons and a nucleus, which is dominant when $\Delta > \omega_{0I} > \delta$. The difference between the two electron Larmor frequencies should be near the nuclear Larmor frequency to achieve maximum polarization transfer between electron and nuclear spins.[73, 75, 79, 84]

$$\omega_{0I} = \omega_{0S_2} - \omega_{0S_1} \qquad (1)$$

To satisfy equation 1 for high-gamma nuclei like ^1H, the polarizing agent needs to have EPR characteristics (EPR spectrum) of a broadline; this is readily seen in nitroxide-based radicals including monoradicals such as TEMPO and TEMPONE, and biradicals such as SPIROPOL, AMUPOL and TEKPOL. The need to have two electrons in close proximity (*i.e.,* dipolar coupled) while minimizing the paramagnetic bleaching of nuclear spins has pushed the field into biradicals.[85, 86] Tethered radicals provide the chemical design to reasonably direct orientation and electron-electron distance so that the dipolar coupling is on the order of 20 to 35 MHz, while enabling the concentration of unpaired electrons to be minimized, typically < 15 mM solution

(*i.e.,* < 30 mM electrons). In contrast to biradicals, monoradicals with a 40 mM electron concentration have significantly reduced dipolar couplings of < 2 MHz when present in a homogenous glassy sample.[85-88] The CE mechanism is often the choice for high-frequency DNP NMR experiments as the mechanism is based on allowable transitions (Figure 2) and loosely scales with the inverse of magnetic field strength. In the past few years, descriptions of the CE mechanism taking into account the level crossing that occurs under magic-angle spinning has shed further light into the spinning rate dependence of the overall enhancement. The reader is referred to the works by the Tycko[89] and Vega[81] groups where they discuss modulations of the energy levels within DNP mechanisms when using magic-angle spinning and the impact it has on polarization transfer.

