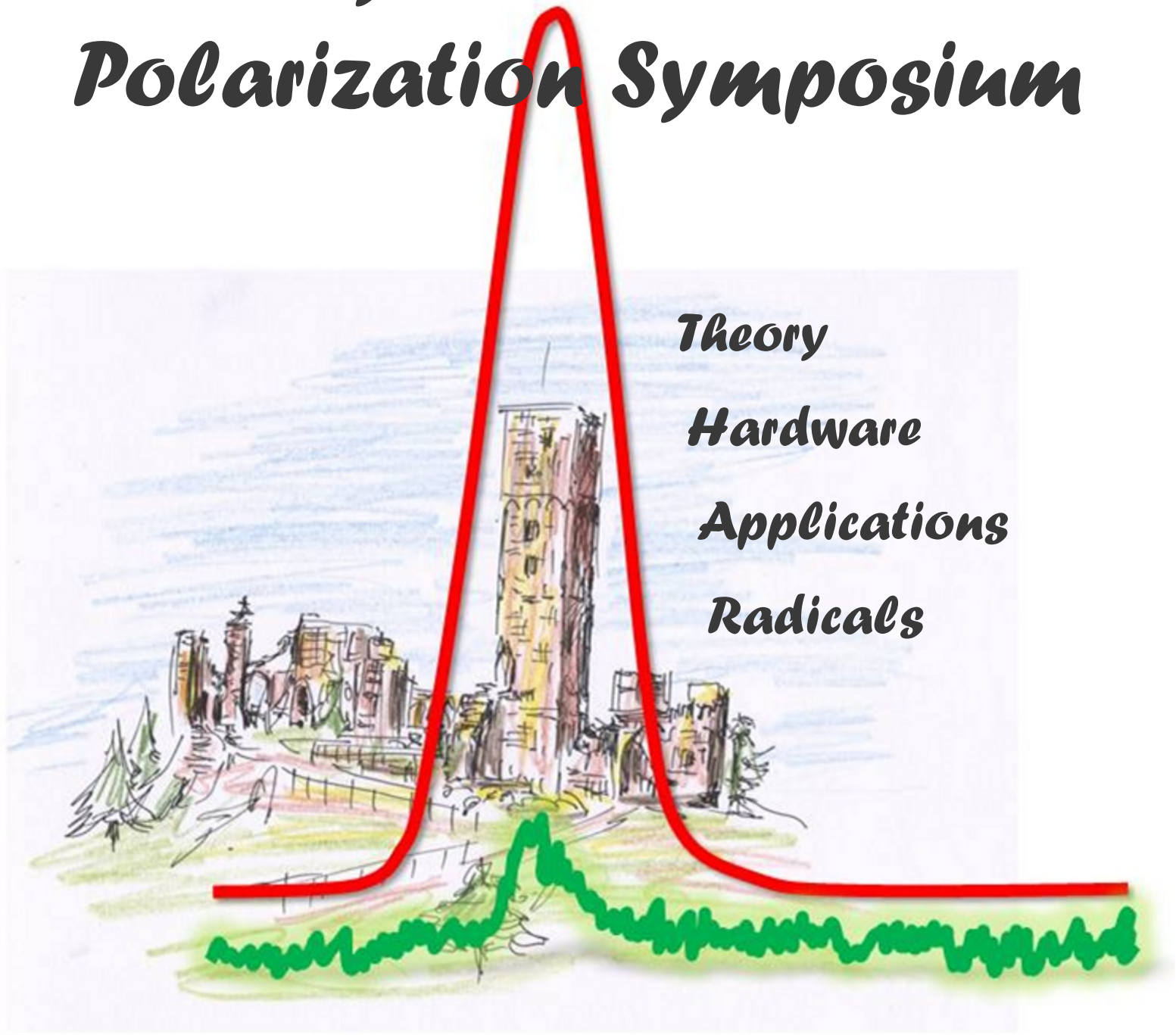
2nd Dynamic Nuclear Polarization Symposium



Königstein, Germany

2nd - 4th September 2009

organized by

Bio-DNP Design Study

GOETHE UNIVERSITÄT FRANKFURT AM MAIN

2nd Dynamic Nuclear Polarization Symposium 2009

Goethe University, Frankfurt

At the KTC Königstein, 2nd – 4th September 2009

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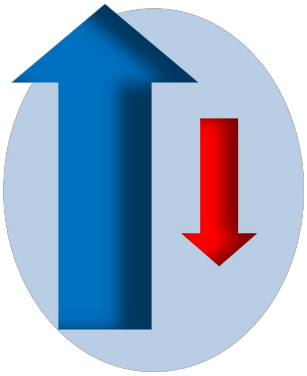

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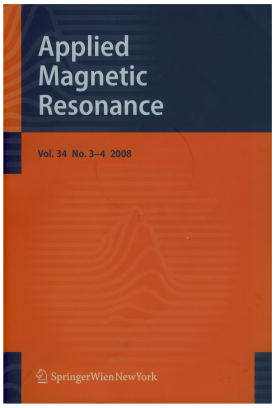


Applied Magnetic Resonance, Springer

Program 2nd DNP Symposium, Königstein

Wednesday 2.9.2009

9:00- **Registration**

10.50-11.00 **Opening remarks** Thomas Prisner, Frankfurt, Germany

Overhauser-DNP *Chair Christian Griesinger, Göttingen, Germany*

11.00-11.30 Songi Han, Santa Barbara, USA
Prospects of Overhauser dynamic nuclear polarization

11.30-12.00 Marina Bennati, Göttingen, Germany
^{1}H and ^{13}C Dynamic Nuclear Polarisation with a Two-Field (0.35/14 T) Shuttle Spectrometer

12.00-12.30 Giacomo Parigi, Florence, Italy
Nuclear relaxometry helps designing systems for solution DNP

12.30-13.00 Deniz Sezer, Frankfurt, Germany
Dynamic nuclear polarization studied with molecular dynamics simulations

Lunch

DNP-Agents I *Chair Paul Tordo, Marseille, France*

14.30-15.00 Kerstin Münnemann, Mainz, Germany
^{13}C DNP of Biomolecules Dissolved in Water and ^{1}H DNP Studies of Spin Labeled Polymers

15.00-15.30 Jan H. Ardenkjaer-Larsen, Hillerod, Denmark
^{13}C DNP with trityl biradicals

15.30-16.00 Thorsten Maly, Cambridge, USA
Optimized Polarizing Agents for High-Field Dynamic Nuclear Polarization

Coffee

DNP-Agents II *Chair Walter Köckenberger, Nottingham, UK*

16.30-17.00 Malcolm H. Levitt, Southampton, UK
Insights from singlet NMR and prospects for DNP applications

17.00-17.30 Maja C. Cassidy, Harvard, USA
Silicon Nanoparticles as Long-T_1 Hyperpolarized Magnetic Resonance Imaging Agents

17.30-18.00 Mathilde Lerche, Malmö, Sweden
Imaging of Elevated Branched Chain Amino Acid Metabolism in Tumors with Hyperpolarized ^{13}C-Ketoisocaproate

19.00 Dinner

20.00-22.00 Session 4: Posters *with Wine and Beer*

Thursday 3.9.2009

SS-DNP *Chair Frank Engelke, Rheinstetten, Germany*

9.00-9.30 Robert Griffin, Cambridge, USA
High Frequency Dynamic Nuclear Polarization in Solids and Liquids

9.30-10.00 Werner Maas, Billerica, USA
Dynamic Nuclear Polarization at 263 GHz and Applications to Biological Solids

10.00-10.30 Tochimichi Fujiwara, Osaka, Japan
Dynamic nuclear polarization experiments at 14.1 T for solid-state NMR

Coffee

Time domain DNP *Chair Daniella Goldfarb, Rehovot, Israel*

11.00-11.30 Steffen Glaser, Garching, Germany

11.30-12.00 Graham Smith, St. Andrews, UK
Improved polarisation transfer using high power pulse techniques

12.00-12.30 Hans-Martin Vieth, Berlin, Germany
Low field time-resolved Dynamic Nuclear Polarization with field cycling and high resolution NMR detection

12.30-13.00 Björn Corzillus, Cambridge, USA
Time domain (pulsed) dynamic nuclear polarization at high magnetic field

Lunch

Methods *Chair Ralf Boelens, Utrecht, Netherlands*

14.30-15.00 Lucio Frydmann, Rehovot, Israel
Indirectly-Detected Ultrafast 2D NMR of Hyperpolarized Solutions

15.00-15.30 Shimon Vega, Rehovot, Israel
Determining factors defining Spin Diffusion during DNP experiments

15.30-16.00 Danuta Kruk, Krakow, Poland
General and flexible theoretical approach to Dynamic Nuclear Polarization

Coffee

Hardware *Chair Mark Prandolini, Frankfurt, Germany*

16.30-17.00 Walter Köckenberger, Nottingham, UK
Dissolution DNP NMR with an integrated system

17.00-17.30 Vasyl Denysenkov, Frankfurt, Germany
New double resonance structures for high field DNP in liquids

17.30-18.00 Marcel Reese, Göttingen, Germany
A Liquid-State Shuttle DNP Spectrometer for 600 MHz NMR: Construction and Results for ^{1}H and ^{13}C Signal Enhancement

19.00 Conference Dinner

Friday 4.9.2009

Dissolution-DNP *Chair Jan H. Ardenkjaer-Larsen, Hillerod, Denmark*

8.30-9.00 Arno Kentgens, Nijmegen, Netherlands
High-Field Dynamic Nuclear Polarization in a Microfluidic Context

9.00-9.30 Christian Hilty, College Station, TX, USA
Chemical and biochemical reactions studied by real-time DNP-NMR

9.30-10.00 Miquel Pons, Barcelona, Spain
Exploring new radicals for solution DNP applications

Coffee

MRI-DNP *Chair Kerstin Münnemann, Mainz, Germany*

10.30-11.00 Arnaud Comment, Lausanne, Switzerland
Dissolution DNP for in vivo brain studies

11.00-11.30 Daniel Vigneron, San Francisco, USA
Towards Clinical Patient Studies of Hyperpolarized Carbon-13 Metabolic Imaging

11.30-12.00 Kevin Brindle, Cambridge, UK
Detecting tumour responses to treatment using hyperpolarized ^{13}C magnetic resonance spectroscopy and imaging

Lunch

13.30 Visit of the Center of Biological Magnetic Resonance Frankfurt (BMRZ)

Oral Presentations

Prospects of Overhauser dynamic nuclear polarization

Songi Han*

*Chemistry and Biochemistry, University of California Santa Barbara, United States of America, *songi@chem.ucsb.edu*

I will present new opportunities enabled by Overhauser dynamic nuclear polarization (DNP) for detecting dynamic intermediate states, mapping out interfacial phenomena at the molecular length scales and capturing other dilute signatures that escape conventional NMR analysis without dedicated signal amplification schemes.

Following are prospects that I will discuss. (1) The quantification of translational dynamics of hydration water in the 5-1000 ps range, and the ability to probing binding interfaces and supramolecular assemblies within molecular (5 Angstrom) length scales and with unprecedented sensitivity. The interfacial solvent dynamics at these timescales is the key parameter modulated in the hydrophobic collapse occurring in protein folding and aggregation of specific protein segments. The dehydration or hydration effects on lipid surfaces have been proposed to drive membrane stabilization and fusion. The solvent diffusivity inside lipid bilayers can reveal the state of water to be lipid-associated or of transient pores, and thus provide important parameters to unravel the mechanism of passive water transport. (2) Using the same theoretical foundation and instrumentation, hyperpolarized water can be employed as an authentic magnetic resonance imaging contrast agent to highlight the perfusion of water through water-saturated media. (3) Discussed is how to re-introduce the strongest asset of NMR to our Overhauser DNP analysis, namely to provide chemical specificity at the atomic or site-specific level. One method is to employ signatures of heteronuclei hyperpolarized through J-mediated transfer from bound protons (1H->13C) or exchanging protons (1H->15N) with hyperpolarized water. The other is to develop methods and instrumentation to perform DNP at higher (7-9 Tesla) magnetic fields.

2

1H and 13C Dynamic Nuclear Polarisation with a Two-Field (0.35/14 T) Shuttle Spectrometer

Marina Bennati[1*], Maria-Teresa Türke[1], Igor Tkach[1], Marcel Reese[2], Christian Griesinger[2], Peter Höfer[3], Thorsten Marquardsen[3], Frank Engelke[3]

[1]Electron Spin Resonance Spectroscopy, Max Planck Institute for Biophysical Chemistry, Germany, [2]Nuclear Magnetic Resonance, Max Planck Institute for Biophysical Chemistry, Germany, [3]Bruker Biospin, Rheinstetten, Germany, [*]bennati@mpibpc.mpg.de

Dynamic nuclear polarization (DNP) permits to increase the NMR signal of nuclei by pumping the electronic spin transitions of paramagnetic centers nearby. This method is emerging as a powerful tool to increase the inherent sensitivity of NMR in structural biology aiming at detection of macromolecules. In liquid solutions, DNP is governed by the Overhauser effect, which depends on various physical parameters but overall looses efficiency with increasing magnetic fields. In aqueous solution, additional technical issues associated with the penetration of microwaves in water and heating effects aggravate the performance of the experiment.
We have recently reported large 1H-DNP enhancements (> 100) on water solutions containing the nitroxide spin label 4-hydroxy-amino-TEMPO (TEMPOL) by using state-of-the-art microwave technology at 9 GHz electron pumping frequencies. To examine the feasibility of low-field (9 GHz/0.35T) DNP in high resolution NMR, we have constructed the prototype of a two-field shuttle DNP spectrometer that polarizes nuclei at 9 GHz/0.35 Tesla and detects the sample polarization at 14 Tesla. We now report our first 1H and 13C DNP results with this spectrometer. The sign of the enhancements gives insight into the acting DNP mechanism. The results provide a proof of principle for the feasibility of a shuttle DNP experiment and open up perspectives for the application potential of this method in solution NMR.

Nuclear relaxometry helps designing systems for solution DNP

Claudio Luchinat, Giacomo Parigi*

*Magnetic Resonance Center, University of Florence, Italy, *parigi@cerm.unifi.it*

The measurement of water proton longitudinal relaxation rates in aqueous solutions of paramagnetic compounds (radicals, paramagnetic metal ions) as a function of magnetic field (relaxometry) may provide three important pieces of information for predicting the efficacy of DNP to be ultimately achieved on protein nuclei: the correlation time for the electron-nucleus interaction, the actual water proton relaxation rate at any magnetic field of interest and the electron relaxation time. Likewise, relaxometry measurements on protein protons themselves, which can be performed in D2O solutions, provide information on the polarization loss occurring on protein protons in DNP-shuttling experiments during the shuttling of the sample from the EPR to the NMR field.
The presence of a paramagnetic compound drastically alters the water proton relaxation rate in all cases when there are water molecules bound to the paramagnetic compound which are in exchange with bulk water protons. Radicals are usually employed for this purpose, although slowly relaxing paramagnetic metal ions can also be considered. The analysis of the relaxation rate profiles as a function of the applied magnetic field can provide a direct estimate of the coupling factor. Relaxation rate measurements as a function of the field have been performed for the TEMPOL and TEMPONE radicals dissolved in water, and from the obtained profiles the coupling factor has been calculated and used for the analysis of the DNP enhancement.

