

# Unprecedented Carbon Signal Enhancement in Liquid-State NMR Spectroscopy

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dynamic nuclear polarization · high magnetic field ·  
NMR spectroscopy · sensitivity enhancement

Dedicated to Hartmut Oschkinat on the  
occasion of his 60th birthday

“The weakness of the nuclear spin interactions, so far described as an advantage, leads on the other hand to severe detection problems. Large number of spins are required to discriminate the weak signals from noise. Under optimum conditions [...],  $10^{14}$ – $10^{15}$  spins of one kind are needed to detect a signal within a performance time of one hour. The low signal-to-noise ratio is the most limiting handicap of NMR. Any increase by technical means will significantly extend the possibility range of NMR application.”<sup>[1]</sup> This quotation from Richard Ernst’s Nobel prize lecture sets the stage for numerous studies and approaches to improve the signal-to-noise-ratio (S/N) in NMR. In recent years, many different methods, including PHIP,<sup>[2]</sup> SABRE,<sup>[2]</sup> hyperpolarized noble gases,<sup>[3]</sup> MAS DNP,<sup>[4]</sup> nitrogen vacancies in diamonds,<sup>[5]</sup> dissolution DNP,<sup>[6]</sup> and DNP in the solid-state,<sup>[7]</sup> have been developed, and a summary of these can be found in a recent review article in *Angewandte Chemie*.<sup>[8]</sup>

A particularly important approach for signal enhancement is dynamic nuclear polarization (DNP), a technique described first by Overhauser and Abragam and pioneered by Bob Griffin over many years. In this technique, the sensitivity of NMR is enhanced by transferring the higher electron spin magnetization to the nucleus through the hyperfine interactions between electron and nuclear spins. The electron spins of the radical are irradiated with microwaves (MW) at/near the electron Larmor frequency.<sup>[9]</sup> In a simple way: the sample is first doped with a stable radical, the electron spin transitions of the radical are irradiated with MW, and afterwards an increased NMR signal is detected.

Up to now, many applications have been published for DNP in the solid state, including the characterization of photo-intermediates for various rhodopsins<sup>[10]</sup> or of functional groups on the surface of catalysts,<sup>[11]</sup> or the detection of cell receptors at native concentrations, to name only a few recent examples.<sup>[12]</sup>

The highlighted publication by the Bennati group focusses on novel methods for DNP in the liquid state.<sup>[14]</sup> Here, the enhancement  $\varepsilon$  depends on a number of different experimental parameters:

$$\varepsilon = 1 - \xi f s \frac{|\gamma_S|}{\gamma_I} \quad (1)$$

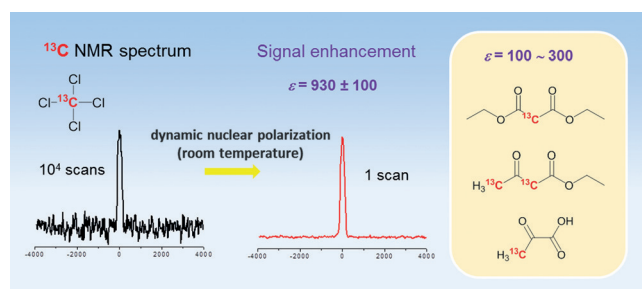
where  $\gamma_{S,I}$  are the gyromagnetic ratios of electron and nuclear spins,  $s$  is the saturation factor of the electron spins,  $f$  is the leakage factor, and  $\xi$  is the coupling factor.  $s$  and  $f$  can range between 0 and 1. The theoretical maximum of enhancement results from the gyromagnetic ratios, which are  $\gamma_e/\gamma_H = -658$  for  $^1\text{H}$  or  $\gamma_e/\gamma_C = -2618$  for  $^{13}\text{C}$ . To understand the underlying mechanism of DNP enhancement at high magnetic field in the liquid state, measurement of the coupling factor  $\xi$  is crucial.

In proton-detected DNP experiments at higher field, only small signal enhancements ( $< 10^2$ ) have been observed up to now.<sup>[7]</sup> The hyperpolarization in this case is dominated by electron–nuclear dipole–dipole interactions, which diminish as field strength increases. Only one high-field application of  $^{13}\text{C}$ ,  $^{15}\text{N}$ ,  $^{19}\text{F}$ , and  $^{31}\text{P}$  has been reported, where significant contribution from scalar interaction was observed.<sup>[13]</sup> The generally used model to predict DNP enhancements assumes spins on spherical molecules and translational diffusion; such treatment leads to the possibility to describe the DNP in a closed analytical form. This model was able to reproduce experimental  $^1\text{H}$  DNP enhancements for different solvents, with different radicals and at different fields.

