Dynamic Nuclear Polarization in Solid State NMR

A Small Step for a Spin, a Giant Leap for Sensitivity

Monu Kaushik[1], Diane Richter[1] and Björn Corzilius[1,]*

Dynamic nuclear polarization (DNP) has been developed more than 60 years ago and increases NMR sensitivity by several orders of magnitude. [1] However, the introduction to modern high-field NMR was delayed and has not occurred until a few years ago. In this short review we will highlight this renaissance and present several examples of DNP in applied solid state NMR.

NMR is widely applied in chemical analysis and industrial quality control. Besides that NMR also plays an important role in applied research, for example, in structural biology or materials science. Here, solution NMR is often approaching its limits due to sample systems being too large for efficient orientation averaging or existing as functional solids. In these cases solid state NMR is utilized, most often in combination with the method of magic-angle spinning (MAS). Fast sample rotation with frequencies up to ~100 kHz along the magic angle of 54.74° with respect to the external magnetic field leads to a collapse of most anisotropies and the occurrence of an isotropic NMR spectrum similar to that in solution.

Unfortunately, NMR is suffering from an inherently small sensitivity due to the small thermal population difference of the spin states (i.e. spin polarization, see fig. 1). In the simple case of a nuclear spin quantum number of $I = 1/2$ this polarization can be described by the Boltzmann distribution:

$$P_I = \frac{N_\alpha - N_\beta}{N_\alpha + N_\beta} = \tanh\left(\frac{\gamma_I \hbar B_0}{2k_B T}\right)$$

Here, N_α and N_β denote the populations of parallel and antiparallel oriented spin moments, γ_I is the gyromagnetic ratio, B_0 is the flux density of the external magnetic field and T is the sample temperature. $\hbar$ and k_B are the reduced Planck's constant and Boltzmann's constant, respectively. As can be seen from Eq. (1) the polarization can be directly enhanced by either increasing the external magnetic field strength or by significant reduction of sample temperature; however, both of these methods are limited to moderate factors due to technical or other reasons.

Dynamic Nuclear Polarization (DNP)

Another possibility to increase nuclear spin polarization – and therefore NMR sensitivity – is the transfer from the much

larger spin polarization of unpaired electrons to the nuclei (fig. 1B). Due to the 660 times larger magnetic moment (in comparison to ^{1}H) that same factor could in theory be obtained as signal enhancement (ε). In experiment, ε of up to ~500 has been achieved within model systems, [3] while for isolated biomolecules factors of up to 250 have been realized [4]. With increasing sample complexity - for example, for cell lysates or whole cells [5] - the enhancement is typically further reduced. Nevertheless, a "small" enhancement factor on the order of ~10 still invaluably increases sensitivity by a factor ~10^2 = 100.

However, this gain in sensitivity does not come for free. For example, unpaired electrons have to be added to the sample in the form of polarizing agents (see fig. 2). While in the pioneering days of DNP relatively simple radicals based on 1,3-bisdiphenylene-2-phenylallyl (BDPA), triphenylmethyl (trityl) or 2,2,6,6-tetramethylpiperidine-1-yl)oxyl (TEMPO) have been used in relatively large concentrations (40-60 mM), this field has been revolutionized in the year 2004 by the development of tethered bis-nitroxide biradicals [6]. By spatially linking two radical moieties efficient dipolar couplings between the electron spins are provided even at small concentrations of 5-20 mM and a much more efficient polarization transfer can be achieved (fig. 1C). At the same time detrimental aspects due to paramagnetic relaxation can be greatly reduced [2]. Furthermore, experiments still have to be conducted under cryogenic conditions with temperatures around 100 K in order to utilize highly efficient DNP mechanisms. Lastly, microwaves with frequencies typically between 263 and 527 GHz have to be generated with large powers in order to efficiently excite certain electron paramagnetic resonance (EPR) transitions responsible for the DNP transfer. These latter aspects not only lead to a significant increase in investment and maintenance and potentially cause inhomogeneous line broadening in the frozen solution but also have posed a hurdle in the development of MAS DNP at high magnetic fields. Solely cyclotron maser devices, gyrotrons, have proven to provide the required output power of ~20 W over continuous operation periods of several days [7, 8]. Additionally, an efficient heat exchanger system is used for cooling of MAS gases with liquid nitrogen (fig. 1D) [9].