1. P. Höfer et al. *J. Am. Chem. Soc.* **130** 3254 (2008)
2. C. Luchinat, G. Parigi, *Appl. Magn. Reson.* **34** 379 (2008)
3. C. Luchinat, G. Parigi, *J. Am. Chem. Soc.* **129** 1055 (2007)

4

Dynamic nuclear polarization studied with molecular dynamics simulations

Deniz Sezer, Mark J. Prandolini, Marat Gafurov, Thomas F. Prisner*

*Institute for Physical and Theoretical Chemistry, Frankfurt University, Germany, *prisner@chemie.uni-frankfurt.de*

The magnetic resonance signal obtained from nuclear spins is strongly affected by the presence of nearby electronic spins. Significant increase of the nuclear polarization can be achieved by driving the electron spin polarization out of equilibrium. This effect finds application in biomedical imaging and structural characterization of large biomolecules. In many of these applications nitroxide free radicals are widely used due to their non-tocicity and versatility as site-specific spin labels. We perform molecular dynamics (MD) simulations to study the electron-nucleus interaction of the nitroxide radical Tempol and water in atomistic detail. Correlation functions corresponding to the dipolar and scalar spin-spin couplings are computed from the simulations. The dynamic nuclear polarization (DNP) coupling factors deduced from these correlation functions are in good agreement with experiment over a broad range of magnetic field strengths. In addition, semiclassical relaxation theory is employed to analyze the electronic polarization of the two-spin system characteristic of nitroxide radicals. Spin-spin and spin-lattice relaxation caused by the rotational diffusion of the radical and by spin-rotation coupling are computed directly from the MD trajectories. Concentration effects on the electron saturation are accounted for by allowing for Heisenberg spin exchange between two nitroxides. Polarization enhancement profiles, calculated from the computed saturation and DNP coupling factor, are directly compared with liquid-state DNP experiments conducted at 9.2 Tesla. Disentangling the contribution of the separate hyperfine lines to the saturation is straightforward with the developed formalism. The approach can be applied to study solute-solvent interactions in general, and to characterize solvent dynamics on the surfaces of proteins or other spin-labeled biomolecules in particular.

13C DNP of Biomolecules Dissolved in Water and 1H DNP Studies of Spin Labeled Polymers

Kerstin Münnemann[1*], Björn Dollmann[1], Lasse Jagschies[1], Konstantin Gruss[1], Matthias J. Junk[1], Laura M. Schreiber[2], Hans W. Spiess[1], Dariush Hinderberger[1]

[1]Polymer spectroscopy, Max Planck Institute for Polymer Research, Germany, [2]Section of Medical Physics, Mainz University Medical Center, Germany, [*]muennema@uni-mainz.de

The limited lifetime of the hyperpolarized state is a major issue in DNP approaches which restricts the application and detection of the hyperpolarized molecules to roughly 3 times T1. In order to overcome this problem, we developed a mobile DNP polarizer based on a tuneable Halbach magnet [1, 2]. Recently, we started to extend our system for the measurement of the Overhauser DNP of 13C. For a sample of 10M 13C enriched urea with 40 mM Tempol dissolved in water we measured a 13C enhancement of -85 (Fig. 1). This demonstrates the feasibility of our approach to polarize 13C in biomolecules with a mobile setup. Currently we are incorporating a second channel in our low field NMR spectrometer (Kea, Magritek, Wellington, New Zealand), because simultaneous irradiation of EPR and 1H transitions should result in a four fold increase of this value [3].

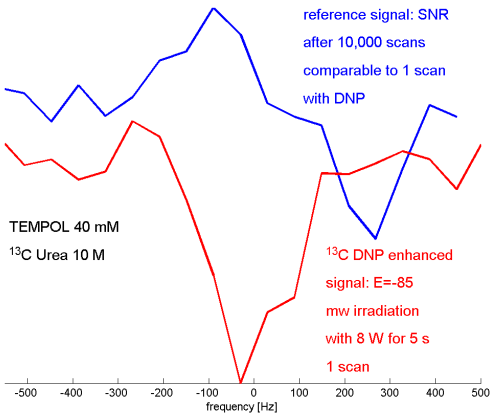


Fig.1: Comparison of 13C DNP (bottom) and reference spectrum (top).

Another part of our research is devoted to the optimization of radical systems for DNP. For medical applications of DNP polarized compounds one major issue is the toxicity of the dissolved radicals. There are mainly two approaches to overcome this obstacle. The first is the subsequent separation of polarizing agent and the solution to be injected. The second is the use of non-toxic radicals. Here we present approaches to both cases. As a system where radicals can easily be separated from the polarized liquids we choose spin-labeled thermoresponsive hydrogels, which were synthesized in our lab [4]. Heating to 40°C result in the fast collapse of the hydrogels and the dissolved water is expelled. Our initial experiments show that these systems are also feasible for DNP. As an example for DNP with biocompatible radicals we present a combined EPR and DNP study of spin-labeled heparin macromolecules with different degrees of labeling, which show comparable high 1H DNP enhancements to free Tempol.

1. K. Münnemann *Appl. Magn. Reson.* **34** 321-330 (2008)
2. C. Bauer *J. Magn. Reson.* **198** 222-227 (2009)
3. J. Potenza *Advan. Mol. Relaxation Processes* **4** 229-354 (1972)
4. M. J. N. Junk *Small* **4** 1485-1493 (2008)

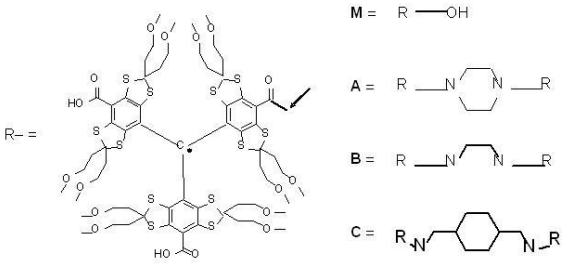
6

13C DNP with trityl biradicals

Jan H. Ardenkjaer-Larsen*, Sven Macholl, Haukur Johannesson

*Global Science and Medical office, GE Healthcare, Denmark, *jan.henrik.ardenkjaer-larsen@ge.com*

Here we report on DNP experiments on a system with three novel trityl biradicals at 3.35 T and 4.64 T. Song et al [1] used biradicals with two TEMPO radicals with different distances between the electron spins or TEMPO/trityl to obtain a suitable broadening of the EPR line allowing electron-electron flip-flops with accompanying 13C flips. The mechanisms of DNP is likely different (Thermal Mixing (TE) versus Cross Effect (CE))



Structures of the studied trityl mono- and biradicals.

The biradicals are made from two units of the trityl monoradical [6] connected with rather stiff linkers of different lengths. The two electron spins are mainly coupled via the magnetic dipolar interaction. The couplings were measured by EPR spectroscopy at X band and average distances between the electron spins of 14.2(2) Å, 15.5(5) Å and 17.9(28) Å were calculated for the three trityl biradicals. The EPR line shape at W band is changed little by the dipolar interaction between electron spins.

The main results are: (1) all three biradicals give a similar maximal DNP enhanced 13C polarization of ca 13 % at 3.35 T and ca 35 % at 4.64 T which is about half of the maximum 13C spin polarization when using the monoradical (ca 27 % at 3.35 T and ca 64 % at 4.64 T), (2) the maximum 13C spin polarization shows only a weak dependence on the biradical concentration and it can be obtained with a 6 to 10 times lower biradical than monoradical concentration. This goes along with a longer build-up time constant.

T1e has been measured by NEDOR [7] and the values are similar to those measured with the monoradical. This is as expected if interactions to phonons are responsible for the relaxation.

T1n for [1-13C]pyruvic acid doped with trityl biradicals is generally shorter than with the trityl monoradical. The dependence on the linker length is not obvious from the data. The observed shortening of the average T1n might be due to a more pronounced shortening of T1n of 13C spins near the two close electron spins of the biradical which reduces the average T1n of the whole sample compared to one with monoradicals.

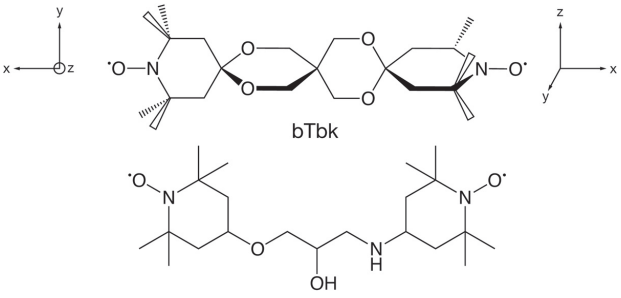
1. Song C *JACS* **128** 11385 (2006)

Optimized Polarizing Agents for High-Field Dynamic Nuclear Polarization

Thorsten Maly[1*], Yoh Matsuki[1], Eric L. Dane[1], Galia Debelouchina[1], Paul Tordo[2], Timothy M. Swager[1], Robert G. Griffin[1]

[1]Department of Chemistry, Massachusetts Institute of Technology, United States of America, [2]CNRS-UMR Chemistry, University of Aix-Marsailles I et III, France, [*]tmaly@mit.edu

For DNP-enhanced solid-state NMR experiments at high magnetic fields (>5T) TEMPO based biradicals are of large interest, since they enable the very efficient cross-effect (CE) as a mechanism to transfer the large electron polarization to surrounding nuclei (typically 1H). The CE is a three-spin mechanism that involves two efficiently dipolar coupled electrons and a nuclear spin and typically yields signal enhancements > 300 [1]. This process is optimized if a sufficiently strong dipolar coupling between the two electrons is present and the difference of the electron Larmor frequencies approximates the nuclear Larmor frequency (matching condition).



Molecular structures of the two biradicals TOTAOL and bis-TEMPO-bisketal (bTbk).

For high-field DNP experiments, currently the most successful TEMPO based biradical is TOTAPOL [2]. However, its flexible tether, linking the two TEMPO moieties, is interfering with its performance, because not all conformations yield the desired matching condition. This can be overcome using a rigid tether, locking the two TEMPO moieties in an optimized conformation [3].

Here we will present different strategies to increase the performance of polarizing agents used for high-field DNP. For TEMPO based biradicals the influence of the relative orientation of the TEMPO moieties on the DNP enhancement will be discussed. Furthermore, we will present first results for a BDPA-TEMPO biradical. In contrast to TEMPO, the inhomogeneous EPR linewidth of BDPA is much smaller, resulting in an increased number of excited orientations. The experimental results are accompanied by numerical simulations of the CE, to gain further insight into the polarizing mechanism and explore possible areas of improvement.

1. Maly, T. et al. *J. Chem. Phys.* **128** 052211 (2008)

2. Song, C. et al. *J. Am. Chem. Soc.* **128** 11385 (2006)

3. Matsuki, Y. et al. *Angew. Chem. in press* 0 (2009)

8

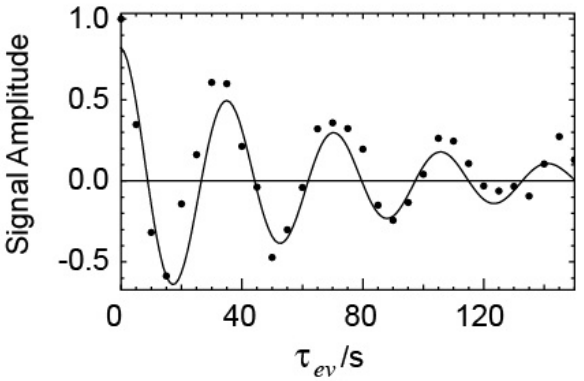
Insights from singlet NMR and prospects for DNP applications

Malcolm Levitt*

*School of Chemistry, Southampton University, United Kingdom, *mhl@soton.ac.uk*

Nuclear singlet states may have lifetimes which are much longer than the spin-lattice relaxation time T1. Long-lived nuclear singlet states have now been observed in samples such as amino acids, entire proteins, saccharides, small molecules such as nitrous oxide, and some natural products, and have already been used to study slow processes such as infrequent chemical exchange, and slow molecular diffusion. In the case of 15N-labelled nitrous oxide, a nuclear singlet lifetime (TS) of 26 minutes has been demonstrated in solution.

Some related phenomena have also been demonstrated, for example the NMR spectroscopy of singlet states conducted outside the magnet using extremely low frequency magnetic fields.



Oscillations of singlet-triplet coherence, obtained by a sequence of extremely low-frequency (8 Hz) pulses applied to a sample of 15N-nitrous oxide, outside the NMR magnet.

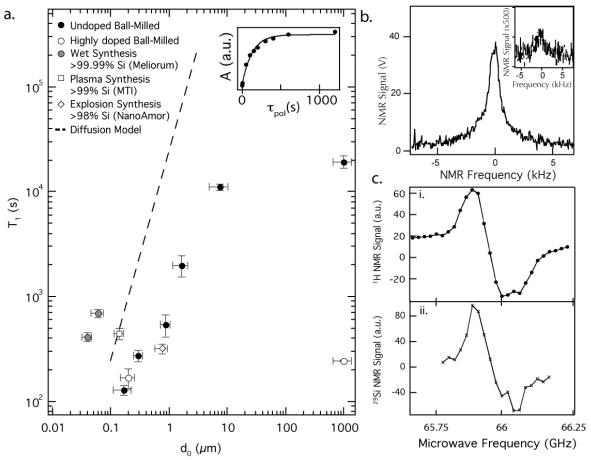
It was realized early on that long-lived states have potential in hyperpolarized NMR, since an extended spin state lifetime could reduce losses of polarization while the sample is transported from the polarizer to the spectroscopic or imaging site, and while the polarized substance undergoes transport or further chemical reactions. However, there are numerous practical and theoretical obstacles to the exploitation of nuclear singlet states for this purpose, and it is not yet clear whether the method will improve on established methods using the long-lived conventional magnetization of selected 13C or 15N sites. I will speculate on the possible combinations of singlet NMR with DNP, highlighting the obstacles, as well as the potential benefits.

Silicon Nanoparticles as Long-T1 Hyperpolarized Magnetic Resonance Imaging Agents

Maja C. Cassidy[1*], Chandrasekhar Ramanathan[2], Ross W. Mair[3], David G. Cory[2], Ronald L. Walsworth[4], Charles M. Marcus[4]

[1]SEAS, Harvard University, United States of America, [2]Department of Nuclear Science and Engineering, Massachusetts Institute of Technology, United States of America, [3]Harvard-Smithsonian Center for Astrophysics, Harvard University, United States of America, [4]Department of Physics, Harvard University, United States of America, [*]cassidy@fas.harvard.edu

Magnetic resonance imaging of hyperpolarized nuclei enables high image contrast with little or no background signal. However, in-vivo applications of pre-hyperpolarized materials such as ^{13}C enhanced biomolecules [1] and noble gases for lung imaging [2] have been limited by short nuclear spin lattice relaxation times (T_1). We propose silicon nanoparticles as a new type of hyperpolarized magnetic resonance imaging agent due to its long bulk (T_1) times and receptivity to hyperpolarization [3]. The low natural abundance of spin-1/2 ^{29}Si nuclei embedded in a lattice of zero-spin ^{28}Si nuclei isolates the active nuclear spins from one another and from the environment, leading to multi-hour (T_1) times and dephasing (T_2) times of up to tens of seconds [4] in the bulk. Additionally, silicon based particles can be easily surface functionalized, potentially enabling them to act simultaneously as a targeted imaging agent and drug delivery device in-vivo.



a. T1 vs mean diameter for a variety of SiNP. Inset: T1 values were extracted from an exponential fit to the signal from a saturation recovery sequence. b. Hyperpolarization of d=60nm SiNP via DNP (T=4K) results in a net polarization of >1000x the polarization at 300K. c. ESR frequency dependence of the 1H and 29Si NMR signal for SiNP in water.

