Dynamic Nuclear Polarization
with
Millimeter Waves and Biradicals
(Why two electrons are better than one!)

Solid State NMR Winter School
Outline

- Background and Rationale
  DNP, EPR, Signal to Noise and bR

- Instrumentation for DNP
  Quadruple Resonance, LT MAS Probes
  Gyrotron Microwave Sources

- DNP Enhanced MAS Spectra
  DNP Enhancements of 50-60 in MAS Spectra @ 90 K
  \( \text{DNP functions quite effectively in broad class of systems} \)

- Polarizing Agents and DNP Mechanisms
  Biradical polarizing agents \( \Rightarrow \varepsilon = 170-340 \)
  Solid effect and cross effect DNP mechanisms

- DNP in Solution (and for Metabonomics)
  Solid state polarization and laser T-jump
  \( \varepsilon^+ = \varepsilon \left( \frac{T_{\text{obs}}}{T_{\text{polar}}} \right) = 130-330 \) for \(^{13}\text{C} \)
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  Biradical polarizing agents ⇒ ε = 170-340
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  Solid state polarization and laser T-jump
  \[ ε^+ = ε \left( \frac{T_{obs}}{T_{polar}} \right) = 130-330 \text{ for } ^{13}\text{C} \]
References -- a disclaimer
This is a partial list of references intended as an introduction to the field. The intent
is to provide a point of departure for the new student. It is lacking many important papers and reviews.

In the solid state NMR community, there was some DNP research in the 1980’s by Wind, Yannoni and
Schaefer, but by the beginning of the 1990’s that had largely ceased. At that point in time,
the MIT group initiated gyrotron based experiments in the millimeter wave regime. Thus, the peculiar
distribution of publications.

There remains a large group of nuclear and particle physicists who are working on polarized targets
using DNP. And the list of groups considering DNP for imaging experiments is expanding rapidly

Original Theory and Experiments


Reviews of DNP


V.A. Atsarkin, “Dynamic polarization of nuclei in solid dielectrics” Sov. Phys. Usp. 21, 725 (1978)

R.A. Wind, M.J. Duijvestijn, C. Vanderlugt, A. Manenschijn, J. Vriend,
“Applications of Dynamic Nuclear-Polarization in C-13 NMR in Solids”

T. Maly, G. T. Debelouchina, V. S. Bajaj, K-N. Hu, C-G. Joo, M. L. Mak-Jurkauskas, J. R. Sirigiri,
P. C. A. van der Wel, J. Herzfeld, R. J. Temkin and R. G. Griffin, “Dynamic Nuclear Polarization at High

**Recent Applications to Polymers**
M. Afeworki, R.A. McKay, J. Schaefer

Afeworki, M.; Vega, S.; Schaefer, J.

**Publications from the Griffin Group @ MIT**

L. R. Becerra, G. J. Gerfen, R. J. Temkin, D. J. Singel, and R. G. Griffin,

G. J. Gerfen, L.R. Becerra, D.A. Hall, D. J. Singel, and R. G. Griffin,

D. Hall, D. C. Maus, G. Gerfen, S. Inati, L. R. Becerra, F.W. Dahlquist, and R.G. Griffin,

V. Weis, M. Bennati, M. Rosay, J.A. Bryant, and R.G. Griffin,


C.T. Farrar, D.A. Hall, G.J. Gerfen, S.J. Inati and R.G. Griffin,

M. Rosay, A. Zeri, N.S. Astrof, S.J. Opella, J. Herzfeld, and R.G. Griffin,
References -continued


M. Rosay, J. Lansing, K. C. Haddad, W. W. Bachovchin, J. Herzfeld, R.J. Temkin, and R. G. Griffin


References -continued


Instrumentation for DNP

"A Spectrometer for EPR, DNP, and Multinuclear High-Resolution NMR"


P.W. Woskov, V.S. Bajaj, M.K. Hornstein, R.J. Temkin, and R.G. Griffin


References (continued)

Pulsed DNP

Integrated Solid effect

NOVEL

RF-DNP


V. Weis, M. Bennati, M. Rosay, and R.G. Griffin,
“Solid Effect in the Electron Spin Dressed State – A New Approach for Dynamic Nuclear Polarization,”