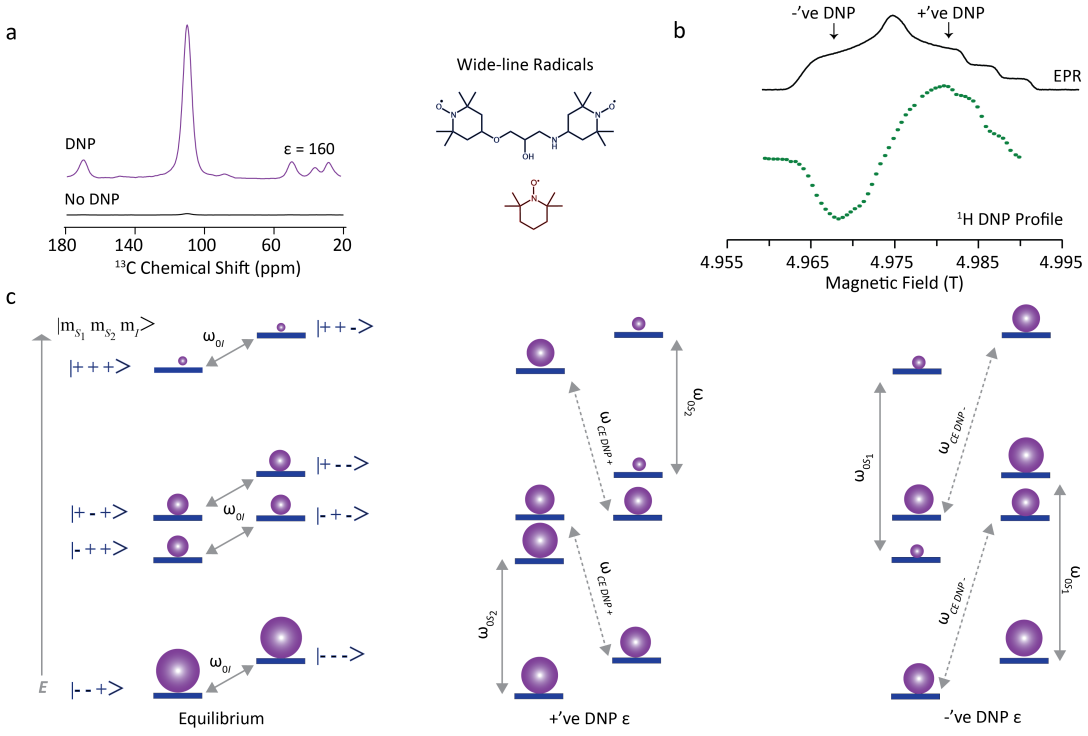


Figure 2: Cross effect DNP: (a) on (DNP) and off (No DNP) ^{13}C[^{1}H] CP MAS NMR experiment on urea using a wide-line nitroxide radical, (b) EPR and ^{1}H DNP NMR field profile for a nitroxide polarizing agent and (c) energy level diagram for CE DNP displaying spin population distribution for a three-spin (two electrons and one nucleus) system at thermal equilibrium, positive and negative CE DNP conditions. Microwave saturation of the electron transition (ω_{0S1} or ω_{0S2}) leads to a three-spin flip-flop-flip process that distributes the population (ω_{CE}), thus increasing the net nuclear polarization.

ii. *Solid Effect DNP mechanism*

The SE mechanism can be described as a two-spin process involving an electron and a nucleus. The SE mechanism is dominant when the nuclear Larmor frequency is larger than the electron homogeneous and inhomogeneous EPR linewidths ($\omega_{0I} > \delta, \Delta$) and microwave irradiation is applied at the electron-nuclear zero- or double-quantum transition as shown in Figure 3.[65, 66, 69, 90] The SE matching condition is satisfied when:

$$\omega_{\mathrm{mw}} = \omega_{0S} \pm \omega_{0I} \qquad (2)$$

where ω_{0S} and ω_{mw} are the electron Larmor and microwave frequencies, respectively. The mechanism may be observed when a narrow-line radical (*e.g.,* BDPA, trityl, *etc*.) is used as the polarizing agent (narrow EPR spectrum) and has an electron spin-lattice relaxation time (T_{1S}) that is optimized to allow for efficient polarization transfer to nearby NMR-active nuclei.

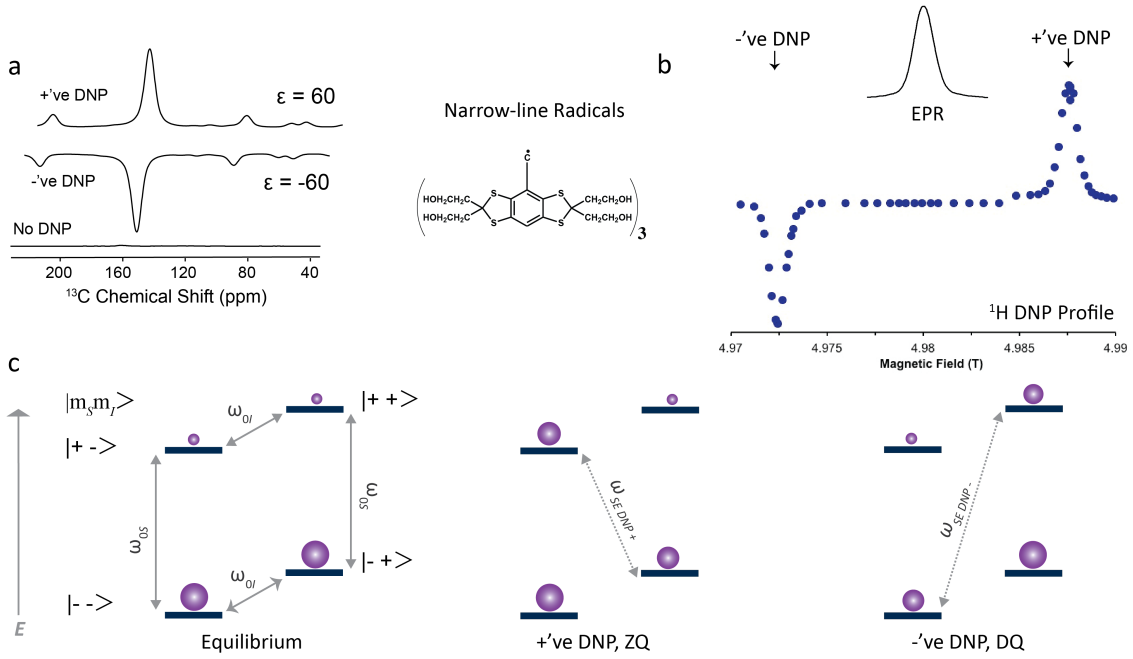


Figure 3: Solid effect DNP: (a) on (DNP) and off (No DNP) experiment showing the positive and negative DNP enhancement (^{13}C[^{1}H] CP MAS NMR experiment on urea using a narrow-line radical), (b) EPR and ^{1}H DNP NMR field profile for a narrow-line polarizing agent and (c) energy level diagram for SE displaying spin-population distribution for a two-spin (one electron and one nucleus) system at thermal equilibrium, positive ($\omega_{0S} - \omega_{0I}$, ZQ) and negative, ($\omega_{0S} + \omega_{0I}$, DQ) DNP enhancements.