We investigate Si particles spanning four orders of magnitude in mean diameter, from 40nm nanoparticles to mm granules. The nuclear spin relaxation times (T_1) of all Si nanoparticles are found to be remarkably long, ranging from many minutes to hours at room temperature (Fig 1a). T_1 is found to be a function of particle size, dopant concentration, and crystallinity, allowing for optimization for specific in-vivo applications.

We demonstrate that these particles can be hyperpolarized using low temperature dynamic nuclear polarization (Fig. 1b). Off-resonant microwave irradiation of paramagnetic electron defects at the silicon-silicon dioxide interface creates a non-equilibrium spin temperature between the electrons and nearby ^{29}Si nuclei, which then undergo hyperpolarization. Polarization is then transferred to the crystalline core of the nanoparticle through nuclear spin diffusion, where it relaxes more slowly. Additionally we show that, when in solution, nuclear polarization can be transferred to other nuclei such as ^1H in the surrounding solution (Fig 1c).

This work is supported by the NIH, the Harvard NSEC and Harvard CNS.

1. Golman *Br J. Radiol* **76** 118-127 (2003)
2. Leawoods *Concepts Magn. Reson.* **13** 277-293 (2001)
3. Dementyev *Phys. Rev. Lett* **100** 127601 (2008)
4. Ladd *Phys. Rev. B* **71** 014401 (2005)

10

Imaging of Elevated Branched Chain Amino Acid Metabolism in Tumors with Hyperpolarized 13C-Ketoisocaproate

Mathilde H. Lerche[1*], Magnus Karlsson[1], Pernille R. Jensen[1], Rene in 't Zandt[1], anna gisselsson[1], georg hansson[1], sebastian meier[2], Jens Duus[2]

[1]Imagnia AB, Imagnia AB, Sweden, [2]Carlsberg Laboratory, Carlsberg Research Center, Denmark, [*]mathilde.lerche@imagnia.se

Powerful analytical tools are vital for characterizing the complex molecular changes underlying oncogenesis and cancer treatment, in particular if information is to be collected in vivo by non-invasive approaches. Hyperpolarized 13C magnetic resonance (MR) spectroscopy has in many cases the potential to deliver the sensitivity and detailed spectral information to report on the chemical fate of tracer molecules in different tissues. In a preclinical study we here show that alfa-ketoisocaproic acid (KIC) can be used to assess molecular signatures of tumors using hyperpolarized MR spectroscopy. KIC is metabolized to leucine by the enzyme branched-chain aminotransferase (BCAT), which is a putative marker for metastasis and a target of the proto-oncogene c-myc. Very different fluxes through the BCAT-catalyzed reaction can be detected for murine lymphoma (EL4) and rat mammary (R3230AC) tumors in vivo. EL4 tumors show a 10-fold higher hyperpolarized 13C leucine signal relative to the surrounding healthy tissue whereas the hyperpolarized 13C leucine signals in R3230AC tumors is similar to the surrounding healthy tissue. The distinct molecular signatures of EL4 and R3230AC tumors were corroborated with biochemical assays ex vivo.

High Frequency Dynamic Nuclear Polarization in Solids and Liquids

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Over the last few years we have developed gyrotron microwave sources that operate at frequencies of 140, 250, and 460 GHz that permit DNP enhanced NMR (DNP/NMR) experiments in magnetic fields of 5-16.4 T (1H NMR frequencies of 211, 380, and 700 MHz, respectively). We review the instrumentation used for these experiments, and discuss two mechanisms that are currently used for DNP experiments in solids at high fields - the solid effect and cross effect -- and the polarizing agents appropriate for each. In addition, we discuss applications of DNP/NMR that illustrate its utility in enhancing signal-to-noise in MAS NMR spectra of a variety of biological systems including membrane and amyloid proteins whose structures are of considerable scientific interest. Presently, enhancements ranging from 40-260 are routinely available depending on experimental variables such as temperature, magnetic field, microwave B1, polarizing agent, etc. Finally, we describe extensions of these experiments that permit observation of 13C liquid state spectra where we have observed enhancements of 140-400 in 2D spectra of small molecules and a protein.

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Dynamic Nuclear Polarization at 263 GHz and Applications to Biological Solids

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Dynamic Nuclear Polarization (DNP) can be used to substantially increase the sensitivity of NMR experiments by transferring the higher Boltzmann polarization of unpaired electron spins to nuclear spins. This polarization transfer is driven by microwave irradiation of unpaired electrons at or near the electron Larmor frequency. We have developed a dedicated spectrometer for solids DNP experiments at 263 GHz microwave frequency, 400 MHz 1H frequency, and have measured DNP signal enhancements of up to a factor of 80 at 100 K using TOTAPOL1 biradical (Song et. al., J. Am. Chem. Soc. 2006, 128, 11385). The microwaves are generated by a high power gyrotron, transmitted to the NMR probe via corrugated waveguide, and irradiated on to a 3.2 mm rotor for magic angle spinning DNP experiments. This contribution focuses on the design of the system and its performance and applications to biological solids. DNP signal enhancements have been measured as a function of sample temperature, microwave power, and sample preparation parameters. Nuclear and electron relaxation times have also been investigated for insight into the DNP temperature dependence. Secondly, a range of samples have been successfully polarized including small peptides, soluble proteins, membrane proteins, and large biological complexes.

Dynamic nuclear polarization experiments at 14.1 T for solid-state NMR

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Dynamic nuclear polarization increases the sensitivity of high-resolution solid-state NMR at high-fields. Since NMR study of large biomolecular systems is primarily limited by the sensitivity, DNP is a promising technique for NMR spectroscopy of systems such as membrane protein complexes.

We have performed DNP experiments with a 600-MHz wide-bore magnet for NMR. For this purpose we have developed a gyrotron system FU CW II that generates 394.5-GHz wave at second harmonics with a power of 50 W in CW operation. The gyrotron tube with a triode electron gun was mounted in an 8-T He-free SCM magnet. The gyrotron has step tunability and oscillates at 394.5 GHz in TE0,6 mode and 392.5 GHz in TE2,6 mode. The submillimeter wave is transmitted to liquid N2 temperature magic-angle spinning (MAS) probe through a rigid circular waveguide system. The DNP-NMR probe was made by modifying a commercially available MAS probe: thermal insulation was improved and a 10-mm circular waveguide was inserted. A submillimeter wave with a power of about 3-W was applied to a sample in a sapphire rotor. The sample temperature was controlled by about 100-L/min N2-gas obtained from the air with a N2 gas separator. The gas temperature was reduced to 90 K by an electric chiller followed by a liq-N2 thermal exchanger.

We have measured 150-MHz 13C-NMR signals of 13C-enriched organic compounds in partially deuterated glass media. The proton polarization was enhanced with mono- or bi-radical compounds by a factor of more than 10. The proton polarization was transferred to 13C spins under a Harmann-Harn condition. The DNP at 14.1 T was confirmed by the field dependence of 13C-NMR signal amplitudes.

Acknowledgement: This research is partly supported by Japan Science and Technology Agency.

1. T. Idehara *Int. J. IR and MM Waves* **28** 433 (2007)

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Improved polarisation transfer using using high power pulse techniques

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One of the key challenges associated with many high field DNP methodologies, is the need to have both rapid and efficient polarisation transfer combined with high NMR filling factors, whilst avoiding excessive thermal heating.

One potential solution is the use of very high pulse power techniques, at relatively low average power levels, in high volume samples. The main problem has always been that the available mm-wave pulse power levels have always been too low to demonstrate this effectively in high magnetic fields. However, at St Andrews a low deadtime kW pulse 94GHz ESR system has recently been developed that allows complex pulse sequences to be specified with timing resolution of a few hundred ps, and can provide $\pi/2$ pulse lengths as short as 5ns in large volume non-resonant sample holders. The system also allows both frequency and phase to be changed on nanosecond timescales, and has recently been adapted to allow low temperature sample loading, which in turn has allowed us to start to evaluate a number of DNP pulse methodologies.

In this paper we experimentally show that the use of high power kW pulses at 94GHz can significantly increase both the rate of polarization transfer and final polarization achieved for the solid state DNP of water/glycerol polarized by 40mM TEMPO at 70K, relative to continuous wave methodologies for the same average power. We show that for equally spaced pulses the optimum polarization transfer occurs when irradiating with short $\pi/2$ pulse lengths of 8ns at average power levels of several watts in our large volume DNP sample holder. The increase is attributed to the greater numbers of spins that are now able to contribute to the polarization transfer process associated with the Cross Effect/Thermal mixing in this system.

We also expect to be able to be report on similar experiments using TOTAPOL and TEMPO/trityl mixtures as polarizing agents, as well as report on new large volume room temperature aqueous (high loss) sample holders that still allow NMR filling factors of around 10%. Experiments that seek to demonstrate coherent polarization transfer in high magnetic fields are also planned.

Low field time-resolved Dynamic Nuclear Polarization with field cycling and high resolution NMR detection

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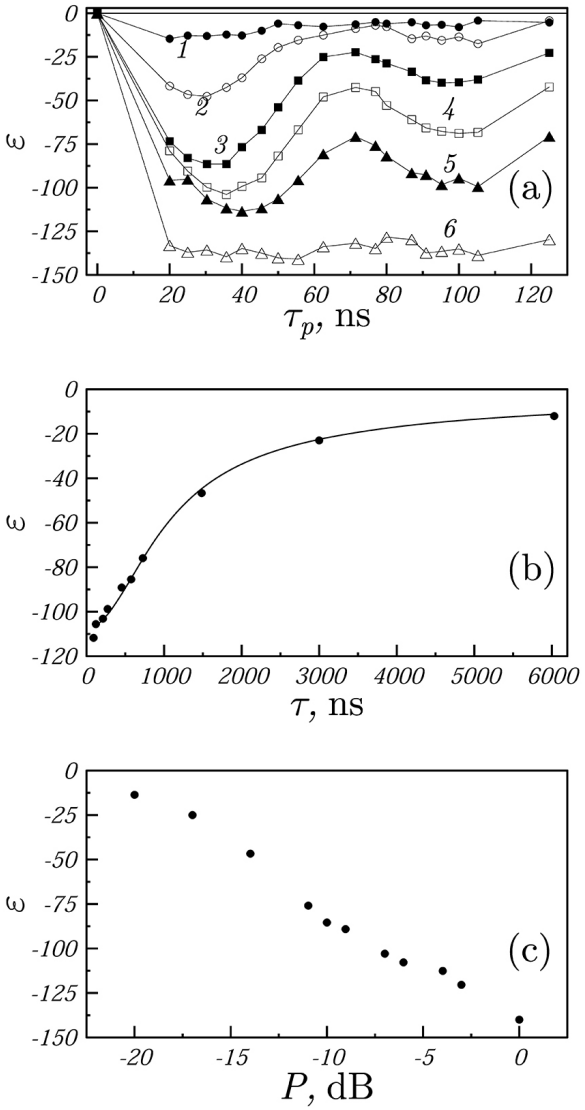
Replacing cw by pulsed pumping of EPR transitions in liquid state DNP is a promising approach to reduce the power deposition in the sample while keeping a high saturation factor. Here the results of our studies on the efficiency of polarization transfer from the electronic to the nuclear spin reservoir by pulsed excitation will be presented. EPR pumping was performed at low field. By means of fast field-cycling the sample was transferred to high field where the high-resolution NMR spectrum was obtained. Fast field-cycling was carried out by utilizing a device that shuttles the whole NMR probe allowing high-resolution NMR detection at high field (7 T). We studied the proton DNP of water and samples containing 3-furoic acid or histidine in aqueous solution doped with stable nitroxide radicals (TEMPOL) at 300 MHz (B=10 mT) and 1400 MHz (B=48.6 mT). The dependence of DNP on the duration of the pumping pulse, RF-power and duty cycle was analyzed. In comparison with cw-pumping a substantial gain in polarization was achievable. When the pulse duration corresponds to flip angles of odd multiples of π the DNP efficiency goes through a maximum showing that coherent electronic spin motion can be exploited. Because of B_1 inhomogeneities and relaxation effects the first π-pulse yields the highest efficiency. For optimum power utilization the pulse repetition rate τ_d^{-1} should be small in comparison with the electronic spin-lattice relaxation rate. From the dependence of the DNP amplitude on τ_d^{-1} we determined the effective relaxation time of TEMPOL at 10 and 47mT. Also, the effect of the TEMPOL concentration on the relaxation times of individual protons and its magnetic field dependence was studied in the field range 0-7 T. Theoretical and numerical models were developed to calculate and optimize the polarization transfer from electron to nuclear spins by cw and pulsed radio-frequency irradiation. Combining these results the strategy for optimal conditions for pulsed DNP at low field was established.

Financial support by the EU via FP6 Bio-DNP grant No. 011721 and Marie Curie Actions program (project MIF2-CT-2006-022008) is gratefully acknowledged.



Optimization of the pulse sequence for gaining higher DNP enhancements ε: (a) at constant total power for six duty cycles: 0.5%(1), 2%(2), 5%(3), 10%(4), 20%(5) 50%(6), and dependence of ε at fixed magnetization flip angle φ=π on (b) delay τ and (c) total power P.

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Time domain (pulsed) dynamic nuclear polarization (DNP) at high magnetic field

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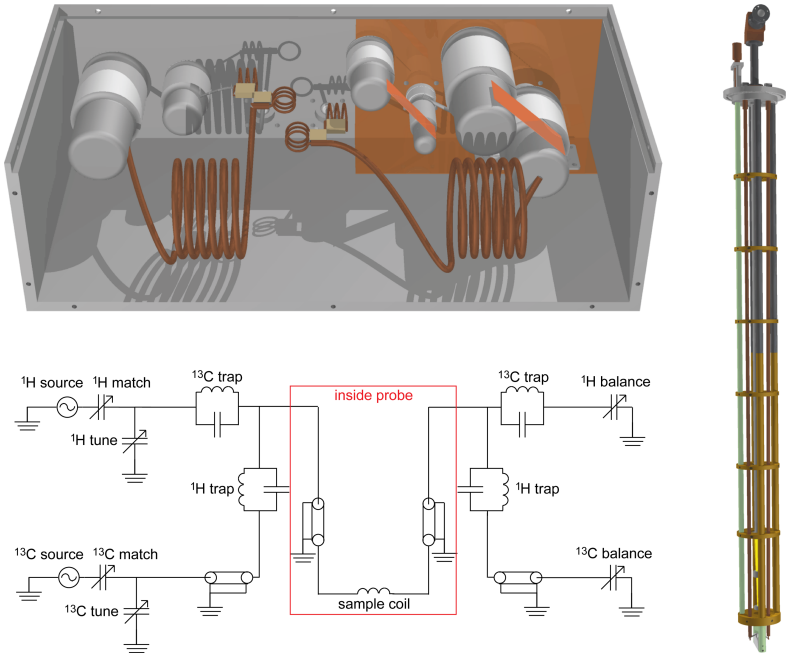
Practically all conventional DNP mechanisms in solid state experiments suffer from the fact that the enhancement decreases at high field. The loss in transferred polarization has two reasons. Either a reduced mixing coefficient of a "forbidden" transition occurs at high external magnetic field or only a small fraction of the electron spins is excited during polarization transfer due to an inhomogeneously broadened EPR line. In the worst case a combination of both reasons applies.

Using time domain DNP by evoking coherent microwave pulses of high field strength, one could overcome that issue in two ways. First, ideally the whole or at least a significant fraction of the EPR line can be excited by the large bandwidth of one short pulse. Second, in coherent transfer pathways the previously "forbidden" transitions can obtain a much higher transition moment, resulting in a significantly increased DNP enhancement. Two promising polarization experiments, *i.e.* nuclear orientation via electron spin locking (NOVEL) [1,2] and dressed state solid effect (DSSE) [3], are briefly reviewed.