In the contribution from the Bennati laboratory highlighted here, enhancements of  $^{13}\text{C}$  solvent nuclei at a magnetic field of 3.4 T (144 MHz proton, 36.4 MHz carbon and 94 GHz electron Larmor frequency) and of  $^{13}\text{C}$ -labelled compounds doped with  $^{15}\text{N}$ -labelled TEMPONE (4-oxo-2,2,6,6-tetramethylpiperidine-*N*-oxyl) are reported.<sup>[14]</sup> In all cases, signal enhancements of at least two orders of magnitude were observed. In case of  $\text{CCl}_4$ , an unprecedented  $930 \pm 100$  signal enhancement was detected (Figure 1). In case of acetoacetate, diethyl malonate, and pyruvic acid with the  $^{13}\text{C}$  labeling pattern shown in Figure 1, 100- to 300-fold enhancements were observed. These enhancements represent the highest enhancement achieved by Overhauser DNP to date, and cannot be explained within the general mechanistic framework developed until now.

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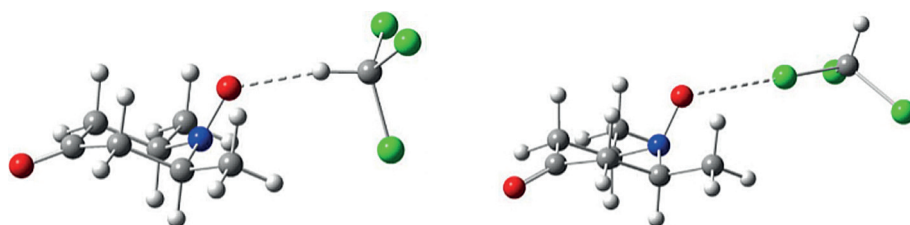
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**Figure 1.** Left:  $^{13}\text{C}$  NMR spectrum of  $^{13}\text{C}$ -labeled  $\text{CCl}_4$  at thermal equilibrium. Center:  $^{13}\text{C}$  NMR spectrum of the former compound with only one scan after MW irradiation; measured enhancement factor =  $930 \pm 100$ . Right:  $^{13}\text{C}$ -labeled compounds with attached protons show 100–300 fold enhancement.

To understand the relying mechanism behind this phenomenon, the authors measured the leakage factor  $f$  and the saturation factor  $s$  separately, and based on Equation (1), they were able to calculate the coupling factor and their temperature dependence. In addition, the  $^{13}\text{C}$  nuclei spin-lattice relaxation  $T_{1n}$  was measured to determine the scalar relaxation probability. To further elaborate on the scalar relaxation dominated electron–nuclei spin interaction, in the Luchinat laboratory in Florence, nuclear magnetic relaxation dispersion (NMRD) was measured of  $^{13}\text{C}$  in  $\text{CHCl}_3$  and  $\text{CCl}_4$  up to 1 T. The experimental results could be reproduced in good agreement with their new model, and from the fitting parameters, it could be concluded that at higher fields,  $^{13}\text{C}$  relaxation is dominated by scalar hyperfine interactions mediated by the chlorine atom and modulated at a very fast time scale around 1 ps. This is in contrast with low-field Overhauser DNP, where mostly the single quantum transitions mediated by dipolar interactions dominate the coupling factor between electron and nuclei spin. The scalar-relaxation-mediated hyperpolarization has favorable scaling with higher magnetic fields in comparison to the dipolar-relaxation-mediated hyperpolarization. To predict the strength of the  $^{13}\text{C}$  hyperfine coupling, the authors calculated the structure of the spin-radical-chloroform complex (Figure 2) using quantum chemical calculations.

The authors then tested the general feasibility of  $^{13}\text{C}$  DNP measurements in biologically relevant molecules with different chemical environment. In all cases, they were able to show 100–200-fold enhancement on carbon nuclei with a attached



**Figure 2.** Two geometry-optimized (using Gaussian09) orientations of the non-methylated TEM-PONE-derivative- $\text{CHCl}_3$  complex used to calculate  $^{13}\text{C}$  hyperfine coupling constants. On the left, the H atom of  $\text{CHCl}_3$  coordinates with the oxygen atom of the radical, on the right, the chlorine atom coordinates with the oxygen atom (unpaired electron) of the radical. Color code: C gray, Cl green, H white, N blue, O red.

proton. For the  $\alpha$ -carbons of diethyl malonate, a positive enhancement was observed that originates from scalar interactions. In conclusion, either proton- or chlorine-mediated hyperfine interactions allow significant DNP signal enhancement through a scalar mechanism.