Polarizing Agents

Dynamic nuclear polarization requires a source of unpaired electron spins, which is typically achieved using exogenous organic-based radicals also known as polarizing agents. Radicals are typically divided into two categories as either narrow-line or wide-line radicals based on their EPR characteristics, since it is the EPR characteristics of the polarizing agent that give insight into what type of DNP mechanism governs the e^- - n^o polarization transfer. For several compelling reasons, including its reduced spin−lattice relaxation, improved sensitivity possible through CP, and a large database of nitroxide radicals, ^{1}H is often the nucleus of choice for initial polarization at cryogenic temperatures using a wide-line biradical polarizing agent. Subsequent to polarization of ^{1}H, a CP step is used to observe other low-gamma nuclei. This indirect polarization transfer method ($e^- \rightarrow {}^1H \rightarrow X$, Figure 4) has been successfully applied to a wide range of solids including biomolecular, materials and surfaces.[18-20, 91] An alternative to indirect polarization is polarizing an NMR active nucleus (X) directly from a source of unpaired electrons, $e^- \rightarrow X$ (*i.e.,* direct polarization, Figure 4).[23, 87, 88, 92-95] This approach is of interest for many chemical systems that do not cross-polarize efficiently by high-γ nuclei (*e.g.,* ^{1}H or ^{19}F), or those where the high-γ nuclei are absent, and the approach may be of assistance in spectral editing to distinguish between protonated and non-protonated chemical environments.

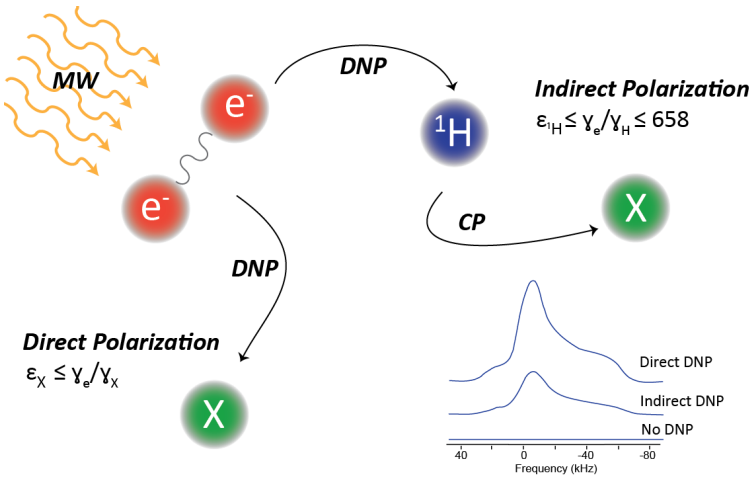


Figure 4: Schematic of indirect (through ^{1}H's) and direct e^{-}-n^{0} polarization transfer pathways in DNP NMR of solids. Indirect polarization (most common) enhances ^{1}H (ε_{1H}), with a theoretical gain in sensitivity of 658, which can then be transferred to some NMR-active nuclei of interest such as ^{13}C, ^{15}N, ^{17}O, *etc*. Direct polarization (less common) enhances NMR active low-gamma nuclei directly (not through a cross polarization step from ^{1}H or ^{19}F). The theoretical gain depends on the NMR active nucleus, such as 2,618 for ^{13}C (ε_{13C}), 3,311 for ^{29}Si (ε_{29Si}), 4,855 for ^{17}O (ε_{17O}), 6,493 for ^{15}N (ε_{15N}), *etc*.