Upper left: Sketch of the open circuit box used in the pulsed DNP intrumentation; lower left: circuit diagram of the doubly balanced rf circuit; right: sketch of the pulsed DNP probe.

In this contribution the instrumentation for pulsed DNP at 140 GHz currently being under development in our laboratory will be presented. The instrumentation includes a low frequency fast switched microwave circuit which is capable to produce sub-ns pulses at 8.75 GHz. The pulses are up-converted and serve as a driving input for a 140 GHz gyroamplifier with a 40-50 dB gain. Also an improved design for a pulsed DNP probe is presented. The probe uses a doubly balanced double resonance NMR circuit for ^{1}H and ^{13}C frequency (211 MHz and 53 MHz, respectively). The NMR coil itself serves as a cylindrical TE_{011} microwave resonance structure. Balancing both NMR channels is compulsory to prevent arcing between the NMR coil and the microwave transmission line coupling the microwaves to the resonator.

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[3] V. Weis, M. Bennati, M. Rosay, and R. G. Griffin, J. Chem. Phys. 113, 6795 (2000).

Indirectly-Detected Ultrafast 2D NMR of Hyperpolarized Solutions

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NMR is a powerful analytical tool for elucidating the nature of organic, biomolecular and biological processes. It is, however, a notoriously insensitive method. This is largely due to the low levels of thermal polarization achivable under conventional conditions. Recent developments have made it possible to reach polarization levels that exceed these thermal equilibrium values by several orders of magnitude. One of the most promising and generally applicable techniques to achieve this hyperpolarization is based on ex situ dynamic nuclear polarization (DNP), which leads to signal-to-noise enhancements of several tens of thousands compared to conventional NMR [1]. In ex situ DNP the hyperpolarization is carried out outside the NMR magnet, in a cryogenic setting. The technique then involves an irreversible melting and transfer of the sample to the NMR spectrometer. Because of ensuing T1 limitations, ex situ DNP is best suited to the acquisition of a single or at most a small number of free induction decays. This makes ex situ DNP ill-suited for collecting an array of transients involving complex pulse sequences, of the kind needed in 2D NMR. The present talk will explore ways to deal with this drawback, based on combining ex situ DNP with spatially-encoded ultrafast 2D NMR methods, capable of yielding 2D NMR spectra in a single-scan [2]. Particularly promising are inverse-detected heteronuclear methods exploiting the hyperpolarization of slow-relaxing nuclei, which is well preserved during the sample transfer from the polarizer to the NMR spectrometer. Using this approach, we show that 2D single-scan heteronuclear NMR spectra of ntural products can be recorded at very low concentrations (≈0.1-1 mM 13C at natural abundance). Additional 2D-oriented features involving 15N-based hyperpolarized NMR experiments within analytical and biochemical settings, will also be presented.

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Determining factors defining Spin Diffusion during DNP experiments

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The major factors determining the magnitude of the nuclear polarization during DNP experiments are the microwave irradiation parameters, the magnetic dipole-dipole interactions and the spin-lattice relaxation times. Depending on the concentration of the unpaired electrons in the sample different DNP mechanisms are considered; the Solid Effect, the Cross Effect and Thermal Mixing. In the first and second case the success of the polarization enhancement depends on the interplay between the effective MW power and the nuclear spin lattice relaxation rates of the bulk nuclei in the system. The intermediate dipolar interaction between these bulk nuclei and part of the core nuclei in the vicinity of the electron initiates the diffusion of polarization into the bulk and the bulk interactions takes care of the rest.
In this presentation the main mechanisms that determine the DNP enhancement of amorphous samples, loaded with a low concentration of stable radicals, will be discussed and results from model calculations will be shown.

General and flexible theoretical approach to Dynamic Nuclear Polarization

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We present simulations of dynamic nuclear polarization in solids based on a perturbation solution of the Liouville von Neumann equation. We have focused on several model systems consisting of various numbers of electrons and nuclear spins. The energy level structure of the combined spin system is determined by a superposition of nuclear and electron Zeeman interactions with dipole-dipole between every pair of participating spins. Under certain conditions, scalar couplings need also to be included. The treatment allows for g-tensor anisotropy effects for electron spins participating in the polarization transfer. The system experiences spin transitions within the complicated energy level structure due various mechanisms: an alternating magnetic field acting selectively or non-selectively on the spin ensemble, electron and nuclear spin relaxation and spin diffusion. Within the proposed approach one can obtain the DNP enhancement factor (the ratio between the nuclear magnetization generated by the DNP effect and the one at thermal equilibrium) depending on molecular geometry, interaction strengths and efficiency of relaxation processes and of spin diffusion.
In this work emphasis was given throughout to the effects which cause most problems in a theoretical analysis of Dynamic Nuclear Polarization, i.e.: how to link the dynamics of electron spins to the transitions of nuclear spins or in other words: how line shapes of Electron Spin Resonance (ESR) spectra mediate the relevant transition probabilities of the nuclear spin subsystem, and how to include into the description of DNP relaxation phenomena.
The proposed approach gives us insight on a quantitative level into the various physical processes responsible for the final enhancement of the nuclear magnetization. It does not require any presumptions on the possible DNP mechanisms. The predicted DNP effect is a consequence of spin interactions and molecular geometry. The theory will be explained in detail and illustrated by numerous examples.

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Dissolution DNP NMR with an integrated system

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Large nuclear spin polarisation in liquid state samples can be generated by first increasing the polarisation of the nuclear spin system in solid state using dynamic nuclear polarisation (DNP) at low temperature followed by a fast dissolution step [1]. After dissolution the sample is rapidly shuttled pneumatically in liquid state to a separate high field NMR magnet. The disadvantage of this strategy is a substantial loss of the non-thermal polarisation of nuclear spins with fast longitudinal relaxation during the shuttling time that requires usually between 2.5 - 4s.

We present here the design and implementation of a novel integrated system in which DNP and high resolution NMR spectroscopy are performed in the same magnetic environment using a unique two-isocentre magnet configuration (3.4T and 9.4T). The system also includes a quasi-optical ESR W-band system using the 3.4T centre that makes it possible to acquire CW ESR spectra and provide enough power for DNP enhancement. The sample can be kept at low temperature (1.4K) using a specially designed cryoinsert in the 3.4T region. A modified flow NMR probehead that makes is possible to inject the solution rapidly into the NMR detection cell is used in the high field part of the magnet. The sample is shuttled between the two centres in solid state using a computer controlled high precision positioning system driven by a stepper motor and dissolved rapidly at a position just above the high field centre.

The novel system will be compared in its performance with the current strategy that is based on the use of a separate magnet for polarisation enhancement followed by dissolution and pneumatic shuttling of the sample in liquid state.

1. J.-H. Ardenkjaer-Larsen *Proc. Natl. Acad. Sci USA* **100** 10158 (2003)

New double resonance structures for high field DNP in liquids

Vasyl Denysenkov[1*], Mark Prandolini[1], Marat Gafurov[1], Alexander Krahn[2], Burkhard Endeward[1], Thomas Prisner[1]

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High field DNP promises to improve high resolution NMR sensitivity substantially. Recently large DNP enhancements have been demonstrated on biomolecules in frozen solution at magnetic fields up to 9 T using thermal mixing and cross polarization mechanisms [1].
In-situ DNP experiments with aqueous solutions at ambient temperatures are a challenging task due to high microwave losses in water causing excessive sample heating. This can be minimized by applying a double resonance structure with well separated E and H components of the microwave (MW) field.
We showed with a helical double resonance structure [2] that indeed substantial enhancements can be achieved at high magnetic fields in liquid water solutions [3]. On the other hand the sample size in the helical resonance structure is restricted to very small volume below 10 nl causing poor NMR signal.
Recently we developed a new type of double resonance structure, which increases the aqueous sample volume significantly. Design, MW and RF performance, and first applications will be presented.

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A Liquid-State Shuttle DNP Spectrometer for 600 MHz NMR: Construction and Results for 1H and 13C Signal Enhancement

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Following the goal of sensitivity enhancement of high resolution liquid state NMR we have developed a field cycling by sample shuttling liquid state DNP spectrometer (Fig. 1). We polarize the sample at low field (0.34T, LF), allowing for relatively large sample diameter and volume with reduced heating, and then pneumatically transfer the sample within 120ms to the high field (14T, HF) for high resolution and high sensitivity NMR detection.

Compared to the 14T Boltzmann signal, enhancement factors of ε_{HF}=/=-3.4 for water protons and ε=+8.4 for carbon in ^{13}C labelled urea (Fig. 1) have been achieved. In experiments using signal accumulation of several scans these enhancements translate into a reduction of measurement time of a factor of 12 and 70, respectively.

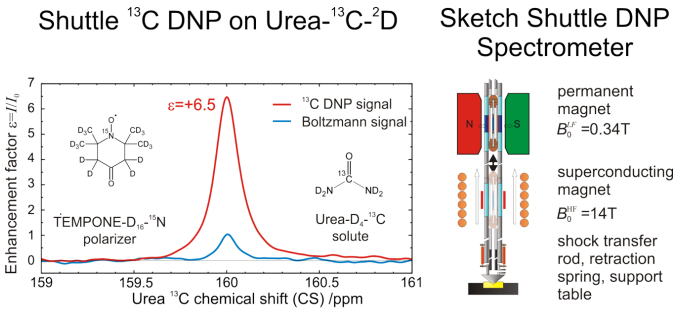

Figure 1. Left: 13C Shuttle DNP result for 4M Urea-13C-2D in D2O with 50mM TEMPONE-15N-2D in comparison to the Boltzmann signal at 14T. For each spectrum 16 scans have been aquired. The highest observed enhancement factor was +8.4 for a single scan. Right: Overview of the setup. On the top the polarizer and on the bottom the HR magnet.

^{13}C shuttle DNP experiments with the radical dissolved in organic solvents, namely ^{13}C labelled chloroform and tetrachloromethane, yield the enhancements ε_{HF}=+21 and ε_{HF}=+9.2, respectively

The highest achieved LF enhancement factors for the protons of water are $\varepsilon_{LF} = I/I_{0,LF} >= -170$ using 25mM TEMPONE-^{15}N-^{2}D. The ^{13}C HF enhancement ε_{HF}=+8.4 translates to positive LF enhancements ε_{LF}>+336 using 4M ^{13}C-Urea in D_2O containing 50mM TEMPONE-^{15}N-^{2}D. For the latter 20s microwave irradiation with 22W has been applied. The temperature increase was measured to be 10K using the temperature dependence of residual H_2O. To our knowledge this carbon enhancement is the so far highest achieved for ^{13}C nuclei of molecules dissolved and polarized in liquid water close to room temperature. Surprisingly we have also observed large negative ^{13}C enhancements under very similar conditions. The reason for this change of sign is under investigation now.

Molecular size dependent losses of magnetization during sample transfer through low stray fields (≥5mT) of the magnets hinder the achievement of large enhancements on large molecules like proteins. To allow for enhanced measurements of proteins we are currently constructing a dual centre magnet system to shorten the transfer distance and transfer time and to assure high magnetic fields on the whole sample track.

Chemical and biochemical reactions studied by real-time DNP-NMR

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Dynamic Nuclear Polarization (DNP) dramatically extends the concentration range of analyte that can be accessed by NMR spectroscopy. However, the instantaneous availability of a large signal also points towards another powerful application of this technology. One-or two dimensional NMR spectra can be recorded without signal averaging of samples under conditions and at concentrations compatible with many chemical or biochemical reactions. Taking advantage of the molecular specificity of chemical shift observations in real-time, DNP-NMR can on one hand be used for the determination of reaction kinetics. On the other hand, since nuclear spin states can be preserved even if the spin carrying atoms undergo a reaction, atomic positions from reactant and product species can be experimentally correlated, potentially leading to the identification of reaction mechanisms. Here, we present applications of this technique to a variety of reactions in the areas of enzyme catalysis, as well as organic chemistry.

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Exploring new radicals for solution DNP applications

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Dynamic Nuclear Polarization (DNP) is attracting considerable attention as a method to increase the NMR sensitivity in selected applications. Non equilibrium nuclear polarization is transferred from electron spin polarization by microwave irradiation at frequencies corresponding to electronic transitions. This is most efficiently carried out in the solid state at low temperatures. For slowly relaxing nuclei, non-equilibrium polarized samples can be transferred to a conventional NMR spectrometer and studied in solution.

The choice of radical is crucial for the success of the experiment. Factors like the width of the EPR line in the solid state compared with the nuclear frequency determine the mechanism of polarization transfer. Additional effects can arise from the different chemical nature of the radical used, including solubility and supramolecular interactions between the molecule to be polarized and the free radical.

We have studied a new series of trityl radicals not previously used for DNP experiments. The new radical tested vary in their substitution leading to different symmetry properties. In all cases we could demonstrate DNP effects. For some of these radicals, we see striking differences in the sign of the nuclear polarization generated in different molecular targets, emphasizing the role of supramolecular interactions in DNP.

Dissolution DNP for in vivo brain studies

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Dynamic Nuclear Polarization (DNP) together with the so-called 'dissolution' procedure to enhance weak NMR signals from molecular tracers is a very promising technique to visualize their bio-distribution and metabolism in vivo [1-3]. The major limitation of the method arises from the short lifetimes of hyperpolarized spin states in liquids. Most applications of the technique have therefore focused on 13C NMR of 13C-enriched tracers containing carboxylic carbons.

Results from in vivo studies in the rat brain after infusion of hyperpolarized 1-13C-acetate will be presented. Since acetate is solely metabolized in astrocytes, it is an interesting tracer to study glial metabolism. We also explored the potential of other hyperpolarized nuclear spins for in vivo brain studies, namely 6Li in LiCl and 15N in choline. Lithium salts are used for the treatment of depressive disorders and choline plays a key role in several critical biological processes, in particular in the synthesis and metabolism of phospholipids in cell membranes, and in cholinergic neurotransmission.

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2. K. Golman *Proc. Nat. Acad. Sci. USA* **100** 10435 (2003)

3. K. Golman *Proc. Nat. Acad. Sci. USA* **103** 11270 (2006)

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Towards Clinical Patient Studies of Hyperpolarized Carbon-13 Metabolic Imaging

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Hyperpolarized Carbon-13 MR is extraordinary new technique that has the potential to become an important new radiological tool for metabolic imaging by directly observing key cellular bioenergetic processes in vivo (1,2). While many studies have focused on utilizing the >10,000 fold signal enhancement provided from this method for basic science studies, the focus of this research is to develop and translate hyperpolarized C-13 methods for future clinical trials. The initial studies were focused on technology transfer of the original methods and instrumentation from the GE facility in Malmo Sweden (3) to the MR research facility at UC San Francisco. New techniques and coils were then developed for the first human studies in prostate cancer patients and tested in canine models with injection of hyperpolarized C-13-pyruvate. A proof-of-concept (POC) DNP polarizer is now functioning in a sterile clean room next to a 3T MR scanner awaiting final approvals and initiation of clinical trials.

In order to investigate this technique in vivo and to develop improved acquisition methods, a number of animal studies in various model systems have been performed. In order to study prostate cancer, we have studied a transgenic model of prostate cancer (4) and observed significantly higher C-13-lactate in primary and metastatic tumors as compared to normal tissues following injection of hyperpolarized C-13-pyruvate. A study correlating hyperpolarized MR data with histologic analyses demonstrated that higher lactate correlated with higher grade (5). Also the potential value of hyperpolarized MR for predicting and detecting early response to androgen deprivation therapy was also shown in this model. Recent work has focused on developing improved MR sequences and investigations of other agents and other applications including liver cancer and brain tumors.