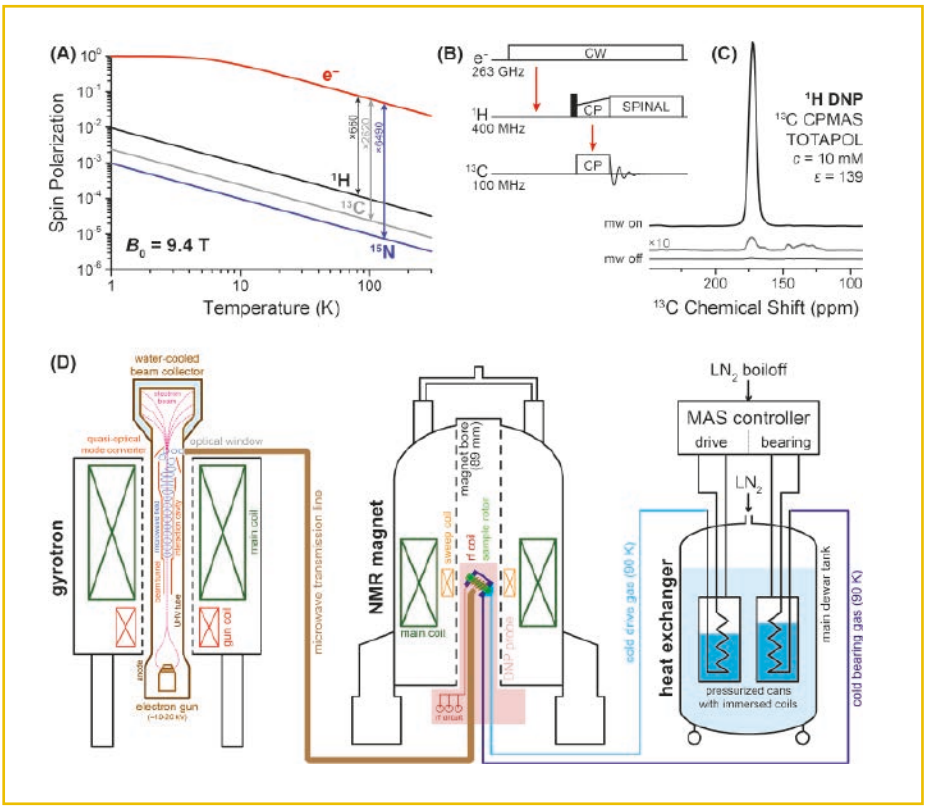

Fig. 1: Spin polarization following Eq. (1) (A); pulse scheme for DNP-enhanced cross polarization (CP-MAS) with depicted transfer pathway of enhanced polarization (red arrows) (B). CPMAS NMR signal of 1 M ^{13}C-urea in d_8-glycerol/D_2O/H_2O (60/30/10 vol.-%) at 5 T and 84 K with (mw on) and without (mw off) DNP enhancement, data from [2] (C); scheme of a typical MAS DNP instrument (D, probe cryostat as well as variable temperature (VT) line are omitted).