V. Weis and R.G. Griffin
“Electron-nuclear cross polarization”

Liquid state DNP experiments

N. M. Loening, M. Rosay, V. Weis, and Robert G. Griffin,
“Solution State Dynamic Nuclear Polarization at High Magnetic Fields”

1950’s -- **Overhauser Experiments in Metals** *(0.00303 T, 84 MHz)*
- A. Overhauser, Phys Rev (1953);
- T. Carver and C.P. Slichter, Phys Rev (1953, 1956) $^7\text{Li}$, $^{23}\text{Na}$, $^1\text{H}$ in $\text{NH}_3$

1960-1980 -- **Liquids and Solids** *(0.3300 T, ~10 GHz)*
- Liquids -- Hauser, Mueller-Warmuth, Richards, and others
- Solids -- Abragam, Goldman, Provotorov, and others
- *Nuclear magnetic ordering, polarized targets for particle physics, liquids at low fields*

1980’s -- **MAS Experiments on Solids** *(1.5 T, 40 GHz)*
- R.A. Wind, C.S. Yannoni, J. Schaefer
- *Polymers, carbonaceous materials, diamond, etc.*

1990 -- **Current** *(5-17 T, 140-460 GHz)*
- *Gyrotron* based high field experiments@ MIT
- J. Schmidt, T. Wenckebach, K. Golman, Jan-Henrik Ardenjkaer-Larsen
- *Amyloid and membrane peptides and proteins, biological samples, liquids*

Melissa Hornstein and her 460 GHz gyrotron oscillator
Polarization of Nuclear Spins in Metals*

T. R. Carver† and C. P. Slichter

Department of Physics, University of Illinois, Urbana, Illinois

(Received August 17, 1953)

\[ ^{7}\text{Li} \text{ w/o DNP} \]
\[ ^{7}\text{Li} \text{ w/ DNP} \]
\[ @ 84 \text{ MHz} \]
\[ ^{1}\text{H} \text{ glycerol} \]

- \(^{7}\text{Li} \text{ NMR} @ \omega_{0}/2\pi = 50 \text{ kHz} (30.3 \text{ Gauss})\)
- \(EPR @ \omega_{0}/2\pi = 84 \text{ MHz}\)

\[ \varepsilon \sim 100 \]

- Initial demonstration of the Overhauser effect -- DNP
- Nuclear Overhauser effect is \textit{important} in solution NMR!

\textit{Carver and Slichter, Phys. Rev. 92, 212-213 (1953)}
\textit{Phys. Rev. 102, 975-980 (1956)}
DNP - brief history

1950's -- Overhauser Experiments in Metals (0.00303 T, 84 MHz)
-- A. Overhauser, Phys Rev (1953); T. Carver and C.P. Slichter, Phys Rev (1953, 1956) $^7$Li, $^{23}$Na, $^1$H in NH$_3$

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Polymers, carbonaceous materials, diamond, etc.

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Gyrotron based high field experiments@ MIT
Amyloid and membrane peptides and proteins, biological samples, liquids

Melissa Hornstein and her 460 GHz gyrotron oscillator
### Sensitivity Enhancement in NMR

<table>
<thead>
<tr>
<th>Technique</th>
<th>Authors</th>
<th>Enhancement (for $^{15}$N)</th>
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<tr>
<td>Fourier Transform NMR</td>
<td>Ernst and Anderson</td>
<td>~10-100</td>
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<td>Polarization Transfer CP and INEPT</td>
<td>Hartmann &amp; Hahn; Pines, Gibby &amp; Waugh; Morris &amp; Freeman</td>
<td>10</td>
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<td>Indirect Detection (HSQC)</td>
<td>Bodenhausen and Ruben</td>
<td>~30</td>
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<td>$B_0$ --200 to 800 MHz</td>
<td>Oxford, Bruker, Magnex</td>
<td>8</td>
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<tr>
<td>Cryoprobes</td>
<td>Peter Styles</td>
<td>2-4</td>
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<tr>
<td>TROSY</td>
<td>Pervushin, et.al.</td>
<td>2-5</td>
</tr>
<tr>
<td><strong>High Frequency</strong></td>
<td><strong>Becerra, Gerfen, Prisner, McDermott, Un, Hall, Farrar, Rosay, Weis, Bennati, Hu, Bajaj, and RG²</strong></td>
<td><strong>~ 50-400</strong></td>
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\[ \mathcal{E} = 50-400 \]