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Detecting tumour responses to treatment using hyperpolarized ^{13}C magnetic resonance spectroscopy and imaging

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We have been developing non-invasive and clinically applicable magnetic resonance-based methods for detecting tumour cell death, since the level of cell death following drug treatment has been shown, in preclinical and clinical studies, to be a good predictive indicator for treatment outcome. Thus by monitoring tumour cell death we may get an indication of whether a particular drug is working very early during treatment, possibly within 24-48 hours, and long before there is any evidence of tumour shrinkage (1).

We have shown that exchange of hyperpolarized ^{13}C label between the carboxyl groups of lactate and pyruvate, in the reaction catalyzed by the enzyme lactate dehydrogenase, could be imaged in tumours and that this flux was decreased in treated tumours undergoing drug-induced cell death (2). We have also shown that we can monitor the conversion of hyperpolarized [5-^{13}C]glutamine to glutamate in human hepatoma cells *in vitro* (3). Since glutaminase activity has been correlated with the rate of cellular proliferation, measurements of its activity *in vivo* could be used to detect the early responses of tumours to cytotoxic and cytostatic drugs.

Alterations in tissue pH underlie many pathological processes, therefore the capability to image tissue pH in the clinic could offer new ways of detecting disease and response to treatment. We have shown that tissue pH can be imaged *in vivo* from the ratio of the signal intensities of hyperpolarized $H^{13}CO_3^-$ and $^{13}CO_2$ following intravenous injection of hyperpolarized $H^{13}CO_3^-$. The technique was demonstrated with a study on a mouse tumour model, which showed that the average tumour pH was significantly lower than the surrounding tissue (4). Since bicarbonate is already used intravenously in humans, we propose that this technique could be used clinically to image tumours and other disease processes that are associated with alterations in tissue pH such as ischemia and inflammation.

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High-Field Dynamic Nuclear Polarization in a Microfluidic Context

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Handling of small samples is simplified using a microfluidic set-up. Using the microfabrication toolbox available nowadays, a completely integrated platform in one chip which can handle and detect raw samples without any preparative laboratory work is within reach. Furthermore there is a growing interest in small volume chemistry performed in dedicated chip sets. These developments ask for analytical methods that can handle the corresponding volumes.

Recently we have introduced a new coil design, based on a stripline configuration[i,ii,iii,iv]. By utilizing the stripline geometry as a detection coil in a microfluidic device, the trade-off between resolution and sensitivity is by-passed. The stripline has the advantage that positioning the coil close to the sample does not decrease the resolution. An optimization study in terms of rf-homogeneity, resolution and sensitivity will be presented and the feasibility of stripline flow-probes for the kinetic monitoring of chemical reactions and the analysis of 600 nL of human cerebrospinal fluid (CSF) will be shown. For the latter application the screening low concentration metabolites requires long signal averaging which prohibits a high throughput of samples for metabolic screening.

These problems can be overcome by increasing the nuclear spin polarization of the spins in the sample by Dynamic Nuclear Polarization (DNP). Therefore we have developed a high-conversion-factor, double-resonance structure for DNP at 3.4 T (95 GHz / 144 MHz) with dimensions compatible to those used in the microfluidic devices[v]. Simultaneous EPR and liquid state ^{1}H NMR experiments are performed on samples of nitroxide radical TEMPO dissolved in pure water or a mixture of water and dioxane. DNP enhancements up to -72 are obtained at a microwave power of 140 mW with a radical concentration of 10 mM in nanoliter sized sample volumes. These results are remarkable in view of the current theory describing the Overhauser effect. Finally variations of the effective proton relaxation are observed depending on the MW power, which call for an evaluation of the existing theory.

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Poster Abstracts

1

A high-conversion-factor, double-resonance structure for high-field Dynamic Nuclear Polarization

Giuseppe Annino[1*], Jorge Villanueva-Garibay[2], Jan van Bentum[2], Adri Klaassen[2], Arno Kentgens[2]

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One of the main problems encountered in the development of instrumentation for high-field Dynamic Nuclear Polarization concerns the construction of a double resonance structure which exhibits very high conversion factors in both the microwave and the radiofrequency response. In this contribution, a novel design of double resonance structure operating at 94 GHz and 144 MHz, in which a miniaturized radiofrequency coil is integrated within a single-mode dielectric resonator based on a nonradiative configuration, will be presented. The proposed system shows a microwave conversion factor Bmw=1.68 mT/W1/2 and a radiofrequency conversion factor of Brf=0.8 mT/W1/2. The microwave conversion factor can be compared with that of the highest quality factor cylindrical cavity constructed so far, for which Bmw=1.21 mT/W1/2.
The developed structure has been employed for simultaneous EPR and liquid state 1H NMR experiments on samples of TEMPO nitroxide radical dissolved in a mixture of water and dioxane. A maximum DNP enhancement of about -17 has been obtained varying the microwave power and the radical concentration. The design principles of the double resonance structure and the obtained results will be discussed, together with possible further improvements and applications. The extension of the proposed design to submillimeter wavelengths will be considered on the basis of some recent results at 360 GHz.

Acknowledgments
The authors kindly acknowledge NOW, the COST action P15 "Advanced paramagnetic resonance methods in molecular biophysics", and the Short-Term Mobility programme of the CNR for the financial support.

A closed cycle helium sorption pump system and its use in hyperpolarized 13C Metabolic MR Imaging

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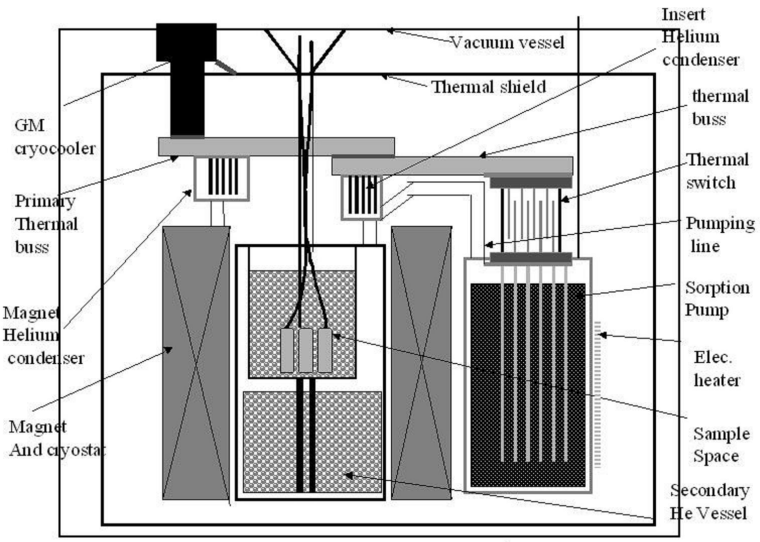
A closed cycle refrigeration system has been designed, built and tested. The goal was to minimize the use of cryogens. The system uses a sorption pump in conjunction with a conventional GM cryocooler to create temperatures below 1.0 K. The low temperature is required for the Dynamic Nuclear Polarization process whereby the nuclear spin polarization is enhanced over thermal equilibrium. The system operates through cycles of pumping to low temperature and regeneration of the sorption pump. The paper focuses on the general design of a prototype clinical polarizer magnet and cryostat and the technical challenges involved.



Schematic of cryogenic system

Dynamic Nuclear Polarization (1) of requires cryogenic temperature to reach close to unity polarization, e.g. below 1.2 K within the 3.35 T magnetic field. The approach used in polarizers to date has been to fill a small helium vessel by either drawing helium from superconducting magnet or by filling directly from an external Dewar (2-4). In either case, low temperatures are reached by using a mechanical pump to reduce the pressure above the liquid helium bath. The liquid then moves down the saturation curve in temperature and pressure. The method is simple conceptually, and has the advantage of being able to run continuously. However, there are important drawbacks: The expensive helium is not recovered and regular (weekly to biweekly) filling of helium is required. Our goal was to create a system that conserves helium and energy and can be operated by hospital personnel in an automated "pushbutton" mode.

Overall heat loads to the system including the superconducting magnet were measured to be 0.45 W. The inner sample space minimum temperature achieved was 0.87 K. The static hold time of the super fluid was measured at just over 84 hours. This data indicates that the sum of the superfluid film creep loss plus the conductive and radiative losses to the sample space add up to 16 mW. Typical microwave power for DNP was 10-20 mW (at source). The system has demonstrated solid-state polarization levels of up to 40 % for [1-13C]pyruvic acid at 0.87 K, i.e. demonstrating the benefit of the lower temperature over similar polarizers.

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3

Overhauser DNP to Study Water Dynamics of Spin Labeled Molecules Applied to Apomyoglobin Folding

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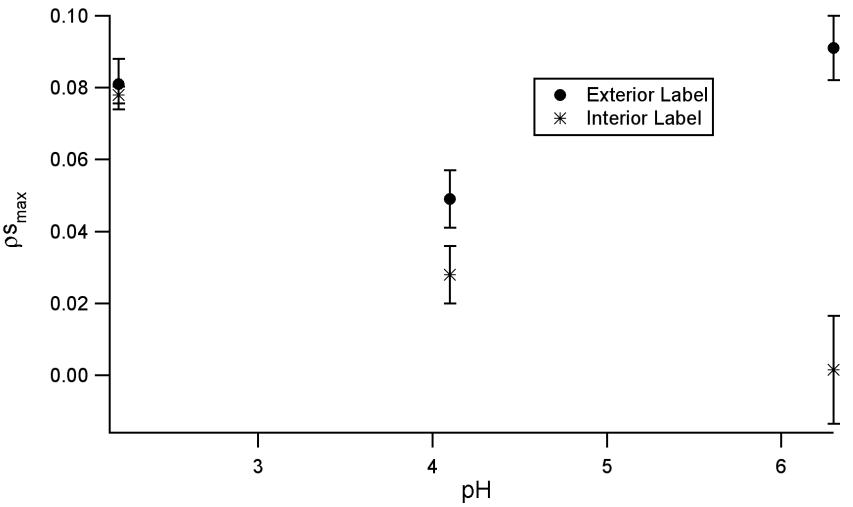
Surface and internal water dynamics of molecules and soft matter is of great relevance to their structure and function, yet the experimental determination under ambient and steady state conditions is very challenging. A potentially powerful approach to measure local dynamics within 10 Å is to utilize the modulation of the nuclear spin relaxation rate of water protons through their time-dependent dipolar coupling to paramagnetic probes, here nitroxide spin labels. The magnitude of the Overhauser DNP signal enhancement critically depends on the translational dynamics modulating the dipolar coupling. The maximum signal enhancement depends on the coupling factor, (ρ), which describes the dipolar coupling between the electron spin and proton spin and contains the dynamic information.



The coupling factor multiplied by the saturation factor for the unfolded, intermediate, and folded states of Apomyoglobin.

The advantage of using DNP on spin labeled molecules is that enhanced 1H NMR signal carries the information allowing the use of sample sizes of only a few μL and sample concentrations of about 100 μM. This presents a great sensitivity gain compared to techniques like field cycling relaxometery or quasi elastic neutron scattering which typically require sample concentrations greater than 1 mM and sample volumes of several hundred μL. Many proteins are insoluble or aggregate at these concentrations. We have applied this technique to the study of apomyoglobin folding. Two different mutants have been spin labeled. One with the spin label at a site on the exterior of the protein (residue 41), and one at a site near the interior of the folded protein (residue 131). By lowering the pH, apomyoglobin populates a molten globule state (around pH 4.1), and by lowering the pH further the protein unfolds. Using DNP we can study the changes in water dynamics between the folded, molten globule, and unfolded states of the protein at the two different residue sites. For the interior site, water mobility increases as the pH is lowered. More mutants are to be examined to study the role of water in the folding of apomyoglobin.

Testing the Limits of Detection: DNP NMR of Mixture of Trace Amount of Metabolic Tracers

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Dynamic Nuclear Polarization (DNP) has gained tremendous popularity in the recent past because of its ability to provide an unforeseen enhancement of signal-to-noise ratio (SNR) for a variety of low gamma nuclei (such as 13C, 15N, etc.). HyperSense is a commercially available automated stand-alone polarizer, which exploits the DNP technology to polarize samples. Use of HyperSense involves polarizing a sample at a very low temperature (1.4 K) in a strong magnetic field, followed by dissolving the sample with hot solvent(s) and rapidly transferring the sample to a Magnetic Resonance (MR) spectrometer for data acquisition.

Use of stable isotopes of metabolic tracers has gained considerable importance in the field of metabolomics because of their ability to trace and identify a given metabolic pathway. 1H NMR spectroscopy, which is the primary analytical technique used in metabolomics is plagued by resonance overlap issues and use of low gamma nuclei (13C, 15N) as diagnostic probes has not been possible, owing to their low natural abundance. In here, we report 13C DNP-NMR studies of a mixture of 50 nmols of several 13C labeled metabolic tracers. The mixture of metabolic tracers is polarized in MeOH-d4, EtOH, and D2O in presence of one or two trityl radicals. The solid-state build-up rates for the individual 13C labeled metabolic tracers revealed slightly different optimal irradiation frequency for each of the 13C labeled metabolic tracers. However, significant SNR enhancement were obtained for all the metabolic tracers when the mixture of all six metabolic tracers was irradiated at the optimal irradiation frequency of 13C-1-Pyruvic acid. In addition, it is shown that a mixture of radicals results in higher SNR for all the metabolic tracers compared to that obtained when only one trityl radical is used. Effect of glassing agents and dissolution solvents were also studied. This study proves the capability of HyperSense in detecting very low concentration of metabolic tracers in a mixture. Such applications can be extremely beneficial in employing heteronuclei (such as 13C, 15N, etc.) as a probe in the field of metabolomics, and also in the field of impurity detection.

5

Development of Sub-THz and THz Electron Devices at the Institute of Applied Physics

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High-frequency electronics has been successfully developed at IAP for many years. One of the most remarkable achievements was the invention of gyrotron whose operation was based on stimulated cyclotron radiation of electrons moving along helical trajectories in homogeneous magnetic field and microwave field of an oversized cavity. Now the most powerful gyrotrons provide the power about 1 MW in the quasi-CW regime at the frequencies up to 0.17 THz. Already in the 1970s-1980s, gyrotrons developed at IAP also generated fairly high power at the frequencies higher than 0.3 THz (submillimeter wavelengths). Recently these results were significantly improved and the maximum frequency of gyrotrons operating at the fundamental cyclotron harmonic increased up to 1.3 THz in the single-pulse regime due to using a very strong magnetic field up to 50 T. It is important that the frequency 1 THz was also obtained in a third-harmonic gyrotron at essentially lower field of 14 T. As for CW operation, modern gyrotrons can use readily available magnetic systems based on liquid-He-free cryomagnets significantly simplifying work with such oscillators. For instance, the CW gyrotron designed at IAP and successfully tested in collaborative experiment at the Far Infrared Center of the Fukui University (Japan) operates with such magnet at the frequency 0.3 THz with the power 2.7 kW. Just recently, a 260-GHz CW gyrotron with a liquid-He cryomagnet was specially designed at IAP for DNP experiments.