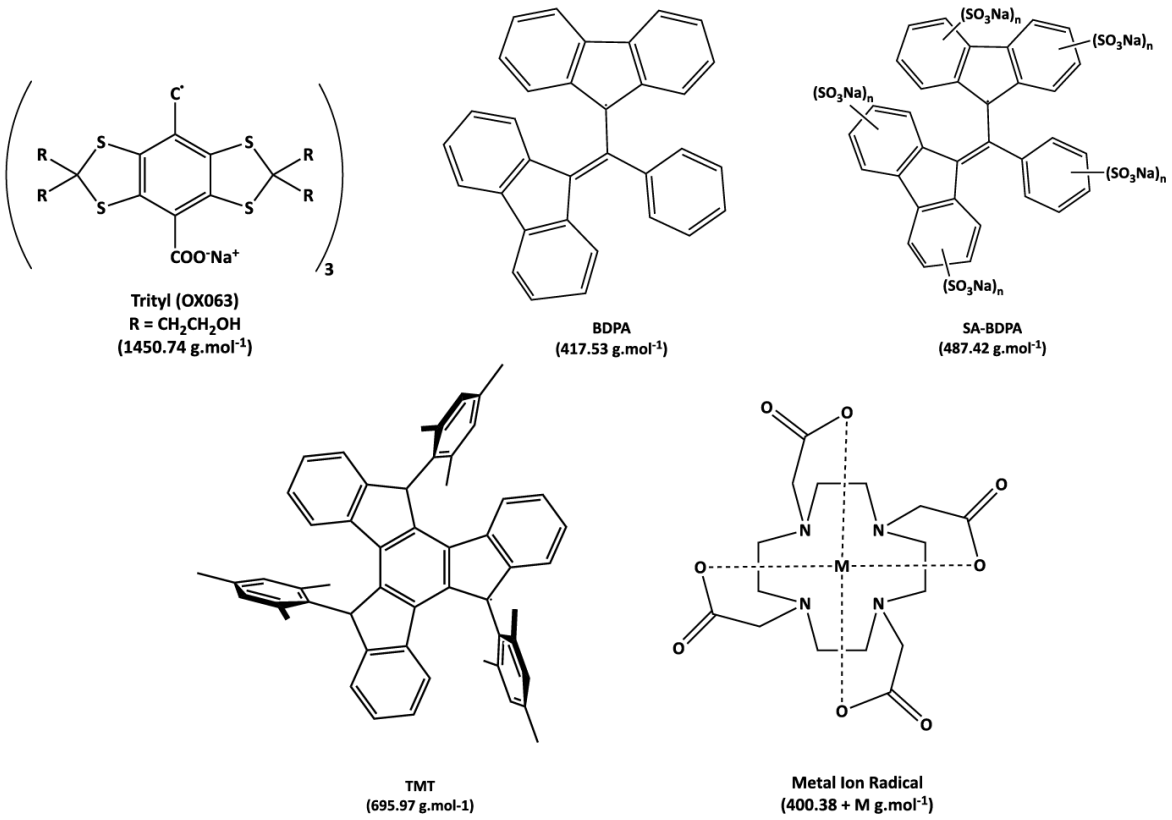
The choices can be vast as many organic radicals exist, particularly with the recent explosive developments in high-frequency DNP NMR and the push for improved biradicals. Below, we highlight a few of the key polarizing agents that are often used for various applications, organized by their characteristic EPR spectra of narrow-line (i) and wide-line (ii) radicals.

i. Narrow-line polarizing agents

Within the DNP NMR field only a handful of narrow-line radicals are currently viable. Although limited in options, these chemically designed radicals provide an interesting array of tunability allowing the selection of different DNP mechanisms and permitting an exploration into various direct and indirect polarization methods. Generally, these narrow-line radicals have been used within applied fields (*vide infra*), and can display the CE or SE DNP mechanism depending on the NMR-active nucleus being probed. In the case of ^{1}H, these radicals exhibit solid-effect characteristics which scale unfavourably with field strength dependence ($\varepsilon \propto B_0^{-2}$).[96] This may

283 change as microwave technology emerges offering higher-output power able to circumvent the
284 losses from increased B_o.