**Significant consequences for NMR -- savings of \(~ 2500 - 160,000\) in time -- NEW SCIENCE !**
What are the THREE most important parameters in magnetic resonance

- Signal-to-noise
- Signal-to-noise
- Signal-to-noise
Nuclear Spin Polarization
Temperature and Field Dependence

Polarization (%)

Temperature (K)

- **Current strategy** -- increase the polarization by increasing $B_0$!

- **Result** -- “modest” increases in sensitivity and resolution!
  Increases in magnet cost are non-linear!
Electron and Nuclear Polarization
Temperature and Field Dependence

4% $e^-$ polarization
@ 700 MHz / 90 K

0.006% $^1H$ polarization
@ 700 MHz / 90 K

POLARIZATION

\[ P = \frac{n^+ - n^-}{n^+ + n^-} = \tanh \left( \frac{\gamma \hbar B_0}{2kT} \right) \]

\[ P = \frac{\gamma \hbar B_0}{2kT} \left( \frac{\gamma_e}{\gamma_{1H}} \right) \sim 660 \]

• Much larger spin polarization is present in the electron spin reservoir
• Transfer the electron polarization to the nuclear spins by irradiating the electrons with *high frequency microwaves*!
An Electron Speaks to Some Nuclear

How do we make electrons talk to nuclear spins?

With ENDOR, ESSEM and DNP

- Bulk nuclear spins
  - DNP
    - Overhauser, '55
    - Carver and Slichter, '55

- Hyperfine coupled spins
  - ENDOR, ESEEM
    - Feher, '56
    - Mims '61, '63, Davies '74

\( \frac{\gamma_e}{\gamma_n} \) \approx 1000
• $^1\text{H}$'s adjacent to the Yb$^{3+}$ are shifted away from the bulk resonance due to coupling with the electron -- strongly and weakly coupled
• $T_{1e}$ is long (~1 s) and therefore hyperfine shifted resonances are observed at $\omega_0 \pm \omega_{hf}$

$^1\text{H}$ resonance $\Rightarrow$ DNP  
Hyperfine shifted $\Rightarrow$ ENDOR

J.P. Wolfe, Phys. Rev. 16, 128(1977)
Transfer the Electron Polarization to

- Increase the sensitivity of NMR by transferring the LARGE POLARIZATION of the electrons to the nuclear spins

DNP
Overhauser, ‘55
Carver and Slichter, ‘55
Bulk nuclear spins

ENDOR, ESEEM
Feher, ‘56
Mims ‘61, ‘63, Davies ‘74
Hyperfine coupled spins

- Increase the sensitivity of NMR by transferring the LARGE POLARIZATION of the electrons to the nuclear spins
Sample Preparation

- 4 amino-TEMPO is soluble in water and stable
- Cryoprotection is critical to minimize inhomogeneous broadening
- Polarization diffuses throughout the macromolecule

Diagram:
- Purple membrane
- Cryoprotected sample
- Bacteriorhodopsin
- Cryoprotectant (e.g. glycerol)
- α-lytic protease

Steps:
1. Resuspension
2. Centrifugation
Dynamic Nuclear Polarization
Solid State Effect

- Irradiate the flip-flop transitions

\[ D_{\text{n.s.}} \frac{|q|}{\omega_n} \frac{e^{-n}}{D_{\text{e-n}}} \]

\[ \text{Enhancement} \sim \left( \frac{\gamma_e}{\gamma_n} \right) \left( \frac{\omega_1}{\omega_0} \right)^2 \left( \frac{N_e}{\delta} \right) T_{1n} \]
DNP Enhanced $^{13}$C MAS Spectra

**TOTAPOL/^{13}C-Urea**

- **Distance** between the two TEMPO radicals in TOTAPOL yields an $e^{-}-e^{-}$ coupling of $\sim 25$ MHz
- Electron concentration $\sim 10 \text{ mM}$
- Enhancements build up over $\sim 40$ seconds to a maximum of $290 \pm 30$