These results point out the limitations of previous models that predicted low DNP enhancement at high field in the liquid state. The elaborate analysis of the mechanism of the DNP interactions using MD and DFT calculations aids in the design of new radicals and an experimental setup at high field, which is standard in high-resolution NMR spectroscopy. Even though a restriction on sample volume still exists, new MW technologies might be able to overcome these limitations, which would enable the application of DNP in standard liquid-state NMR measurements. Such methodological improvements would find widespread application: In the case of metabolomics, on-chip NMR, microfluidic device detection, or experiments coupled to HPLC, where the sample amount is limited, the DNP enhancement achieved by Bennati et al. could allow NMR to become the method of detection. In metabolomics so far, the lower limit of detection is around the micromolar range. With DNP, it might be possible to reach nanomolar range, which would be required to detect small molecules at their physiological concentrations and for metabolomic flux analysis.

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### Conflict of interest

The authors declare no conflict of interest.

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- [1] R. R. Ernst, *Angew. Chem. Int. Ed. Engl.* **1992**, *31*, 805; *Angew. Chem.* **1992**, *104*, 817.
- [2] R. A. Green, R. W. Adams, S. B. Duckett, R. E. Mewis, D. C. Williamson, G. G. R. Green, *Prog. Nucl. Magn. Reson. Spectrosc.* **2012**, *67*, 1–48.
- [3] B. Goodson, *J. Magn. Reson.* **2002**, *155*, 157–216.
- [4] Q. Ni, E. Daviso, T. V. Can, E. Markhasin, S. K. Jawla, T. M. Swager, R. J. Temkins, J. Herzfeld, R. Griffin, *Acc. Chem. Res.* **2013**, *46*, 1933–1941.
- [5] D. Suter, F. Jelezko, *Prog. Nucl. Magn. Reson. Spectrosc.* **2017**, *98–99*, 50–62.
- [6] J. H. Ardenkjær-Larsen, B. Fridlund, A. Gram, G. Hansson, L. Hansson, M. H. Lerche, R. Servin, M. Thaning, K. Golman, *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 10158–10163.

- [7] C. Griesinger, M. Bennati, H. M. Vieth, C. Luchinat, G. Parigi, P. Höfer, F. Engelke, S. J. Glaser, V. Denysenkov, T. F. Prisner, *Prog. Nucl. Magn. Reson. Spectrosc.* **2012**, *64*, 4–28.
- [8] J. H. Ardenkjær-Larsen, G. Boebinger, A. Comment, S. Duckett, A. Edison, F. Engelke, C. Griesinger, R. Griffin, C. Hilty, H. Maeda, G. Parigi, T. Prisner, E. Ravera, J. Van Bentum, S. Vega, A. Webb, C. Luchinat, H. Schwalbe, L. Frydman, *Angew. Chem. Int. Ed.* **2015**, *54*, 9162–9185; *Angew. Chem.* **2015**, *127*, 9292–9317.
- [9] T. Maly, G. Debelouchina, V. Bajaj, K.-N. Hu, C.-G. Joo, M. Mak-Jurkauskas, J. Sirigiri, P. van der Wel, J. Herzfeld, R. Temkin, R. Griffin, *J. Chem. Phys.* **2008**, *128*, 052211.
- [10] a) M. Mak-Jurkauskas, V. Bajaj, M. Hornstein, M. Belenky, R. Griffin, J. Herzfeld, *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 883–888; b) J. Becker-Baldus, C. Bamann, K. Saxena, H. Gustmann, L. Brown, R. Brown, C. Reiter, E. Bamberg, J. Wachtveitl, H. Schwalbe, C. Glaubitz, *Proc. Natl. Acad. Sci. USA* **2015**, *112*, 9896–9901; c) K. Frederick, V. Michaelis, B. Corzilius, T.-C. Ong, A. Jacavone, R. Griffin, S. Lindquist, *Cell* **2015**, *163*, 620–628.
- [11] A. Zagdoun, G. Casano, O. Ouari, G. Lapadula, A. Rossini, M. Lelli, M. Baffert, D. Gajan, L. Veyre, W. Maas, M. Rosay, R. Weber, C. Thieuleux, C. Coperet, A. Lesage, P. Tordo, L. Emsley, *J. Am. Chem. Soc.* **2012**, *134*, 2284–2291.
- [12] M. Kaplan, S. Narasimhan, C. de Heus, D. Mance, S. van Doorn, K. Houben, D. Popov-Čeleketić, R. Damman, E. Katrukha, P. Jain, W. Geerts, A. Heck, G. Folkers, L. Kapitein, S. Lemeer, P. van Bergen en Henegouwen, M. Baldus, *Cell* **2016**, *167*, 1241–1251.
- [13] N. Loening, M. Rosay, V. Weis, R. Griffin, *J. Am. Chem. Soc.* **2002**, *124*, 8808–8809.
- [14] G. Liu, M. Levien, N. Karschin, G. Parigi, C. Luchinat, M. Bennati, *Nat. Chem.* **2017**, <https://doi.org/10.1038/nchem.2723>.

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