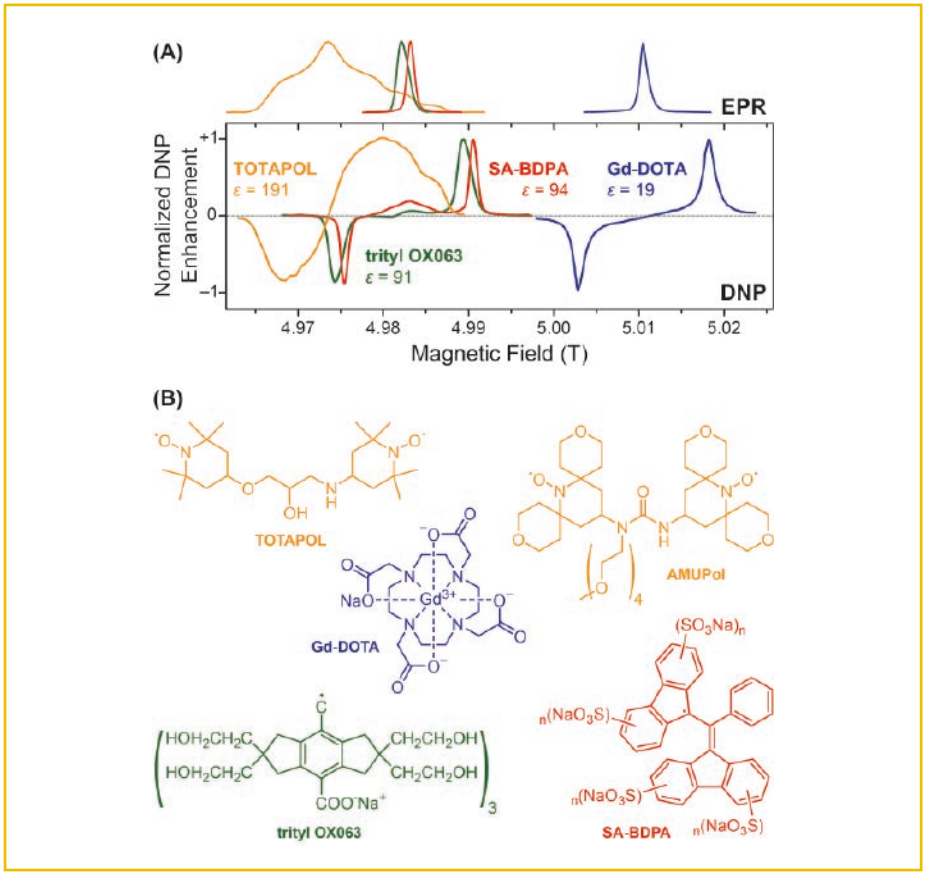

Fig. 2: EPR spectra and field dependent enhancement (normalized to given ε) of TOTAPOL [10] (10 mM), OX063 [11] (40 mM), SA-BDPA [12] (40 mM) and Gd-DOTA [13] (10 mM) at a microwave frequency of 140 GHz (A) as well as their respective chemical structures (B). Structure of AMUPol from Sauvée *et al.*[14]

Modern applications of MAS DNP

By combining all of the aforementioned developments in the laboratory of Robert G. Griffin at MIT it has been possible

to investigate the late stages in the photocycle of bacteriorhodopsin [15] as well as the structurally important elements of amyloid fibrils [16, 17]. These successes finally led to the successive development and distribution of the first commercial MAS DNP spectrometer operating at 9.4 T or 400 MHz in the year 2009 by Bruker Biospin [18]. Recently this has been followed up by two newer developments at 14.1 T/600 MHz and 18.8 T/800 MHz. Besides the applications in structural biology the advantages of DNP have had a tremendous impact in materials sciences. Even though NMR is ideally suited for the investigation of surface functionalized materials for heterogeneous catalysis, the small sensitivity often impedes the observation of the active sites due to their rather small volume concentration. Lesage *et al.* succeeded in polarizing ^{1}H nuclei within the pores of a powdered support material by DNP; an enhancement factor of ~50 allowed for the first time the detection and analysis of ^{13}C MAS NMR signals of the organic functional groups in natural isotope abundance (1.1%) [19].