285 Figure 5 illustrates a series of narrow-line radicals that have been successfully applied to
286 various chemical problems. They are promising candidates for low-gamma polarization including
287 ^{13}C,[88, 94, 97] ^{2}H,[93] ^{17}O[23, 87] and ^{29}Si,[98] as these begin to satisfy the CE DNP mechanism of low-
288 gamma nuclei via direct polarization. This may be attractive for materials that do not contain
289 high-gamma nuclei or solids that suffer from extremely long ^{1}H T_1's. Recently, research has
290 begun to emerge adopting the Overhauser DNP mechanism for ^{1}H at high magnetic fields using
291 narrow-line radicals.[67, 68, 99] As the Overhauser effect is the only DNP mechanism that improves
292 with magnetic field strength and requires little microwave power to saturate the electrons,[99] it
293 may offer significant advantages including permitting the use of low-power microwave sources
294 (*e.g.,* EIK). As the field turns to higher and higher magnetic fields (>800 MHz), alternative
295 radicals will surely be developed.
296



297

298 **Figure 5: Common narrow-line radicals used as polarizing agents in high-frequency DNP NMR.**

299 *ii.* *Wide-line polarizing agents*

300 The most prominent radicals used in DNP NMR applications are wide-line polarizing
301 agents, typically comprised of nitroxide moieties. These are chosen due to the range of offerings
302 within the literature and/or commercial sources, synthetic tunability and ease in which they
303 satisfy the CE DNP mechanism for ^{1}H. In most applications of DNP NMR, polarization transfer
304 occurs indirectly through high-polarized protons and a CP step to lower gamma nuclei such as
305 ^{13}C, ^{15}N or ^{29}Si. As outlined above, targeting ^{1}H is advantageous; in addition, in some cases this
306 approach allows one to tune the ^{1}H spin-lattice relaxation and ^{1}H-^{1}H spin-diffusion behavior
307 through ^{2}H exchange (*i.e.,* adjusting the ^{1}H to ^{2}H ratios within the solvent and/or solid of interest).

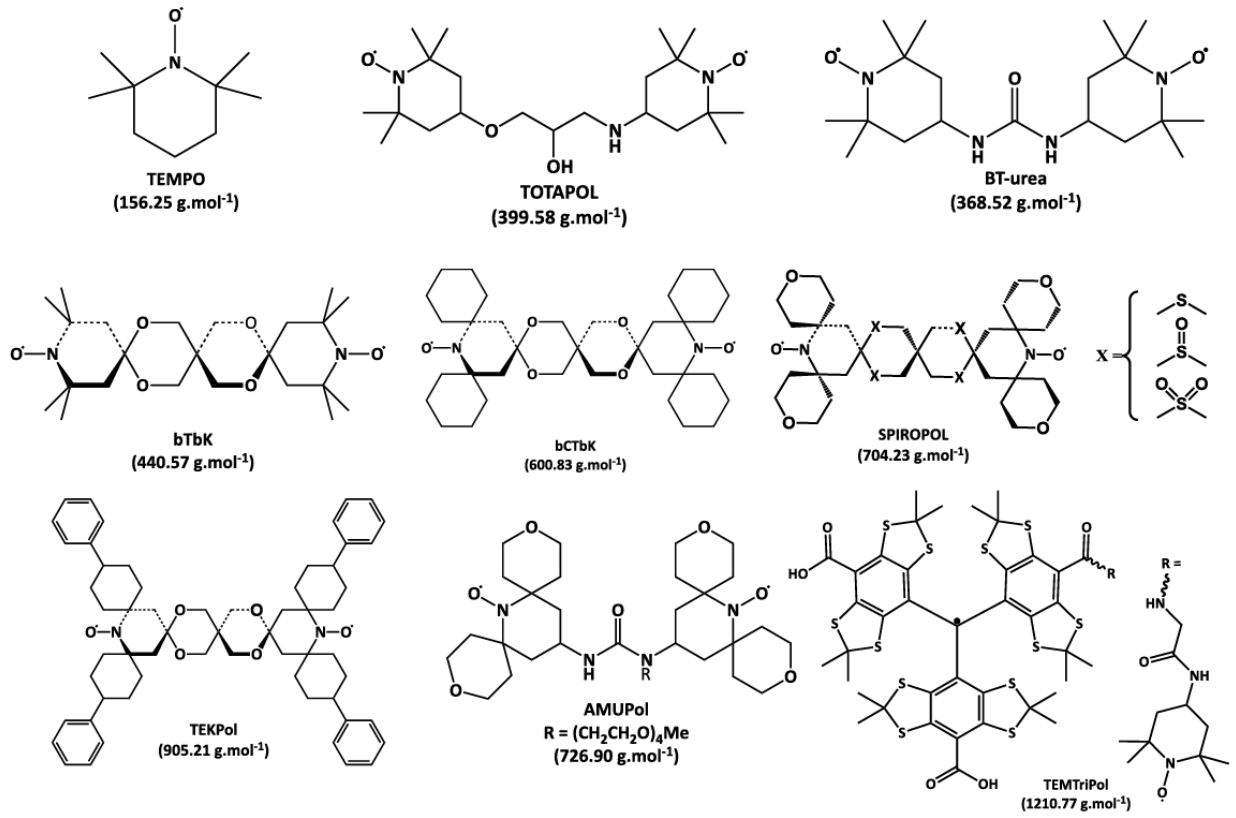


Figure 6: Common wide-line radicals used as polarizing agents in high-frequency DNP NMR.

