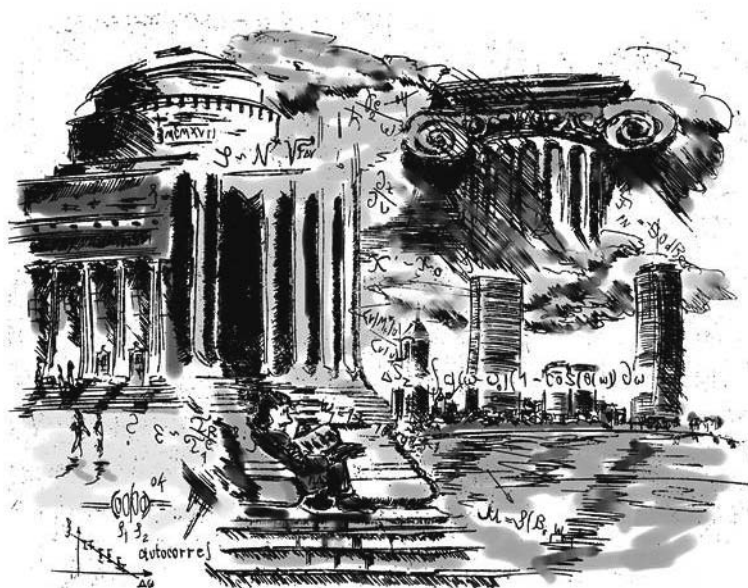


## Dynamic Nuclear Polarization: New Experimental and Methodology Approaches and Applications in Physics, Chemistry, Biology and Medicine

This special issue of *Applied Magnetic Resonance* provides an overview of recent advances in the use of dynamic nuclear polarization (DNP) to enhance signal intensities in nuclear magnetic resonance (NMR) and magnetic resonance imaging (MRI).



*This etching dates back to 1990 when Thomas Prisner worked as a postdoc in the laboratory of Robert Griffin at Massachusetts Institute of Technology.*

NMR is an extremely powerful tool for the identification of chemical composition and the determination of molecular structure. Because of its versatility it is widely applied in chemistry, materials sciences, and biomedical research. MRI provides noninvasive spatial and functional information that is superior to any other imaging technique particularly in the field of medical diagnostics. The major drawback of the methods is their low sensitivity caused by the small magnetic

moment of the nuclear spins which results in a small Zeeman splitting of the nuclear spin energy levels and therefore in only a small Boltzmann polarization. Various strategies have been designed to overcome this sensitivity limitation.

The invention of pulse methods has drastically improved the sensitivity of NMR spectroscopy and MRI. Pioneering research developing this idea and applying it to biological and medical applications was recognized by the award of three Nobel prizes.

Furthermore, within the recent years hardware developments such as magnets operating at very high fields and low detector noise temperature using cryoprobes led to further improvements in sensitivity.

Not only these hardware-driven advancements in NMR signal-to-noise ratio but also quantum mechanical couplings of the nuclear spin system to other transitions can be employed to obtain large signal enhancements. They all rely on a specific coupling of the nuclear spin system to other highly polarized quantum states.

The transfer of angular momentum of photons in polarized laser light onto He or Xe atoms gave rise to high spin polarization [1, 2]. Similarly, the high electron spin polarization of photoexcited radical pairs or triplet states have been used to polarize nuclear spin states [3–6]. Another strategy takes advantage of the correlation between rotational and spin states in diatomic hydrogen [7]. The pairwise transfer of the diatomic hydrogen in the rotational “para” state during a hydrogenation reaction allows generation of highly polarized nuclear spin systems. Another method, known as the Haupt effect, involves the conversion of rotational energy bound in molecular rotors during a fast temperature jump into nuclear polarization [8].

In all of these cases, the mechanisms are restricted to a rather small class of molecules. An exemption is DNP. It permits a broad range of applications both in solids and liquids, solely requiring the presence of a paramagnetic species in the sample. The polarization of the more than 600 times larger electron spin moment (as compared to the proton nuclear spin moment) can be transferred onto the nuclear spin system by microwave irradiation of electron transitions. Interestingly, this idea already originated in the early days of NMR, when Overhauser [9] predicted the enhancement of the nuclear spin system polarization by electronic cross relaxation at a conference of the American Physical Society in 1953. Leading NMR experts of that time, such as Bloch, Purcell, Rabi and Ramsey, received this proposal initially with deep scepticism. However, shortly afterwards Carver and Slichter [10, 11] succeeded in experimentally verifying Overhauser’s prediction in metallic lithium using a static magnetic field  $B_0 = 30.3$  G (corresponding to an NMR frequency of 50 kHz and an ESR frequency of 84 MHz). Overhauser’s idea was quickly extended to other systems such as dielectric solids and electron–nuclear spin systems in the liquid state.