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Joo, Hu, Bryant and Griffin (2006)

$T = 90 \text{ K}$

$\nu_r = 8 \text{ kHz}$

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No DNP x 10

[Graph showing $^{13}$C Chemical shift (ppm) over Microwave irradiation (s)]
DNP Enhanced $^{15}$N MAS Spectra of U-$^{13}$C,$^{15}$N-bR

- DNP enhancements permit observation of high S/N in short acquisition periods
- bR is a membrane protein MW~32 kD (protein + lipid)
- Schiff base $^{15}$N well resolved from the other $^{15}$N signals

Bajaj, Mak, Belenky, Herzfeld (2008)
DNP Enhanced Selective 3D $^{15}\text{N}-^{13}\text{C}-^{13}\text{C}$ Experiment

- CW irradiation with 250 GHz microwaves (stability <1%)
- Select the Schiff base $^{15}\text{N}$ resonance with a Gaussian
- Transfer to the $^{13}\text{C}$ with SPECIFIC CP and RFDR
Assignment of Nζ-C15-Cx and Nζ-Cε-Cx

• SPECIFIC-CP transfer from Schiff base $^{15}$N to the ....
  • Lys-216 sidechain $^{13}$C’s
  • retinal polyene chain $^{13}$C’s
• DNP enhancement of single $^{13}$C’s in a uniformly labeled membrane protein in short (< 1 hour) periods of time.

Bajaj, Mak, Belenky, Herzfeld (2007)
Bacteriorhodopsin is a heterogeneous protein.

Low temperatures quench dynamics

Bajaj, Mak, Herzfeld, Griffin (2007)
Bacteriorhodopsin Trimer

- Are the three members of the trimer equivalent?

- Sheves et al. Biochem 42, 11281(2003) suggest they are not!

- $^{13}$C-$^{15}$N spectra of bR$_{555}$ indicates that the trimer is heterogeneous!
Heterogeneity in K iso-K transient

- Iso-K intermediate observed with non-linear sampling - decays over a period of hours
- K-shifted upfield from bR$_{568}$
Heterogeneity in L - the predischarge state

- Four L different L cross peaks are observed
- L distribution similar whether produced from K or by 640 nm (red) illumination of LA
Three QM/MM pathways all equally consistent with kinetics*

(1) Direct transfer:
SB-Ret → D85

(2) Indirect transfer
SB-Ret → T89 → D85

(3) Indirect transfer
SB-Ret → D212 → W402 → D85

* Bondar, et al Structure 12, 1281 (2004); simulations-- http://www.iwr.uni-heidelberg.de/groups/biocomp/fischer
Requirements for High Field DNP Experiments

- Low Temperature Multiple Resonance NMR Probes
  *Typically four frequencies --$^1$H, $^{13}$C, $^{15}$N and electrons -- performing MAS at < 90 K*

- Millimeter wave microwave sources
  *10-100 watts in the 100-600 GHz regime  
  -- gyrotrons (cyclotron resonance masers)*

- Polarizing agents that are widely applicable and stable
  *TEMPO (nitroxides), Biradicals, metal ions*
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  Biradical polarizing agents \( \Rightarrow \varepsilon = 175 \)
  Solid effect and cross effect DNP mechanisms

• DNP in Solution (and for Metabonomics)
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  \( \varepsilon^+ = \varepsilon \left( \frac{T_{obs}}{T_{polar}} \right) = 130-330 \) for \( ^{13}\text{C} \)
High Frequency DNP Spectrometer

Three basic components

• Microwave source — Gyrotron oscillator

• Transmission line — Corrugated waveguide

• NMR probe w/ waveguide
  Schaefer/McKay transmission line probe

Bajaj, Kreischer, Woskow, Temkin
High Frequency DNP Spectrometer

Three basic components

• Microwave source — Gyrotron oscillator

• Transmission line — Corrugated waveguide

• NMR probe w/ waveguide Schaefer/McKay transmission line probe

Bajaj, Kreischer, Woskow, Temkin
• Gyrotrons provide 10-100 watts of CW power (100 hours of operation)