Early work focused on monoradicals, typically involving TEMPO-based derivatives which are easily sourced. To reach maximum CE DNP enhancements, quantities of 40+ mM electrons were required as at this level favourable $e^- - e^-$ dipole interactions on the order of a few MHz were obtained, but this caused significant paramagnetic relaxation, resulting in a loss of signal as well as reduction in T_2, affecting resolution. In 2006, Song and Hu, graduate students within the Swager and Griffin groups (MIT), respectively, synthesized a highly effective and water soluble biradical known today as TOTAPOL.[86] This began the movement to utilize biradicals which permit a reduction of the concentration of the polarizing source (5 to 15 mM biradical or 10 to 30 mM electrons) and consequently reduce signal quenching[100] while yielding larger enhancements as the two electrons required within the three-spin CE DNP mechanism are highly coupled due to the chemical tether. Current research suggests that a dipolar coupling of

between 25 and 35 MHz, the relative orientation between the two organic radical moieties and the exchange interaction appear to be important factors.[96, 101-103]

For years two biradicals, bTbK[104] and TOTAPOL[86] (Figure 6) were the benchmarks for high-frequency DNP, but with the introduction of commercial instrumentation, advances in both organic and water soluble radicals has exploded since 2013. The nature of biradicals have varied using a host of species; some of the most efficient radicals that have emerged include AMUPOL (hydrophilic biradical)[105] and TEKPOL derivatives (hydrophobic biradical),[103, 106] among many others.[86, 102, 104-111]

Emerging areas in radical development include a report highlighting the benefit of using a mixed biradical that tethers a tempo and trityl moiety. The report demonstrates the excellent high-field enhancements, surpassing conventional biradical nitroxides as well as reducing the signal quenching of the sample, adding another dimension worth exploring in this exciting field.[101] There have also been advances in the use of paramagnetic metals as DNP polarizing agents including homogeneous solids, biomolecular and inorganic chemical systems.[64, 112-115] There is no doubt that radical development will continue to advance along with high-frequency DNP.

Sample Preparation

The vast majority of high-frequency DNP NMR experiments applied to chemical systems utilize an *ex situ* polarizing agent with a cryoprotectant. A DNP sample will consist of a polarizing agent, typically a biradical in 10 ± 5 mM concentration, a solvent mixture and the sample of interest. The cryoprotectant (*i.e.*, solvent mixture) is important for several reasons including allowing the homogeneous dispersion of the polarizing agent within the sample, assisting in providing uniform polarization across the sample and protecting the sample from cryogenic temperatures (particularly important in biological specimens). When choosing a

344 solvent mixture one should consider whether the solvent is more likely to have an amorphous-
345 like consistency or to crystalize, as this has drastic effects on the enhancement efficiency as well
346 as on radical homogeneity.[109] It is also important to ensure the solvent does not react with the
347 sample of interest. Typically, a glycerol-water (60:40, v/v) mixture is the most effective solvent,
348 forming an excellent glass at cryogenic temperatures. Glycerol has been used for decades as a
349 cryoprotecting medium in a host of research projects requiring low-temperature experiments.
350 Other solvent mixtures that appear in the DNP literature include DMSO/water (60:40, v/v),
351 dichloroethane/methanol (95:5, v/v), o-terphenyl, *etc.*[23, 50, 109, 116-120] Typically, hydrophilic
352 solvents are preferred for biomolecular solids while hydrophobic solvents are preferred for
353 inorganic solids.

354 One further step in choosing the appropriate solvent mixture is the ability to exchange
355 isotopes. In general, a ^{1}H concentration of approximately 10% ^{1}H within a solvent has been
356 found to be an effective ^{1}H spin-bath reservoir. Thus, for example, the ^{1}H concentration in the
357 glycerol/water mixture (colloquially referred to as *DNP Juice*) is diluted to approximately 10 %
358 using a combination of glycerol-d_8, D_2O and H_2O (60:30:10, v/v/v).