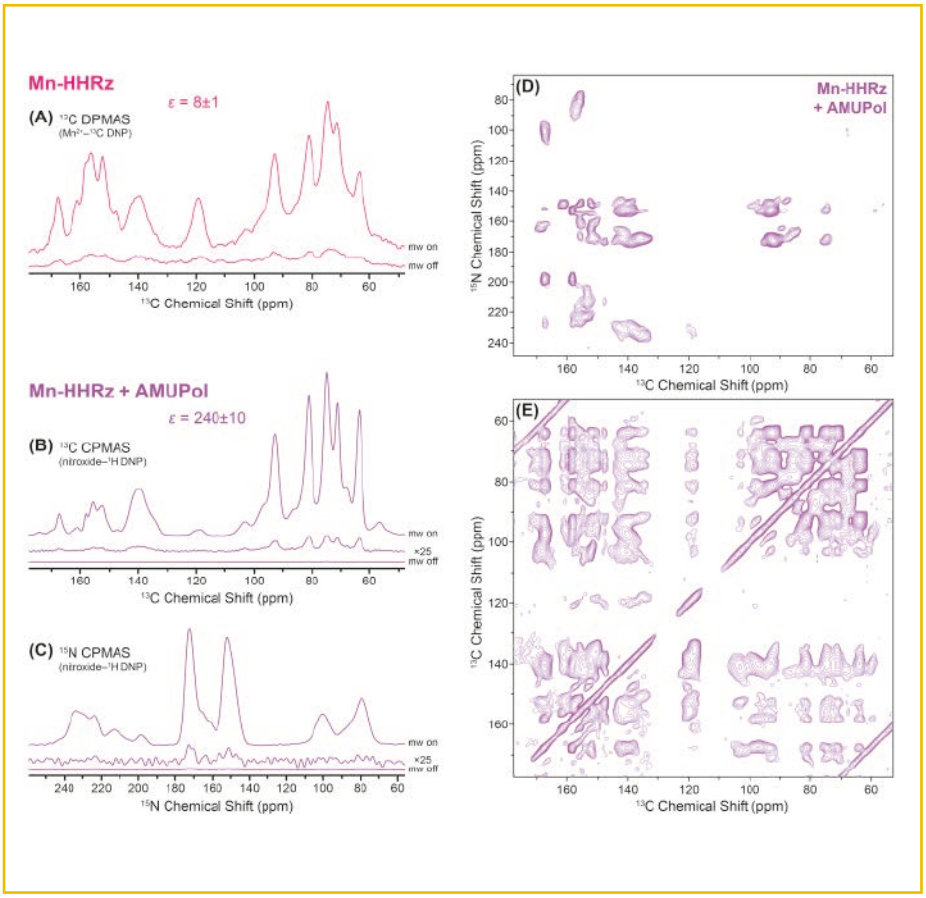

Fig. 3: MAS NMR spectra of Mn-HHRz, direct polarization (DPMAS) read-out by a single 90° pulse and DNP-enhanced by Mn^{2+} (A). ^{13}C (B) and ^{15}N (C) CPMAS spectra as well as ^{15}N-^{13}C (D) and ^{13}C-^{13}C (E) correlation spectra of Mn-HHRz, DNP-enhanced by AMUPol (ε = 240 in all cases). All data from Wenk *et al.* [4].

Ongoing methods development on the basis of endogenous electron spins

Up to this point all discussed applications utilized exogenous radicals which have been added to the sample. However, many biomolecules already contain paramagnetic sites – especially metalloproteins or redox-active enzymes. Maly *et al.* have recently demonstrated the use of an endogenous radical for DNP of a protein [20]. Besides that, the utilization of paramagnetic metal ions is a very exciting and promising aspect which attracted the main focus of our research group. Several years ago we have been able to prove that Gd^{3+} as well as Mn^{2+} can act as polarizing agents [13]. This allows the utilization of natural or substituted metal ions within the active sites of biomolecules for DNP. Following this, we have been able to polarize a hammerhead ribozyme (HHRz) with an endogenously bound Mn^{2+} for the first time (fig. 3A) [4]. This RNA molecule is generally difficult to access via solution NMR due to its size and internal dynamics. Furthermore we have obtained an enhancement factor of 240 by addition of a biradical (fig. 3B-E). This is an important step towards the development of powerful methods for RNA structural biology based on solid state NMR, an experimental field which is practically still in its infancy status and is highly impeded by limited sensitivity [21].

Acknowledgment

We thank the Deutsche Forschungsgemeinschaft (DFG) for funding within the Emmy Noether program (grant CO802/2-1) and the center for biomolecular magnetic resonance (BMRZ) for financial support and access to instrumentation.

Literatur

All References are available online for your convenience at http://bit.ly/GLJ-Corzilius

Affiliations

[1]Institute of Physical and Theoretical Chemistry, Institute of Biophysical Chemistry and Center for Biomolecular Magnetic Resonance (BMRZ), Goethe University, Frankfurt am Main, Germany

More references are available from the authors.

Contact

Dr. Björn Corzilius
Institute of Physical and Theoretical Chemistry
Institute of Biophysical Chemistry and Center for Biomolecular Magnetic Resonance (BMRZ)
Goethe University of Frankfurt
Germany
corzilius@solidstatednp.com