• *Continuously* frequency coverage in the 100-1000 GHz range

• *Long lifetimes* -- no slow wave structures

• EIO/EIA’s and diodes all have disadvantages for DNP -- short lifetimes and low output power

• Terahertz lasers are not tunable and the CW output power is low
• Superconducting magnet provides the external magnetic field

• Electron gun emits electrons

• High voltage accelerates the electrons through the magnetic field

• μwaves are generated in the cavity region and brought out through the cross bore
250/460 GHz Gyrotron

A: Electron emission from an annular ring

B: Bunching in the cavity and emission of microwaves

C: Quasi optic coupling of the microwaves out to the sample. Electrons continue to the collector.

D: Electrons are collected in the collector
A: Electron emission from an annular ring

B: Bunching in the cavity and emission of microwaves

C: Quasi optic coupling of the microwaves out to the sample. Electrons continue to the collector.

D: Electrons are collected in the collector
460 GHz/ 700 MHz Gyrotron Oscillator

460 GHz Gyrotron Operation

- Time stability -- several hours of CW operation
- Record high powers -- \( \leq 8 \text{ watts} @ 460 \text{ GHz} \)

8 watts CW power

stable CW operation

Melissa Hornstein and her 460 GHz gyrotron oscillator
Power Dependence of Thermal Mixing DNP

• Enhancements of 10-30 can be achieved with a low power Gunn or Impatt diode at these temperatures -- 30 to 100 mW.

• Larger enhancements and faster rates of polarization build up can be observed with a gyrotron source -- 10-100 watts.
Thermal Mixing/Cross Effect DNP

High frequency (140 GHz) dynamic nuclear polarization: Polarization transfer to a solute in frozen aqueous solution

G. J. Gerfen, L. R. Becerra, D. A. Hall, and R. G. Griffin
Francis Bitter National Magnet Laboratory and Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139
R. J. Temkin
Plasma Fusion Center, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139
D. J. Singel
Department of Chemistry, Montana State University, Bozeman, Montana 59717
(Received 16 February 1995; accepted 20 March 1995)


• DNP in aqueous media — biological samples
• Three spin mechanism applicable at high fields
Microwave Resonators

Solid state source with microwave resonant cavity

- TE\textsubscript{011} resonator with 30 mW Impatt diode source
  \[ V = 0.5 \mu l \]

- Rotor dimensions are comparable to \( \lambda \) -
  \[ V \sim 40-60 \mu l \]

- MAS sample size complicates design of high-Q cavity
- Smaller volume decreases S/N by \sim 200-300 \}

\[ \lambda \]

4mm rotor
- - - - \( \rightarrow \) 95 GHz
- - - - \( \rightarrow \) 140 GHz
- - - - \( \rightarrow \) 250 GHz
- - - - \( \rightarrow \) 460 GHz
Corrugated Waveguide

- Wall thickness 300 microns
- \( \lambda/4 \) Corrugation depth
- 1.3 m

- Very low insertion loss (0.01dB/m)
- Cryogenic Operation
- Excellent mode and polarization characteristics
Quadruple Resonance DNP/MAS Probe w/ Optical Irradiation of the Sample

- Quadruple resonance -- $^1\text{H}$, $^{13}\text{C}$, $^{15}\text{N}$, and e$^-$
- *Routine* low temperature spinning at 85-90 K, $\omega/2\pi \sim 10$ kHz
- Optical irradiation (532 nm) of samples to generate photochemical intermediates

Vik Bajaj, Jeff Bryant
Challenges for cryogenic sample exchange:

- Magic angle adjustment
- Limited space
- Seals at low temperature
- Physical restrictions under the magnet
- Prevent damage to rotor

*Alexander Barnes*
140 GHz Amplifier - Pulsed DNP & EPR

- Provides phase shifted pulses @ 140 GHz for *pulsed DNP and EPR*
  - The gyrotron approach can be applied to higher frequencies such as 250 GHz and 460 GHz.

- The physics challenges are:
  - High gain
  - High efficiency
  - Wide bandwidth
  - Short pulse operation
  - Low electron beam voltage and current
  - Novel overmoded circuit.