The application of this concept to obtain highly polarized nuclear spin targets for neutron scattering initiated a number of theoretical and experimental approaches to investigate the underlying physics of polarization transfer in an electron–nuclear spin system. Abragam published numerous papers on the solid

effect involving a coupled electron and nuclear spin in a dielectric system [12, 13]. In the 1960s various publications described the interaction in a system consisting of two coupled electrons and one nuclear spin – nowadays conventionally called cross effect [14, 15]. Goldman and others subsequently extended the DNP theory to systems containing many coupled electron and nuclear spins by introducing the concept of spin temperature [16]. Despite the success of particle scientists generating up to 90% spin polarization using DNP [17] it is interesting to note that this strategy initially did not find wide use in the field of NMR application. Only a few rather exotic, but in hindsight important applications by Wind et al. [18] and Yannoni [19] took advantage of DNP to enhance the NMR polarization in charcoal and diamonds. It is noteworthy that they combined for the first time signal enhancement by DNP with magic-angle spinning (MAS) to narrow the line width in solid-state NMR spectra. Schaefer et al. studied polymers by using DNP at 1.4 T, which corresponds to 40 GHz ESR and 60 MHz  $^1\text{H}$  NMR frequency [20, 21].

The enhancement of nuclear polarization in liquids by the Overhauser effect was investigated in detail by Hausser and Stehlik [22] and later by Müller-Warmuth [23]. They achieved high polarization at low magnetic fields (0.1–1 T), which rapidly diminished when the microwave frequency exceeded the inverse rotational or translational correlation time of the radical-solvent mixture.

Only just during recent years achievements of the Griffin group at MIT renewed the interest in DNP for enhancing the spin polarization in high-field MAS NMR experiments [24, 25]. Their advances in hardware technology are mainly related to the use of high-power high-frequency microwave sources which are needed for DNP at high magnetic field strengths. They are the result of more than a decade of research spent on optimising conditions for DNP MAS NMR as evidenced by their first paper published in 1993 [26]. In addition, they could demonstrate that signal enhancements up to 330 are achievable even at high magnetic fields using the cross effect with biradicals as DNP agents. This signal enhancement improvement was a breakthrough for high-resolution biomolecular structural studies by MAS NMR [27].

Another recent development by Golman's group (based at GE Healthcare) involved in the generation of highly polarized spin systems in the solid state at very low temperatures in a purpose-designed polarizer followed by a rapid dissolution process and sample transfer to the NMR/MRI spectrometer during which the very high nuclear spin polarization of  $^{13}\text{C}$ - or  $^{15}\text{N}$ -labelled small molecules is largely maintained [28]. Especially the medical MRI community became excited by the prospects of using DNP to increase sensitivity to determine the spatial distribution and conversion rates of metabolites *in vivo*. The feasibility was demonstrated in a number of *in vivo* experiments using hyperpolarized  $^{13}\text{C}$ -labelled pyruvate [29–31].

The interest generated by DNP MAS NMR and biomedical MRI (using a prepolarizing procedure based on DNP) has recently stimulated wide-ranging activities in the field of hardware development, theoretical studies of the underlying physical principles of DNP and search for novel applications.

The strategy of dissolution DNP has been extended to *in vitro* applications to gain high-resolution liquid-state NMR spectra from prepolarized small molecules. Currently, a number of research groups are investigating potential applications of this enhancement strategy for metabolomics and molecular dynamics studies as well as *in vitro* metabolic reactions and perfused cell systems. Strategies for rapid multidimensional NMR spectroscopy techniques using single sample excitations have been recently published [32, 33]. They are ideally suited for experiments with nonthermal high nuclear spin polarization created by prepolarization. For spin systems with specific symmetry properties theoretical and experimental evidence for the existence of long-lived spin states with relaxation rates much slower than for longitudinal relaxation were published [34, 35]. Such states could be used to store and preserve the polarization. Driven by the huge interest in the design of novel strategies for medical diagnostics, in particular in oncology, a number of *in vivo* projects have been initialised and first human applications using externally prepolarized  $^{13}\text{C}$ -labelled pyruvate will be carried out in the near future.