Another problem being solved now at IAP in collaboration with the Federal State Unitary Enterprise VNIIFTRI and the Institute of Spectroscopy of the Russian Academy of Sciences is the advancement of relatively low-power devices based on stimulated Smith-Purcell radiation of rectilinear electron beams in open cavities containing periodic slow-wave structures, namely, orotron oscillators and frequency multipliers. Such devices provide pulse generation at the frequencies up to 0.4 THz with broadband electro-mechanical frequency tuning and higher power than that provided by the most frequently used Backward Wave Oscillators. These oscillators can be presumably employed in ESR and DNP-NMR spectroscopy.

Combined DNP and Magnetization Transfer in Low-Field MRI

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In our study we decided to combine DNP and MT to improve the images in low-field MRI.
A pulsed DNP has been chosen because the electron spin saturation and FID nuclear detection can occur at two different time intervals like making it in a gated nuclear Overhauser experiment. The pulse sequence consists of a h.f. pulse of variable magnetic field of the amplitude B1 and the length τ to saturate the electron spins followed by a 90° l.f. pulse to interrogate the protons.
In the MT technique RF pulses are used to selectively saturate protons in the bound pool (biomacromolecules). This saturation effect is transferred subsequently (by dipolar and chemical exchange interactions) to protons in free water, and is proportional to the relative sizes of the pool, individual proton relaxation rate, and cross-relaxation rate. In biological materials, MT or cross-relaxation is commonly modeled by two spin pools identified by their different T2-relaxation times. The free water proton pool with a long T2 gives rise to a narrow spectral line detected in imaging. The bound proton pool with very short T2 gives rise to a broad spectral line which is not directly observed in MR imaging. We used an off-resonance pulse technique, where multiple, short duration, high intensity RF pulses irradiate a sample at a frequency offset on several kilohertz from the free water resonance.
MT imaging has been quantitatively investigated and computer-simulated as a function of the number, amplitude, offset and duration of the off-resonance pulses using a special computer simulated program.
In our DNP-MT studies we used simple phantoms that model biopolymer-water systems, containing some paramagnetic stable radicals, and reproducing many of the cross-relaxation features, found in tissues and biological samples.
All experiments have been performed on home-made low-field MRI scanner at the Department of Physics, Saint-Petersburg University.
The application of combined DNP and MT in low-field MRI permits to improve the contrast of images for biopolymer-water systems.

7

Biocompatible, spin-labeled heparins as polarizing agents for dynamic nuclear polarization (DNP)

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A versatile and biocompatible class of spin-labeled macromolecules was investigated by continuous-wave (CW) electron paramagnetic resonance (EPR), double electron-electron resonance (DEER) and dynamic nuclear polarization (DNP) that can be utilized for in vivo magnetic resonance imaging (MRI DNP enhanced) and EPR imaging (EPRI)[1]. The distance distributions of the spin labels were experimentally obtained and compared with the crystallographic structure of heparin. All presented heparin radicals show reasonably high ^{1}H DNP enhancement factors up to E = -91. The heparin radicals for which the dipolar coupling frequency ν_{dd} between the electron spins matches the proton Larmor frequency showed the best enhancements. One striking result concerning the very broad lines of the heparin-nitroxides in the EPR spectra is the fact that the achievable enhancements are comparable to free TEMPOL. Usually, for free radicals broad EPR lines correspond to low achievable enhancement factors. This "extra-polarization" effect may be due to the broad range of intramolecular and intermolecular radical-radical distances and hence of dipolar couplings between electron spins that can be expected because of the statistical nature of the labeling and the conformations that a polysaccharide in solution may adopt. In this respect, the spin-labeled heparin can be seen as a "broad-band" polarizing agent that might also be suitable for an efficient hyperpolarization of ^{13}C (^{15}N, ^{19}F) containing molecules[2,3]. Furthermore the ^{13}C nuclei of the heparin backbone themselves could be hyperpolarized and spin-labeled heparin could be utilized directly as active agent for molecular imaging since it is known to bind to the endothelium and to hundreds of different proteins[4]. Consequently, if the ^{13}C spins of the spin-labeled heparin could be polarized sufficiently a new field would emerge in molecular imaging and metabolic processes could be studied.

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A Combined NMR (144 MHz) and Pulsed EPR (95 GHz) Spectrometer For DNP

Akiva Feintuch*, Yonatan Hovav, Vijayasarathi Nagarajan, Yaacov Lipkin, Yehoshua Gorodetzky, Koby Zibzener, Shimon Vega, Daniella Goldfarb

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The recent development of dissolution DNP and its potential for biological and clinical applications has led to an increasing interest in the field. However, a quantitative theory of the effect and the role played by the different suggested mechanisms involved (i.e. solid effect, cross effect, and thermal mixing) is still incomplete. We report here on a homemade DNP spectrometer capable of NMR and 95 GHz pulsed EPR detection built for the purpose of probing the basic DNP mechanisms. The description of the spectrometer and initial results from TEMPO in 50:50 H2O:glycerol at 40K are shown.

9

High-Field Overhauser Effect of Nitroxide Radicals: Maximum Enhancement and Electronic Saturation in Aqueous Solutions

Marat Gafurov*, Deniz Sezer, Vasyl Denysenkov, Mark J. Prandolini, Burkhard Endeward, Sevdalina Lyubenova, Thomas F. Prisner

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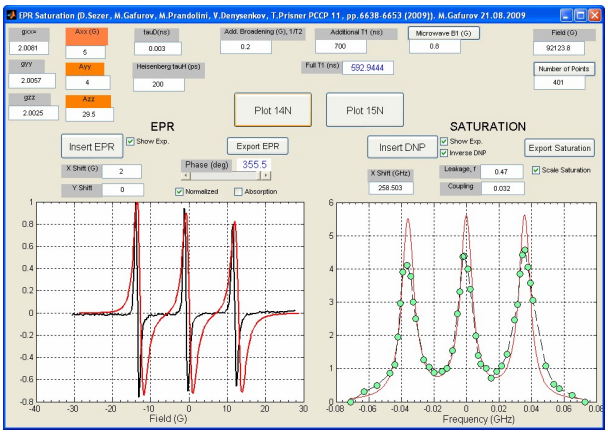
Large dynamic nuclear polarization (DNP) enhancement in liquids at high magnetic fields opens up the possibility of overcoming the current NMR sensitivity limits and allows the study of macromolecules complexes at low concentrations. Our approach is to polarize liquid samples in-situ at high magnetic fields using a double-resonance structure at both NMR and microwave EPR frequencies [1]. The structure has two important features: firstly, it drastically reduces the microwave electrical field strength at the sample position, thus avoiding excessive heating of the liquid sample; and secondly, it strongly enhances the MW magnetic field strength at the sample position, which allows significant DNP enhancements already with a very low incident MW power of less than 45 mW.



Graphical User Interface of Matlab based program to simulate EPR and DNP spectra

In this work we compare experimentally the DNP efficiencies of various organic and inorganic nitroxides in aqueous solutions at 9.2 T (400 MHz of 1H NMR frequency and 260 GHz EPR frequency). Unexpected high DNP enhancements of more than 10 have been achieved on protons in liquid water samples [2]. As it is well known, the magnitude of DNP enhancement depends upon the degree of electron saturation and relative nitroxide-solvent motion. To disentangle these effects the saturation has been modeled within the Redfield approximation [3]. A Matlab-based implementation of the formalism allows us to extract the DNP coupling factor from the observed data (Fig.1). Such understanding is the first important step towards the application of DNP to biomolecules under physiologically relevant conditions.

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DNP and Spin Diffusion- a Quantum Based Description

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While the Solid Effect DNP process occurs between an electron and its neighboring core nuclei, the observed nuclei are typically far away from the electron. The detection of hyperpolarized signals depends therefore on the 'diffusion' of the polarization from the close nuclei to remote bulk nuclei. Here we focus our attention on those core nuclei that effectively interact with the bulk nuclei, and enable the spin diffusion process. We use a full QM calculation and three model systems to study the mutual influences of the MW irradiation, the dipolar interactions, and the relaxation processes, on the bulk nuclei polarization.

A QM calculation of the nuclear polarization has been performed in Liouville space on a system composed of an electron, a core nucleus, and N additional dipolar coupled nuclei. Spin-lattice and spin-spin relaxation times of all the spins in the system are taken into account. However, using this calculation we are limited to $N = 3$.

To extend the number of bulk nuclei to $N > 3$ it is suggested to simplify the problem by performing all calculations in Hilbert space. To do so we perform a stepwise integration of the Liouville-von Neumann equation for the density matrix, again considering the MW irradiation, the spin-spin relaxation, spin diffusion via the nuclear dipolar interaction, and the spin-lattice relaxations. Using this we succeeded to evaluate the spin diffusion process considering $N = 8$ nuclei.

To enlarge the number of nuclei even further we combined all spin states of the bulk nuclei into groups of equal total spin number. Doing so, we could characterize the global effects of the MW irradiation and the different spin-lattice relaxation parameters on the bulk polarization. To get a better understanding of the spin diffusion mechanism a semi-classical model is constructed that shows the spin diffusion process in a large bulk spin system with $N > 100$. Here the nuclear spins are treated as two level systems coupled by dynamic processes, presenting the MW irradiation, the dipolar interaction and the spin-lattice relaxation rates.

11

High power pulsed DNP using the HIPER 94GHz spectrometer

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The development of high field DNP methodologies requires rapid and efficient polarisation transfer, high NMR filling factors and minimal thermal heating of the sample.

One possible approach to this is the use of very high power pulsed techniques, although up until recently the available mm-wave pulse power levels have always been too low to demonstrate this effectively in high magnetic fields. However, at St Andrews, a high performance kW pulsed 94GHz ESR system has recently been developed. With the ability to use high power pulses at relatively low average power levels, in high volume samples, we now have the potential to evaluate a number of DNP pulse techniques.

In this poster we present results showing that significant increases in the final polarization are achieved for the solid state DNP of water/glycerol polarized by 40mM TEMPO at 70K, relative to continuous wave methodologies for the same average power. This result has also been repeated with a similar water/glycerol sample polarized using 10mM TOTAPOL. With both of these samples we have also found that for high power kW pulses at 94GHz, the optimum polarization transfer occurs when irradiating with short $\pi/2$ pulse lengths of 8ns at equal spacing, with average power levels of several watts in our large volume DNP sample holder. The increase is attributed to the greater numbers of spins that are now able to contribute to the polarization transfer process associated with the Cross Effect/Thermal mixing in this system.

We will also report on a new large volume room temperature aqueous sample holder (for use with high loss samples) that still allows NMR filling factors of around 10%.

Microwave-based Dynamic Nuclear Polarization of proteins in solution.

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DNP enhancement of ^{13}C and ^{1}H spectra in proteins has been studied starting by applying microwave irradiation of a water-glycerol glass at 1.4 K containing the free radical TEMPOL. After the solvent-ice mixture is rapidly heated up to room temperature, the hyperpolarized solution is quickly transferred to the protein sample already waiting inside the NMR spectrometer. This allowed us to observe by NMR in real-time the saturation transfer and solvent exchange from polarized water to the protein. In another method, the TEMPOL / glycerol / protein mixture has been polarized directly in the glassy state at ultra low temperature, subsequently rapidly heated up under pressurized boiling water and fast-shuttled (700ms-3s) to the NMR magnet, in order to finally measure the net polarization on the protein.

13

Producing Hyperpolarized gases by Sublimation Dynamic Nuclear Polarization

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We have developed a method to produce hyperpolarized gases by *Sublimation* Dynamic Nuclear Polarization (S-DNP), in analogy to *dissolution* Dynamic Nuclear Polarization [1], as an alternative to the usual optical pumping method [2]. It has the advantage to allow the production of large amounts of polarized gas in a relatively short time. As an example, we used this technique to hyperpolarize ^{129}Xe nuclear spin in natural abundance xenon gas. A 0.3 mL sample of a liquid (1:1) mixture of xenon:isobutanol was doped with TEMPO free radical (30 mM), frozen and dynamically polarized at 1.2 K and in a field of 3.35 T by irradiation at 93.89 GHz with a microwave power of 30 mW. A steady-state ^{129}Xe nuclear spin polarization of about 3% was quickly obtained with a build-up time constant T=140 s. The solid xenon was subsequently sublimated, and 35 mL of the resulting hyperpolarized xenon gas was solidified in a 5 mm NMR tube pre-cooled in liquid nitrogen and placed in a 0.1 T permanent magnet. The ^{129}Xe nuclear spin-lattice relaxation time under these conditions exceeds 10^4 seconds [3]. The tube was then placed in the room temperature coil of a 300 MHz high resolution NMR spectrometer (Bruker) to measure the hyperpolarized ^{129}Xe nuclei in the gas phase. The ^{129}Xe nuclear spin polarization was estimated to be 2.7 %. The enhancement of the polarization compared to the Boltzmann thermal equilibrium polarization at 7.05 T and 25°C exceeds a factor 4000. By using a larger solid-state sample [4] and by optimizing the sample loading/sublimation process (with a cycle time of 10 min) this technique would allow the production of up to 30 L per hour of atmospheric-pressure hyperpolarized xenon. The new S-DNP method could also easily be extended to other gas like nitrous oxide ($^{15}N_2O$).

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Development of an LT MAS NMR Probe for DNP use at 395GHz / 600MHz

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A probe has been developed to allow low-temperature (LT) high-resolution DNP-enhanced NMR of solid samples to be performed at high magnetic field (14.1 T). The probe is a modified Doty LT MAS probe with additional optics that allow microwaves to be transmitted along the axis of the rotor. The design allows it to be used in HXY NMR mode at 14.1 T and HX mode at 6.7 T (285 MHz NMR) to match the gyrotron's second and fundamental harmonics. Spinning samples at low temperature results in increases in conventional NMR signal strengths from the Boltzmann factor and an increase in the quality factor of the coil. Stable spinning rates for this probe are 5 kHz at 90 K, increasing to 8 kHz at 110 K. Measurements of the low temperature characteristics of the probe using both $Pb(NO_3)_2$ and $Sm_2Sn_2O_7$ have shown that the temperature differential across the sample is approximately 1 K throughout the temperature range. ^{119}Sn NMR of $Sm_2Sn_2O_7$ was found to have a number of advantages over ^{207}Pb NMR of $Pb(NO_3)_2$ for thermometry.

NMR at low temperatures can yield improved sensitivity but it also increases T_1 relaxation times in diamagnetic organic samples. The relaxation in such systems is disadvantageous to DNP experiments and is one reason why DNP is usually performed at low temperatures where unwanted magnetisation losses are reduced. However, this weak relaxation can result in long or impractical experimental times in conventional NMR. The presence of paramagnetic ions in organic samples that is essential for DNP enhancements also increases the nuclear T_1 relaxation rates and can therefore be beneficial to conventional NMR. Preliminary results of MAS NMR conducted at low temperature on various undoped diamagnetic and paramagnetically doped solid samples will be presented.