Sketch of experimental tube in magnet.
140 GHz Gyro-Amplifier for Pulsed DNP

- 140 GHz Gyro-amplifier and MIT Grad Student Colin Joye
- First operation Feb., 2007
- Currently generating 400 watts
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**CW Dynamic Nuclear Polarization**

**Mechanisms**

**Overhauser Effect (OE)** -- applicable to systems with mobile electrons -- i.e., metals, liquids, 1D conductors (*discussed later wrt liquid state DNP*)

**Solid Effect (SE)** -- insulating solids (organic, biological systems) when ....

\[ \delta \sim \Delta \ll \omega \]

\(\delta\) = homogeneous linewidth of the EPR spectrum
\(\Delta\) = breadth of the EPR spectrum
\(\omega\) = nuclear Larmor frequency \(\left( {^{1}H, ^{13}C, ^{15}N} \right)\)
**CW Dynamic Nuclear Polarization Mechanisms**

*Thermal Mixing (TM)* -- insulating solids, but ....

\[ \delta, \Delta \gg \omega \]

*TM* -- dominates when the g anisotropy is small, and/or the EPR line is *homogeneously* broadened, and \( \omega \) is small.

*Cross Effect (CE)* -- insulating solids, but ....

\[ \Delta > \omega > \delta \]

*CE* -- operative at high fields where \( \Delta g \gg \delta \), the line is *inhomogeneously* broadened.
Time Domain DNP Mechanisms

**Pulsed DNP** -- *in progress* [Weis and Griffin, SSNMR 29 105-117 (2006)] mostly at 9 GHz (0.3 T).

*Integrated Solid Effect* -- Wenckebach (CPL, 1988)
\[ \pi \text{ -- CW on the electrons} \]

*NOVEL* -- Wenckebach (CPL, 1988)
rotating frame / lab frame
\[ \omega_{1e} = \omega_0 \]

*RF DNP* -- Wind and Co. (AMR, 1985)

*High frequency microwave amplifiers are just becoming available.*
**CW Dynamic Nuclear Polarization Mechanisms**

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**Solid Effect (SE)** -- insulating solids (organic, biological systems) when ....

\[ \delta \sim \Delta \ll \omega \]

\( \delta = \) homogeneous linewidth of the EPR spectrum
\( \Delta = \) breadth of the EPR spectrum
\( \omega = \) nuclear Larmor frequency (\( ^1\text{H} \), \( ^{13}\text{C} \), \( ^{15}\text{N} \))
Paramagnetic Centers for DNP

- EPR lineshapes are dominated by $g$-anisotropy
- BDPA linewidth ~21 MHz
  ---- *Solid effect*
- TEMPO powder pattern
  ~600 MHz
  ---- *Thermal mixing or cross effect*

$\omega/2\pi = 28$ GHz/T
Dynamic Nuclear Polarization
Solid State Effect

- Irradiate the flip-flop transitions

Equilibrium
Positive
Enhancement
No
Enhancement
Negative
Enhancement

Enhancement $\sim \left( \frac{\gamma_e}{\gamma_n} \right) \left( \frac{\omega_1}{\omega_0} \right)^2 \left( \frac{N_e}{\delta} \right) T_{1n}$
DNP with Gyrotrons

Dynamic Nuclear Polarization with a Cyclotron Resonance Maser at 5 T

Lino R. Becerra,1 Gary J. Gerfen,1 Richard J. Temkin,2 David J. Singel,3 and Robert G. Griffin4
1Francis Bitter National Magnet Laboratory and Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139
2Plasma Fusion Center, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139
3Department of Chemistry, Harvard University, Cambridge, Massachusetts 02138
(Received 26 July 1993)

DNP (dynamic nuclear polarization) experiments at 5 T are reported, in which a cyclotron resonance maser (gyrotron) is utilized as a 20 W, 140 GHz microwave source to perform the polarization. MAS (magic angle spinning) NMR spectroscopy with DNP has been performed on samples of polystyrene doped with the free radical BDPA (a,γ-bis diphenylene-β-phenylallyl) at room temperature. Maximal DNP enhancements of ~10 for 1H and ~40 for 13C are observed and are considerably larger than expected. The DNP and spin relaxation mechanisms that lead to these enhancements at 5 T are discussed.
Trityl Radical
Structure and FT EPR Spectrum

- Small g-anisotropy yields a solid effect enhancement mechanism
Solid Effect with Trityl Radical

- Soluble in aqueous media

- Frequency dependence shows a well resolved solid effect

- Peaks in the enhancement curves at $\omega_{e} \pm \omega_{n}$

$$\delta_{e} < \omega_{n}$$

90 MHz < 211 MHz

- Enhancements are significant but modest only $\pm 15$!