359 A homogenous sample is one where the radical and chemical sample is readily dissolved
360 in the solvent-matrix and upon quenching a homogeneous glass is formed. This approach often
361 leads to the most effective polarization transfer as the radical is effectively distributed throughout
362 the sample. DNP NMR of a homogeneous, amorphous chemical system can be limited in
363 resolution due to line-broadening stemming from a distribution of chemical shifts, a commonly
364 observed occurrence for many organic and inorganic amorphous materials, as well as from
365 slower side-chain dynamics at cryogenic temperatures. A heterogeneous sample is one where the
366 solvent matrix (radical and solvent) are in contact with the chemical sample, although the sample
367 of interest does not readily dissolve in the matrix. Large enhancements are achievable for these

systems although they are typically reduced due to inhomogeneous radical distribution, solvent selection, glassing ability, domain size, pore size, radical-sample interaction and the limitations in effective e^- - ^1H and ^1H - ^1H spin-diffusion across the solid.[23, 66, 83, 121-126] Dynamics and hydrogen concentrations within the sample can also affect the recorded enhancement. An added benefit of heterogeneous DNP within nano- and microcrystalline solids is that resolution is typically unhampered at cryogenic temperatures enabling both a savings in acquisition time as well as high-resolution spectra that provide detailed structural information. It has also been our experience that using low radical concentrations (< 10 mM biradical) tends to diminish the quenching effect on spin-spin relaxation (T_2), providing higher resolution spectra, although the enhancement is reduced (a trade-off between sensitivity gain and spectral resolution). DNP NMR spectroscopy has been successfully applied to a diverse range of homogeneous and heterogeneous biomolecular[18, 29, 123, 127-140] and inorganic[18, 20, 23, 121, 123, 125, 126, 141, 142] solids.

Other sample preparations have also proven effective for high-frequency DNP NMR including ones that are cryoprotectant-free. An approach that does not need a solvent, or instead one that disperses the radical with a solvent that is subsequently removed. For example, a radical that is introduced onto or into a chemical system such as cellulose or a porous material then followed by evaporation has recently shown promise for natural-abundance systems.[143-145] A self-cryoprotecting solvent-free approach using *in situ* or *ex situ* sedimented (SED) DNP within an apoferritin complex (480 kDa) and BSA has recently been described. [23, 146, 147]

Although these methods lead to a more heterogeneous distribution of radicals and hence polarization is not uniform within the samples, they maintain excellent sensitivity and produce excellent spectral resolution from an overall smaller effect from paramagnetic broadening. More recently a series of *in situ* methods have appeared using either paramagnetic metals within inorganic solids[115] or tagging organic radicals[148] or metals[112, 114] onto biomolecular solids. These

unique approaches to chemically engineering the polarizing agent within the system are interesting avenues that could improve homogenous radical dispersion within the host material such as crystalline solids (for example, alleviate domain size issues in solids) or incorporating radicals as synthetic tags with proteins (for example, incorporating the radical in the lipid within membrane proteins, or using alternative labelling procedures to tag the protein itself).

Summary and Future Outlook

High-frequency DNP NMR will continue to revolutionize our ability to explore the most challenging spectroscopic questions. The balance between radical concentration and resolution is a critical concept that must be carefully evaluated while sample preparation is critical to one's ability to obtain the highest quality spectra. Due to the access of biradicals, glass forming solvents and ease in implementing CE DNP NMR, this area will surely lead in future DNP NMR applications and development for solids. To circumvent some of the enhancement issues at high fields and/or access to high power microwave sources, mixed and narrow-line radical development will surely continue, with the latter turning the attention to Overhauser DNP NMR.

As for the next technological challenges we face, DNP NMR growth will continue with development beyond 800 MHz / 527 GHz on the horizon, faster magic-angle spinning (*i.e.,* > 40 kHz), reducing the cryogen-usage footprint and method development (*e.g.,* electron decoupling, pulsed vs. CW DNP NMR, frequency tunable microwave devices, *etc.*) to improve the sensitivity gains and resolution at higher magnetic fields and/or temperatures. One can foresee the day where a DNP NMR spectrometer will be as routine in research facilities as liquids and solid-state NMR spectrometers are today.

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