The pioneering high-field solid-state DNP work of the MIT group also started a number of new technological and methodological projects. Stable high-power and high-frequency microwave sources such as gyrotrons are currently available, allowing DNP experiments at magnetic fields that provide sufficient chemical shift resolution for NMR spectra of proteins and complex metabolite mixtures [36, 37]. Furthermore, research in modulating the frequency output of these sources and generating very short pulses has been initialised with the likely outcome of a new generation of microwave sources with optimised properties for DNP experiments. On the other hand, low-temperature NMR systems were designed and the feasibility of MAS NMR at very low temperatures was demonstrated [38]. It also initiated research activities to quantitatively calculate and optimize polarization transfer mechanisms. An entire new field of spin manipulations using principles based on optimal-control theory has been established within the last few years [39, 40]. Another activity is to extend the method to high-resolution liquid-state NMR. In these cases the liquid protein sample is either polarized at low magnetic field values and rapidly shuttled to the NMR field or the polarization transfer is performed *in situ* directly at the required NMR field.

This revival of DNP launched a number of new academic and commercial activities. In various countries academic DNP initiatives were formed such as in Germany, Switzerland, Israel and the UK. In particular, an EU-wide network DNP for NMR in Structural Biology funded by the Framework 6 program was established between ten European research groups investigating the feasibility of liquid-state DNP strategies in biomolecular research. A commercial polarizer is currently available from Oxford Instruments Molecular Biotools Ltd which can be used to prepolarize molecules and pneumatically shuttle the sample after dissolution to high-resolution NMR instruments. Furthermore, developments are underway for both the design of a commercial integrated dissolution DNP NMR system by Oxford Instruments and a commercial DNP MAS NMR spectrometer including a high-power microwave source by Bruker BioSpin GmbH.

The recent interest in DNP has brought together scientists from a wide range of different fields of magnetic resonance research: links between the ESR hardware experts and high-resolution NMR specialists were established and discussions between radical chemists and medical MRI scientists were initiated. With this objective in mind, the first international symposium on dynamic nuclear polarization was organised at the University of Nottingham in August 2007. The recent rapid developments in the field of DNP research deserved a platform for scientific discussions and exchange of ideas. The collection of publications in this special issue is the outcome of this symposium. They demonstrate the full breadth of ongoing research in DNP hardware, theory, experimental optimization and a variety of applications. Additionally they also provide a summary of the many challenges this research field is currently facing. We hope that this special issue may serve the newcomers as an introduction into the current state of DNP research and for the hardware and application experts in the ESR, NMR and MRI communities to get more details on particular aspects of DNP.