**CW Dynamic Nuclear Polarization Mechanisms**

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\[ \delta, \Delta \gg \omega \]

**TM** -- dominates when the \( g \) anisotropy is small, and/or the EPR line is *homogeneously* broadened, and \( \omega \) is small.

**Cross Effect (CE)** -- insulating solids, but 

\[ \Delta > \omega > \delta \]

**CE** -- operative at high fields where \( \Delta g \gg \delta \), the line is *inhomogeneously* broadened.
- EPR lineshapes are dominated by \(g\)-anisotropy
- BDPA linewidth \(~21\) MHz
  ---- Solid effect
- TEMPO powder pattern
  \(~600\) MHz
  ---- Thermal mixing or cross effect
Cross Effect DNP mit TEMPO

- Note different field profiles for the solid and cross effects
- Smaller enhancements (~± 15) obtained with the solid effect
- Larger enhancements (~± 45) observed with the cross effect and monomeric TEMPO
1. **μwave irradiation** and e-e cross-relaxation burn a hole in the EPR line.

2. **Two electrons** separated by $\omega_n$ flip-flop and the difference in energy is used to flip a nucleus.

$$ (\tau_{een})^{-1} = 4 |g|^2 \frac{1}{T_{2e}^{SS}} \int_{-\infty}^{\infty} g(\omega)g(\omega - \omega_n) g(0) $$

3. The EPR intensity is given by:

$$ \omega_{2e} - \omega_{1e} = \omega_n $$

4. **Enhancement**

$$ \varepsilon \sim \frac{\gamma_e}{\gamma_N} $$

657 for $^1H$

2615 for $^{13}C$
• NO

• EPR spectrum is ~600 MHz wide

• Thermal mixing and the cross effect are …

\textit{three spin process}

involving the irradiation of a dipolar coupled electron spin system

• Flip two electrons and then a nuclear spin.

C.F. Hwang and D.A. Hill, PRL18, 110-112 (1967)
• \( \omega / 2\pi = 4.6 \text{ kHz}, \ RD = 4.8, \ 32 \) shots, 1 watt of 140 GHz at the top of the probe

• Significant enhancements are possible at LN\(_2\) temperatures where MAS is relatively straightforward

Rosay, Weis, Kreischer, Temkin and Griffin *JACS* (2002)
DNP Enhanced $^{13}\text{C}$-MAS Spectra

U-$^{13}\text{C}$, $^{15}\text{N}$-Proline

- $\omega_r/2\pi = 4.6$ kHz, RD = 4.8, 32 shots, 1 watt of 140 GHz

- Significant enhancements are possible at LN$_2$ temperatures where MAS is straightforward

40 mM 4-amino TEMPO

\[ \text{H}_2\text{N}-\begin{array}{c} \text{N} \\ \text{O} \end{array} \]

2.4% efficient

Rosay, Weis, Kreischer, Temkin and Griffin JACS (2002)
DNP Enhanced $^{15}$N MAS Spectra

$\varepsilon$-$^{15}$N -Lys Labeled bR

Sans DNP, 317 MHz
5 mm rotor, 160 µl
25,856 scans, 14.4 hours

DNP, 211 MHz
4 mm rotor, 40 µl, T=90K
1280 scans, 1 hour

$\varepsilon = 55$

M. Rosay, et. al. JACS (2003)
**CW Dynamic Nuclear Polarization Mechanisms**

**Thermal Mixing (TM)** -- insulating solids, but ….

\[ \delta, \Delta \gg \omega \]

*TM* -- dominates when the *g* anisotropy is small, and/or the EPR line is *homogeneously* broadened, and *\omega* is small