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Liquid state DNP on metbolites at 260GHz EPR/ 400MHz NMR frequency

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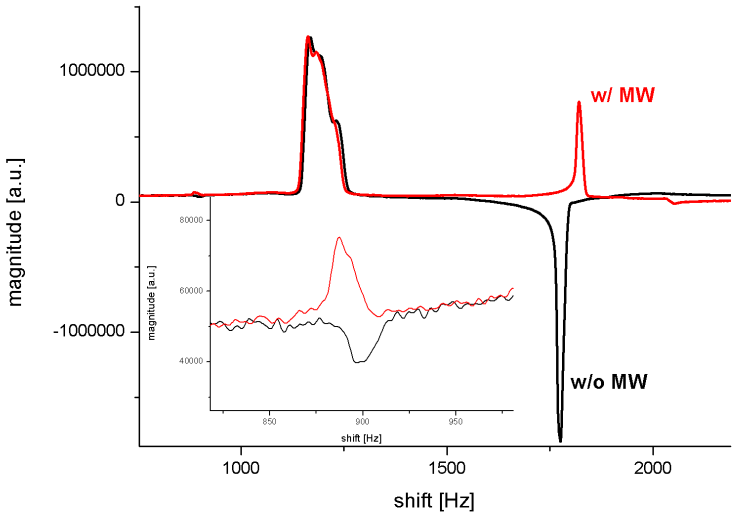
A common method to improve NMR sensitivity is to increase the external magnetic field. However, large NMR magnets are not only expensive but will reach practical and technical limits at around 23.5 T (proton freq. 1000 MHz). Another way to greatly improve NMR sensitivity is a method called Dynamic Nuclear Polarization (DNP). By resonating unpaired electron spins with microwaves DNP mechanisms transfer the larger Boltzmann polarization of these electrons to nuclear spins, thereby improving the NMR signal-to-noise ratio. In the case of dipolarly coupled protons at low magnetic fields NMR enhancements can theoretically be as high as 330, however at higher magnetic fields this value is greatly reduced. In the liquid state the dominant DNP mechanism is the Overhauser Effect. It relies on the coupling and therefore relative motion between the unpaired electron spins and the nuclear spins on the target molecule, thus the DNP effect greatly depends on the size and mobility of the molecules in question. In here we present direct Overhauser enhancement of protons on different metabolites in water solution at 9.2 T magnetic field. The experimental results yield coupling factors for the respective protons and can therefore be used as benchmarks for molecular dynamics simulations modeling the process.



Direct liquid state DNP polarization on pyruvate CH3 protons at 9.2 T magnetic field

Overhauser proton DNP in water and toluene at 94 GHz

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Liquid state DNP NMR enhancements for water at room temperature and toluene at temperatures between 340 and 160 K were measured using a Bruker DNP probe with a dielectric cylindrical cavity. A 150 mm bore NMR magnet with a room temperature sweep coil was used to enable facile variation of the magnetic field. The microwave radiation from a pulsed W-band EPR console (50 mW maximum power) was applied to the central line of the EPR triplet of the TEMPOL nitroxide radical. An enhancement of -30 in a 24 nL water sample with 10 mM radical concentration was achieved. Toluene is a less polar solvent and therefore allowed samples sizes as large as 1.2 μL to be used with the same cavity. The enhancement for the ring protons in toluene containing 5 mM of TEMPOL was found to increase strongly with temperature rising from near zero at 160 K to -14 at room temperature. The enhancement for the methyl sites was smaller, being -8 at room temperature. This difference is probably due to a shorter 1H relaxation time for methyl protons in pure toluene which reduces the leakage factor. Removal of diffused oxygen from toluene samples is critical if maximum enhancements are to be achieved, so a freeze, pump and thaw method was utilised for sample preparation. The reciprocal of the enhancement varies linearly with the inverse of the applied microwave power, and extrapolation of the experimental data to higher powers indicates that the enhancement could be increased to > -50 at room temperature. Above RT the EPR line becomes broader and it is more difficult to saturate the line so that the enhancement no longer increases with temperature in the present set-up. To aid understanding of the variation of enhancement with temperature CW EPR spectra of TEMPOL in toluene were obtained (at X-band as well as W-band) over this temperature range to determine the electron correlation time. The proton spin-lattice relaxation time was also measured.

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Performance of an integrated dissolution DNP spectrometer for liquid state NMR spectroscopy

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Recently it was demonstrated that large nuclear spin polarisation in liquid state samples can be generated by first increasing the polarisation of the nuclear spin system in solid state using dynamic nuclear polarisation (DNP) at low temperature followed by a fast dissolution step [1].

A two-centre, integrated 3.4 T DNP polariser and 9.4 T liquid state NMR spectrometer has previously been presented [EUROMAR 2008]. Due to the proximity of the two magnetic centres in such a system, the polarised sample can be rapidly transferred and, furthermore, this can be done in the solid state followed by subsequent dissolution immediately above the NMR centre. This significantly reduces T1 relaxation loss, as well as eliminating cross-relaxation that can arise when liquid-state samples are shuttled through a varying magnetic field. Consequently it is possible to observe signals from very short T1 species in both natural abundance 13C spectroscopy and low concentration 1H spectroscopy.

Here we present results obtained using low molecular weight peptide samples. The acquired liquid state enhancements depend exponentially on the T1 values. For the proton groups used, the T1 value varies between 0.4 and 2.5 s. A comparison of the enhancement factor, ε, is provided between the integrated system and a system consisting of a stand-alone polariser connected to a separate 9.4T magnet. In natural abundance 13C spectroscopy experiments using the stand-alone polariser, it was found that 3 of the 8 Ala-Gln di-peptide carbon resonance frequencies could not be observed due to the short T1 of these lines. However, using the dual-centre system it was possible for all resonance lines to be detected with significant enhancement. It is found that fast shuttling of the sample in the solid state allows for maximal exploitation of the DNP enhancement.

Acknowledgements:
We are very grateful for support from G Smith and R Hunter from the University of St Andrews, Scotland, UK. The project was funded by an EPSRC instrument development grant and a technology development grant from the BBSRC.

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EPFL Design of a Frequency-Tunable Gyrotron for High Frequency Dynamic Nuclear Polarization

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We report on the design of a modular low-power (10-50W) high-frequency gyrotron (265-530GHz) for DNP enhanced Solid-State NMR spectroscopy. With the view of covering a wide range of frequencies, a 9.7T helium-free superconducting magnet is planned for the gyrotron operation on either the fundamental or second harmonic of the electron cyclotron frequency. The gyrotron design is based on a triode electron gun (Vk=15kV, Ib=100mA, Va= 6-8kV), which is very flexible for adapting the electron beam properties to a wide variety of cavities operating at the fundamental or at the second harmonic. The gyrotron is designed for a lateral output with an internal Vlasov-type converter. The reference parameters for application of DNP-enhanced NMR spectroscopy on a 400MHz (1H) spectrometer are optimized with a RF frequency tunability corresponding to twice the proton NMR frequency. The modularity of the construction of the gyrotron allows for the possibility of changing only some elements like the cavity-uptaper system in order to adapt to the wide range of NMR spectrometers existing at EPFL.

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Spectral properties of hyperpolarized xenon detected without rf excitation: maser emissions and spin-noise detection

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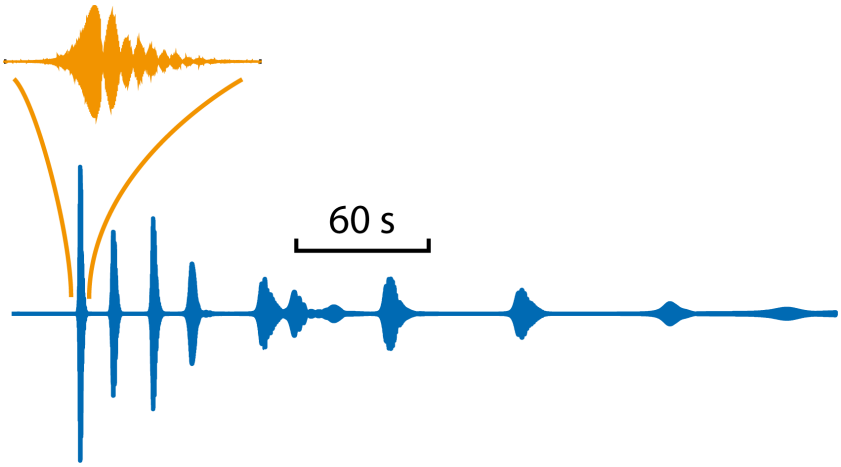

Multiple maser emissions observed without rf excitation when laser-polarized xenon with negative spin-temperature is used.

In liquid-state NMR, resorting to a dissolved substance polarized at a high level (>20%) induces the appearance of new physical phenomena due to distant dipolar fields (DDF) and non-linear coupling between the magnetization and the rf coil (radiation damping, RD). As a consequence a reassessment of the conclusions drawn for thermal equilibrium systems is required and new perspectives appear. We report here our latest results obtained simply by monitoring the NMR signal of laser-polarized xenon without coherent rf excitation. When negative xenon spin temperature is selected during the optical pumping step, multiple maser emissions appear spontaneously. Their analysis indicates a linear correlation between their radiative energy and a rate characteristic of their life-time, tending to validate the key importance of DDF in this chaotic behavior [1]. On the other hand, for positive xenon spin temperature, the first detection of nuclear spin-noise of an hyperpolarized species is reported. We show that conversely to situations encountered for thermal equilibrium, this approach gives directly access to the spectral and dynamic properties of Xenon. The conditions of detection are fully renewed, making this approach particularly promising for a very small number of spins, even at low static magnetic field. This concept is validated by spin-noise detection of a number of spin smaller than what can be detected by a one-pulse experiment at thermal equilibrium [2].

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Dinitroxides as polarizing agents for dynamic nuclear polarization

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NMR sensitivity can be enhanced orders of magnitude by dynamically polarizing the nuclear spin system via a coupled electron spin reservoir.[1] A prerequisite for Dynamic Nuclear Polarization (DNP) is the presence of paramagnetic centers in the sample. On irradiation close to the frequency of the EPR transition, the large polarization of the electron spin system can be transferred to the nearby nuclei through the dipolar interaction between the electron and nuclear spins.

To date, beside the large efforts dedicated to improve the instrumentation and to complete the DNP mechanistic understanding, works are undertaken for developing new polarizing agents with superior enhancement. Different kinds of free radicals (Cr^V complexes, BDPA, trityls, nitroxides) have been employed, and it appears that TEMPO-like nitroxides are one of the most promising polarizing agents. Nitroxide have been shown to have strong dipolar coupling to water. Moreover, they have the advantage of being easily available and non-toxic at the concentration used for biological applications. In solid sate DNP experiments, dipole-dipole coupled dinitroxides have demonstrated to be the more effective source of polarization than monomeric nitroxides.[2,3] In these systems, the polarization mechanisms rely on a three-spin coupling (electron - electron- nucleus) process via thermal mixing and cross effect.[4]

We will report the structure, the EPR characterization and the DNP enhancement factor of a series of dinitroxides.[5]

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Dissolution DNP-NMR for peptide studies

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The sensitivity enhancement arising from DNP makes it possible to significantly reduce the NMR averaging time, in fact very often single scan acquisition provides sufficient a signal-to-noise ratio. The fact that sufficient information on the system may be obtained in milliseconds in liquid state makes dynamic sample studies possible such as protein folding and interactions, ligand binding or chemical reactions provided that the time constant of the dynamic process is bigger than 10ms.

C-13 and H-1 DNP polarisation was applied to a range of organic samples (amino acids, peptides). The DNP liquid state signal enhancement, and relaxation characteristics for both a stand alone polariser and an integrated DNP-NMR instrument based on a dual iso-centre 3.4/9.4T magnet were acquired.

The short acquisition time and the single scan capability of ultrafast multidimensional spectroscopy make it possible to acquire 2D NMR spectra of polarized spin systems in the liquid state using DNP. The work on the application of single scan 2D sequences in conjunction with DNP will be presented. The sample transfer and settling process has been studied and optimised for the demands of fast 2D spectroscopy and a variety of DNP enhanced amino acids and peptide samples have been successfully detected. A slice-selective version of the single-scan COSY sequence was implemented with which two consecutive spectra can be acquired from two different regions of the same sample. This strategy may be useful for monitoring dynamical changes in the highly polarised spin system.

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DNP-Enhanced NMR at 3.4 and 14.1 Tesla with High-Power Microwave Sources

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Two dynamic nuclear polarisation NMR spectrometers are presented that have been integrated into solid-state NMR equipment. Each demonstrates an experimental method that is critical to the development of DNP-NMR as an analytical tool for solids: strong magnetic fields and pulsed microwave sources.

The first system uses a 94 GHz (3.4 T, 143 MHz), state of the art Bruker pulsed EPR system with a 100 W extended-interaction klystron. Initial studies of DNP mechanisms in liquid-state samples utilised a DNP probe that was optimised for use with a vertical magnetic field, small sample volumes and small filling factors, with low incident microwave power levels (50 mW). Solid-state samples and high-power pulsed DNP require a different approach with different probe hardware needs to those for continuous wave and liquid-state DNP.

The second system uses a CW gyrotron operating with a 394.5 GHz second-harmonic as a microwave source (approx. 30 W). Microwave hardware is presented that treats the waves quasi-optically and ensures mode purity. The NMR system includes a 14.1 T (600 MHz) magnet with a superconducting sweep coil, which has a persistent-mode switch, and a triple-channel probe with MAS ability at temperatures down to 90 K designed such that DNP experiments at the first harmonic of the gyrotron (187 GHz, 285 MHz, approx. 200 W) can also be undertaken. The aim of the system design is to ensure that experimental parameters critical to DNP operation are known accurately and are stable.

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Optimal Control Study of Dynamic Nuclear Polarisation

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Dynamic Nuclear Polarisation (DNP) allows to increase the polarisation of nuclear spins by polarisation transfer from unpaired radical electrons [1]. Recently Weis and Griffin showed analytical matching conditions for cross polarisation (CP) transfer modified for the DNP case, namely the electron nuclear cross polarisation (eNCP) [2].

Optimal control theory was applied to study DNP for spin systems in the absence and presence of relaxation [3]. First prototypes of combined ESR and NMR spectrometers have become available and the numerically optimized pulse sequences can be implemented. Our results obtained by optimal control theory exceed the limits of cw radiation experiments and reach the maximum polarization transfer from the electron to the nucleus.

Optimized DNP sequences for two- and three spin model systems with auto, cross and cross-correlated relaxation will be presented for different coupling topologies and relaxation rates. Simulations are based on on the homogeneous form of the quantum-mechanical master equation. Non-trivial polarisation transfer mechanisms were found, such as combinations of hyperfine coupling transfer, Overhauser transfer and very fast electron spin relaxation acting as a polarization source.

For more realistic simulations, the parameters have been adjusted considering limited rf and mw amplitudes, finite switching times of the hardware equipment, limited radiation bandwidths as well as measured relaxation times for radicals (malonic acid) leading to theoretical curves for maximum achievable transfer efficiency depending on external (transfer time, rf-and mw-limits, finite switching times, pulse bandwidth) and intrinsic system parameters (relaxation, coupling).

Furthermore some progress towards the pulse sequence simplification was made by Cartan decomposition of the operator for DNP time scale setting [4] which have been prooved experimentally on a pure NMR system.

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Liquid-State DNP at 400 MHz / 260 GHz: Limits and applications to biological samples

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The early development of liquid-state dynamic nuclear polarization (DNP) was performed at low magnetic fields, where the Overhauser Effect (OE) was found to be most effective [1]. Recently, unexpected high DNP enhancements of more than 10 have been achieved in liquid water samples at room temperature and magnetic fields of 9.2 T (corresponding to 400 MHz 1H NMR frequency and 260 GHz EPR frequency) [2]. The liquid samples were polarized in-situ using a double-resonance structure, which allows simultaneous excitation of NMR and EPR transitions and achieves significant DNP enhancements at very low incident microwave power of only 45 mW [3]. These results are compared to predictions of the DNP coupling factor from nuclear relaxation data [4]. Finally, these results demonstrate the first important step towards the application of DNP to high-resolution NMR, increasing the sensitivity on biomolecules with small sample volumes and at physiologically low concentrations; some potential application will be demonstrated.