#### References

1. Goodson, B.: *J. Magn. Reson.* **155**, 157–216 (2002)
2. Leawoods, J.C., Yablonskiy, D.A., Saam, B., Gierada, D.S., Conradi, M.S.: *Concepts Magn. Reson.* **13**, 277–293 (2001)
3. de Kanter, F.J.J., den Hollander, J.A., Huizer, A.H., Kaptein, R.: *Mol. Phys.* **34**, 857–874 (1977)
4. Zysmilich, M.G., McDermott, A.: *J. Am. Chem. Soc.* **118**, 5867–5873 (1996)
5. van Kesteren, H.W., Wenckebach, W.T., Schmidt, J.: *Phys. Rev. Lett.* **55**, 1642–1644 (1985)
6. Brunner, H., Fritsch, R.H., Hausser, K.H.: *J. Phys. Sci.* **42**, 1456–1457 (1987)
7. Bowers, C.R., Weitekamp, D.P.: *Phys. Rev. Lett.* **57**, 2645–2648 (1986)
8. Horsewill, A.: *Prog. Nucl. Magn. Reson. Spectrosc.* **35**, 359–389 (1999)
9. Overhauser, A.W.: *Phys. Rev.* **92**, 411–415 (1953)
10. Carver, T.R., Slichter, C.P.: *Phys. Rev.* **92**, 212 (1953)
11. Carver, T.R., Slichter, C.P.: *Phys. Rev.* **102**, 975–980 (1956)
12. Abragam, A., Proctor, W.G.: *C. R. Acad. Sci.* **246**, 2253 (1959)
13. Abragam, A., Goldman, M.: *Nuclear Magnetism: Order and Disorder*. Clarendon, Oxford (1982)
14. Hwang, C.F., Hill, D.A.: *Phys. Rev. Lett.* **18**, 110 (1967)
15. Kessenikh, A.V., Manenkov, A.A., Pyatnitskii, G.I.: *Sov. Phys. Solid State* **6**, 641–643 (1964)
16. Goldman, M.: *Spin Temperature and Magnetic Resonance in Solids*. Clarendon, Oxford (1970)
17. de Boer, W.: *Nucl. Instrum. Methods* **107**, 99–104 (1973)
18. Wind, R.A., Duijvestijn, M.J., Vanderlugt, C., Manaenschijs, A., Vriend, J.: *Prog. Nucl. Magn. Reson. Spectrosc.* **17**, 33–67 (1985)
19. Singel, D.J., Seidel, H., Kendrick, R.D., Yannoni, C.S.: *J. Magn. Reson.* **81**, 145–161 (1989)
20. Afeworki, M., Schaefer, J.: *Macromolecules* **25**, 4092–4096 (1992)
21. Afeworki, M., Vega, S., Schaefer, J.: *Macromolecules* **25**, 4100–4105 (1992)
22. Hausser, K.H., Stehlik, D.: *Concepts Magn. Reson.* **3**, 79–139 (1968)
23. Müller-Warmuth, W., Meise-Gresch, K.: *Adv. Magn. Reson.* **11**, 1 (1983)
24. van der Wel, P.C.A., Hu, K.N., Lewandowski, J., Griffin, R.G.: *J. Am. Chem. Soc.* **128**, 10840–10846 (2006)
25. Weis, V., Bennati, M., Rosay, M., Griffin, R.G.: *J. Chem. Phys.* **113**, 6795–6802 (2000)
26. Becerra, L.R., Gerfen, G.J., Temkin, R.J., Singel, D.J., Griffin, R.G.: *Phys. Rev. Lett.* **71**, 3561–3564 (1993)
27. Hu, K.N., Yu, H.H., Swager, T.M., Griffin, R.G.: *J. Am. Chem. Soc.* **126**, 10844–10845 (2004)
28. Ardenkjaer-Larsen, J.H., Fridlund, B., Gram, A., Hansson, G., Hansson, L., Lerche, M.H., Servin, R., Thaning, M., Golman, K.: *Proc. Natl. Acad. Sci. USA* **100**, 10158–10163 (2003)

29. Golman, K., Olsson, L.E., Axelsson, O., Mansson, S., Karlsson, M., Petersson, J.S.: *Br. J. Radiol.* **76**, Spec. No. 2, S118–S127 (2003)
30. Golman, K., Petersson, J.S.: *Acad. Radiol.* **13**, 932–942 (2006)
31. Golman, K., in't Zandt, R., Lerche, M., Pehrson, R., Ardenkjaer-Larsen, J.H.: *Cancer Res.* **66**, 10855–10860 (2006)
32. Frydman, L., Lupulescu, A., Scherf, T.: *J. Am. Chem. Soc.* **125**, 9204–9217 (2003)
33. Frydman, L., Blazina, D.: *Nat. Phys.* **3**, 415–419 (2007)
34. Carravetta, M., Johannessen, O.G., Levitt, M.H.: *Phys. Rev. Lett.* **92**, 153003 (2004)
35. Carravetta, M., Levitt, M.H.: *J. Chem. Phys.* **122**, 214505 (2005)
36. Hornstein, M.R., Bajaj, V.S., Griffin, R.G., Temkin, R.: *IEEE Trans. Plasma Sci.* **34**, 524–533 (2006)
37. Saito, T., Nakano, T., Hoshizuki, H., Sakai, K., Tatematsu, Y., Mitsudo, S., Ogawa, I., Idehara, T., Zapevalov, V.E.: *Int. J. Infrared Millimeter Waves* **28**, 1063–1078 (2007)
38. Samoson, A., Tuherm, T., Past, J., Reinhold, A., Anupold, T., Heinmaa, N., in: Klinowski, J. (ed.) *New Techniques in Solid-State NMR. Topics in Current Chemistry*, vol. 246, pp. 15–31. Springer, Berlin (2005)
39. Khaneja, N., Reiss, T., Kehlet, C., Glaser, S.: *J. Magn. Reson.* **172**, 296–305 (2005)
40. Khaneja, N., Brockett, R., Glaser, S.J.: *Phys. Rev. A* **63**, 032308 (2001)

Thomas Prisner and Walter Köckenberger