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Dynamic Polarization of 13C Nuclei in Solid 13C Labeled Pyruvic Acid

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We studied the dynamic nuclear ^{13}C polarization in solid [1-^{13}C]pyruvic acid doped with the trityl radical AH111501 at different temperatures and magnetic fields. The measurements were performed in a ^{4}He evaporation refrigerator operated inside a superconducting solenoid system. Working points at temperatures between 900mK and 1350mK have been achieved and the polarization measurements have been performed at magnetic fields of 2.5 T, 3.5 T and 5.0 T, respectively. This set of measurements allows to draw a clear picture of the temperature and magnetic field dependency of the ^{13}C polarization within the given range. The highest polarization measured was 74.7% at a temperature of 900mK in a magnetic field of 5 T.

Hyperpolarised combretastatins and pyruvate: potential bio-markers for vascular targeting of tumours.

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Vascular targeting of tumours represents a proven complementary approach to conventional cancer therapy[1,2]. However, effective clinical evaluation of new agents in this field requires the development of imaging bio-markers that can determine tissue pharmacokinetics (PK), early pharmacodynamic (PD) response and measures of resistant tumor phenotypes in early clinical trials.

Hyperpolarised $^{13}C_1$-pyruvate, has previously been shown as a metabolic marker for studying tumor growth[3, 4]. We propose to use this methodology to study glycolytic metabolism in BD9 rat P22 carcinosarcoma models.

Vascular targeting agents, such as combretastatin A-4-phosphate (CA-4-P), are potentially useful in the treatment of tumours and clinical studies of tumour oxygenation status have predicted prognosis in a number of tumour sites. We will investigate the relationship between $^{13}C_1$-pyruvate flux and acute changes in oxygenation within tumours, (measured by luminescence decay of oxygen-sensitive probes and elicited by changing the oxygenation of inspired gases and by administration of CA-4-P) to determine the feasibility of monitoring glycolytic metabolism as a useful bio-marker of tumour microenvironment oxygenation. In addition, we show that CA-4-P can be hyperpolarised, and discuss its potential use as an MRIS metabolic marker.

Funded by Cancer Research UK and EPSRC, with additional funding from MRC and the Department of Health, England (Programme Grant C1276/A10345)

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Radiation Damping Effects in Hyperpolarised 13C MR

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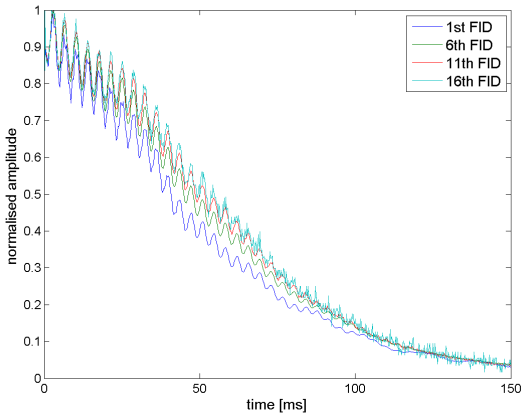
Radiation damping (RD) describes an interaction between sample and RF coil [1-3], where the detection current in the coil is strong enough to induce an opposing flip upon the sample magnetisation, which can distort the FID or relaxation measurements. RD is visible usually only for high sample magnetisation and good coil-sample coupling (high Q on-resonance). RD is usually negligible in normal MRI, but it is well-known in high-field liquid-state NMR with high polarisations and small, well-coupled coils. However, RD can potentially be observed on MRI scanners with hyperpolarised samples [4]. Here, detection of RD in hyperpolarised ^{13}C MR is demonstrated, theoretically explained and possible implications on experiments are discussed.



Normalised FIDs distorted by RD for experiment b (127°). The apparent decay is faster, particularly for the first FID, where the hyperpolarisation is not yet depleted and magnetisation density is highest. The small beating pattern stems from interconversion between pyruvate and pyruvate-hydrate.

RD is described by a characteristic time constant τ_{RD} and its impact on the measurement depends on the relation of τ_{RD} relative to T_1 and T_2^s. RD can be effective both during RF pulses and acquisition. RD leads to a faster, effective decay of the detected signal by partly recycling magnetisation. An FID distorted by RD is composed of an exponential (T_2^s) decay and a sech function (Eq 12 in [2]). For quantification, a time-domain based, non-linear fitting algorithm was implemented to extract T_2^s and τ_{RD}. Hyperpolarised $^{13}C_1$-pyruvate (230mM; 10% polarisation; HyperSense) was filled into a 3ml glass sphere. FIDs (hardpulse; 22.5°(a)/127°(b); TR=5s; BW=5kHz; n=2048) were acquired on a 3T GE HD scanner with a solenoid coil (Q≈500).

RD is indeed detectable with hyperpolarised Pyr, leading to a mild FID distortion. Measured and fitted τ_{RD} were 59ms(a)/55ms(b), corresponding well with the theoretical τ_{RD}≈40ms. This is weak (T_2^s

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Fast DNP enhanced NMR spectroscopy in conjunction with rapid mixing

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To extend dissolution DNP spectroscopy to the study of dynamical changes on the molecular level it is frequently necessary to rapidly mix two liquids immediately before the NMR signal is acquired. While the first liquid contains the highly polarised spin system, which may be a ligand or a protein, the other solution could contain either receptor molecules or small molecules that trigger a particular dynamical process of the hyperpolarised molecule. Combined with techniques for fast data acquisition this strategy could make measurement of molecular dynamics with time constants in the millisecond regime possible. For this strategy, there are two time constraint. First, the decay of the non-thermal high polarisation with the characteristic T1 time constants and second the time constant of the molecular dynamic process to be monitored.

Here, we demonstrated the potential of time-resolved NMR spectroscopy by using DNP polarised uniformly ^{13}C-labelled glucose for monitoring the mixing process. After DNP using the trityl derivative OXO63 and dissolution in a stand-alone polariser the ^{13}C spins were polarised up to $\varepsilon+ = 1500$ in liquid state depending on their relaxation time constants. Additional full deuteration of the ^{13}C labelled glucose made a built up of polarisation with $\varepsilon+ = 10000$ possible.

Our mixing strategy for the two liquids is based on pneumatic injection of a small volume (100µl) into the solution arriving from the stand-alone polariser immediately before the fluid is allowed to settle for a short period of time in the NMR sample tube. We have investigated the effect of pressure variation and injection timing on the quality of the acquired spectrum. With optimised experimental parameters it is possible to acquire spectra of the ^{13}C labelled glucose with a linewidth of 20Hz in 500ms after combining the two solutions.

We also demonstrate a number of fast 2D COSY, HSQC and TOCSY sequences using ^{13}C labelled glucose and other small molecules in conjunction with DNP enhancement.

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Gyrotron FU CW VII for 600 MHz and 285 MHz DNP-NMR

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High-field dynamic nuclear polarisation using electron spins is a promising technique for biological systems which can significantly enhance the sensitivity of high-resolution solid-state NMR. For high-field DNP experiments, high-power and high-frequency microwave sources are required to excite electron spins. Conventional microwave devices for this frequency region such as an extended interaction oscillator (EIO) and a backward wave oscillator (BWO) are limited to low power levels since the resonator sizes scale down with increasing frequency. Unlike these devices, the cavity size of a gyrotron can be much larger than the operating wavelength and high-power microwaves are generated. A 187- and 395-GHz gyrotron source suitable for dynamic nuclear polarisation at ^{1}H NMR frequencies of 285 and 600 MHz is presented.

The gyrotron tube, which consists of the cavity designed for operation at TE_{13} fundamental and TE_{16} second harmonic modes, and a triode electron gun, is mounted in a 9.2 T sweepable superconducting magnet to enable choice of mode. The frequencies were measured using a heterodyne detection system and were found to be 187.1 GHz and 394.5 GHz, respectively. They were slightly lower than the design values suggesting that the fabrication error of the cavity radius is ≈10 μm. Output powers at these frequencies were measured by a water load. The gyrotron oscillator generates a CW power of ≈200 W in the TE_{13} mode and ≈30 W in the TE_{16} mode. These modes are converted into a Gaussian beam using a Vlasov antenna for transmission by the microwave optics to the DNP NMR probe. The power level is sufficient to overcome losses in the transmission system and saturate electron spins for sensitivity enhancement of NMR.

Solid-State NMR of Magnetic Systems and Nuclear Polarization by Use of Conventional CW-ESR Equipment and Micro-coil NMR

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Stable nitroxide radicals are useful to construct molecular magnetic systems. Particularly, radicals substituted by -COOH and -CONH2 can be coordinated to magnetic metal ions and may be used as cladding reagents of gold nano-particles for modifying magnetism. Nitroxide molecules with unsaturated five-member ring have almost planner structure and electron spin delocalization can be expected. We determined the hyperfine coupling constants (hfcc) of 1H, 13C and 15N of a series of nitroxide radicals, 1-pyrrolidinyloxy,3-carboxy-2,2,5,5-tetramethy , 2,2,5,5-tetramethyl-3-pyrrolin-pyrrol-1-oxy-3-carboxylic acid, pyrrol-1-yloxy,3-(aminocarbonyl)-2,5-dihydro-2,2,5,5-tetramethyl,
2,2,5,5-tetramethyl-3-imidazoline-3-oxide-4-carboxylic acid. Experimental values of hfcc were compared with those deduced from calculations based on density functional theory.
We are trying to characterize the dynamic properties of a giant vesicle and a helical tube composed of surfactants with nuclear polarization and micro coil NMR technique by use of organic radicals described above and others.
The magnetic behavior of nano particle of antiferromagnet ND4MnF3 with 30nm diameter is compared with that of bulk. To elucidate the low temperature property of a magnetic nano-particle, we characterized bulk sample of ND4MnF3, nano-particle of ND4MnF3 with ca. 30 nm diameter, and magnetically diluted compound of ND4Mn0.5Zn0.5F3 in bulk with using powder X-ray diffractometry, SEM, TEM, SQUID magnetometry, and particularly with solid-state deuterium NMR. The magnetic properties of these materials are discussed from internal magnetic field determined by deuterium NMR spectra in the antiferromagnetic phase. It was found that critical behavior of the nano-particles with approaching the Neel temperature is clearly different from the bulk sample, while internal magnetic field of the nano-particles in the low temperature limit is almost the same as that of bulk sample. Near surface spins of nano-particles in the antiferromagnetic phase were distinguished from the inner spins by wide-line deuterium NMR spectrum.

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^{1}H and ^{13}C Low Field Dynamic Nuclear Polarization for High Resolution NMR in Liquid Solutions

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Dynamic nuclear polarization (DNP) is a powerful tool to enhance the sensitivity of NMR by transferring the larger electron spin polarization to nuclei of interest. In liquid state, DNP is governed by the Overhauser mechanism which rapidly loses efficiency with increasing magnetic field. One possibility to exploit DNP for high resolution NMR in solution is to polarize the sample at low field and subsequently shuttle it into a high field magnet for NMR detection. To test this concept a prototype of a liquid state shuttle DNP spectrometer (0.34 T / 14 T) has been constructed.[1]

In order to investigate the mechanism of polarization transfer in aqueous solution and to further optimize the pumping conditions for the shuttle spectrometer a separate low field DNP spectrometer (0.34 T / 15 MHz ^{1}H detection / 9.6 GHz electron pumping) has been set up additionally.[2,3] It is based on a Bruker ELEXSYS X-band EPR spectrometer and a Bruker minispec coupled to an ENDOR cavity. An option for nitrogen gas cooling of the sample has been implemented. With this setup large ^{1}H signal enhancements up to $\varepsilon(0.34T) = / = -170$ on water samples containing ^{15}N-^{2}H-TEMPONE as polarizer have been achieved. Saturation studies have been performed and Overhauser parameters have been evaluated.

With the prototype of a shuttle DNP spectrometer ^{1}H water enhancements up to $\varepsilon(14T) = -2.6$ have been observed which translates into $\varepsilon(0.34T) = (14T/0.34T)\ \varepsilon(14T) = -110$ at 0.34 T. The discrepancy to the low field setup is presumably due to differences in instrumentation and relaxation losses during sample transfer. ^{13}C shuttle DNP experiments with the radical dissolved in ^{13}C labelled chloroform and tetrachloromethane yield $\varepsilon(14T) = +15$ and $\varepsilon(14T) = +10$, respectively. As a first test towards DNP on biological samples the ^{13}C enhancement on urea dissolved in aqueous solution alongside the radical was measured to be -4 ± 1. Currently, a dual center magnet is being constructed to minimize relaxation losses on the pathway and thus allow high field NMR enhancements on aqueous protein samples.

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Fast 2D Spectroscopy Experiments Using Multiple RF Coil And Receivers

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Gradient-assisted single scan 2D spectroscopy is well suited to provide information about intramolecular connectivity, especially when used in conjunction with hyperpolarisation techniques such as dynamic nuclear polarisation [1]. One possibility to extend this spectroscopy technique so it can also provide dynamical information about the sample, involves the acquisition of a set of 2D spectra from various locations in the sample.

This strategy is particularly useful for samples with high non-thermal polarisation since the relaxation of the sample to thermal equilibrium after the first fast 2D experiment would otherwise require time intensive repolarisation before a new experiment can be started.

To test this idea a NMR probehead with two saddle radio-frequency coils tuned to the 1H frequency of 400 MHz was built. [A.v.d.Drift, R.Panek, W.Koeckenberger, Poster 446, Euromar 2008]

Fast 2D COSY spectra of Ethanol from two positions of the sample were successfully acquired using a BRUKER AVIII spectrometer console with a dual receive and transmit setup.

A weakness identified in this method was the difficulty of achieving good shimming and hence narrow lineshapes for both of the coils at the same time. Thus a strategy for automated simultaneous shimming was developed and implemented.

It is based on acquiring B_0 field maps of the two coils and calculating the required shim currents by multiplying the pseudoinversion of a matrix describing the shim fields with the field required to minimise the deviation from the homogeneous field. [2]

A very good agreement between the predicted and the obtained shimmed fieldmaps has been achieved. The lineshape and signal intensity could be improved. This makes it possible to extend the multicoil acquisition strategy to samples where high resolution is needed.

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Microsecond kinetics of light-induced DNP and its application to peptides and proteins

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The application of light induced Chemically Induced Dynamic Nuclear Polarization (CIDNP) to the study of proteins allows for the determination of their structural and dynamic properties. CIDNP manifests itself in the anomalous intensities (enhanced absorption or emission) of signals in the NMR spectra of products of the termination of radicals formed via reaction of reversible electron (hydrogen) transfer between a triplet excited dye and CIDNP-active amino acid residues in a protein. Tryptophan, tyrosine, histidine, and methionine give rise to a significant hyperpolarization. CIDNP generated in the spin-selective decay of the transient radicals provides information about magnetic resonance parameters of elusive radicals allowing for unambiguous assignment of hyperfine coupling constants to particular nuclei in radicals. The time-resolved version of the CIDNP technique utilizes the structural information available from NMR spectroscopy for monitoring the chemical and structural changes associated with particular atoms of proteins during fast radical reactions on a microsecond time-scale. The protein ubiquitin was chosen as a test system forming the partially folded A-state and two types of unfolded states with a single CIDNP-active residue, tyrosine. For these three non-native states and the native state, the correlation times of intramolecular mobility were determined under assumption of isotropic motion.One of the processes that may be important in oxidized proteins is intramolecular electron migration involving tyrosine, tryptophan and histidine residues. The radicals of His, Trp and Tyr in different dipeptides were generated in the reaction of hydrogen atom transfer to the photoexcited triplet dye. The CIDNP kinetics is very much sensitive to the rates of radical reactions, and proved to be a versatile tool in establishing the mechanism and determining the rates of intra- and intermolecular electron transfer reactions.

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