**Cross Effect (CE)** -- insulating solids, but ….

\[ \Delta > \omega > \delta \]

*CE* -- operative at high fields where *\Delta g* \( \gg \delta \), the line is *inhomogeneously* broadened.
Biradical Polarizing Agents

Bis TEMPO \( n \)-ethylene glycol

\[ (BTnE) \]

- Biradical consists of two stable radicals (TEMPO) tethered together by a linker (ethylene glycol).
- Used extensively in the 70’s for the study of dynamics with EPR.
- Currently employed in pulsed EPR investigations of distances in a variety of systems.
Inter-Electron Distances in BTnE CW EPR Lineshapes

Simulation of 9 and 140 GHz EPR spectra
\( ^{15}\text{N}, {^2}\text{H}-\text{labeled} \)

- Relative g-tensor orientations permitted to vary.
- \( R_{e-e} \) determined by regression.
- Distance in 40 mM TEMPO \( \sim 35 \text{ Å} \)

DNP enhancement vs. \( (R_{e-e})^{-3} \)

- Decreasing \( R_{e-e} \) increases e-e- dipole coupling & \(^1\text{H} \) enhancement

45 \( \Rightarrow \) 175!
**Distance** between the two TEMPO radicals in BT2E yields an e⁻-e⁻ coupling of ~25 MHz.

- Electron concentration -- **40 mM to 10 mM**!
- Enhancements build up over ~15 seconds and the maximum appears to be ~ **175**!
• Optimal enhancements of $\pm 175$ are observed with biradicals -- BT2E at a factor of four lower electron concentration!
Solid and Cross Effect DNP

- Smallest enhancements (≈± 15) obtained with the solid effect
- Optimal enhancements of ± 175 are observed with biradicals -- BT2E at a factor of four lower electron concentration!
DNP Enhanced $^{13}$C MAS Spectra

TOTAPOL/$^{13}$C-Urea

- **Distance** between the two TEMPO radicals in TOTAPOL yields an e$^-$-e$^-$ coupling of ~25 MHz
- Electron concentration $\sim 10 \text{ mM}$
- Enhancements build up over ~40 seconds to a maximum of $290 \pm 30$!

Joo, Hu, Bryant and Griffin (2006)
Can we improve on the enhancements obtained with monomeric TEMPO?

Thermal mixing and the cross effect are three spin processes involving the irradiation of a coupled electron spin system -- flip two electrons and then a nuclear spin.

TM and the CE are inherently inefficient since only a fraction of the spins in a powder have the correct distance and relative orientations to contribute to DNP!

\[ \omega_{2e} - \omega_{1e} = \omega_n \]

C.F. Hwang and D.A. Hill, PRL18, 110-112 (1967)
• Correct relative orientations of the TEMPO molecules are required to yield two lines separated by

\[ \omega_{2e} - \omega_{1e} = \omega_n \]

efficient thermal mixing/cross effect polarization transfer

• Simulations suggest that the TEMPO molecules in BTnE’s are oriented at approximately 90° with respect to one another

• g-tensor orientations yield two lines separated by \(~ \omega_n/2\pi\)?
DNP Enhanced $^{13}$C MAS Spectra
BT2E$/^{13}$C-Urea

• **Distance** between the two TEMPO radicals in BT2E yields an e$^-$-e$^-$ coupling of ~25 MHz
• Electron concentration drops from 40 mM to 10 mM!
• Enhancements build up over ~15 seconds and the maximum appears to be ~ 175!
Paramagnetic Centers for DNP

- EPR lineshapes are dominated by \( g \)-anisotropy
- BDPA linewidth \( \sim 21 \) MHz ---- *Solid effect*
- TEMPO powder pattern \( \sim 600 \) MHz ---- *Thermal mixing or cross effect*
Dynamic Nuclear Polarization
Solid State Effect

$|q| \propto D_{n.s.}^{e-n} / \omega_n$

Enhancement $\sim \left( \frac{\gamma_e}{\gamma_n} \right) \left( \frac{\omega_1}{\omega_0} \right)^2 \left( \frac{N_e}{\delta} \right) T_{1n}$

Electron Zeeman Bath

Nuclear Zeeman Bath

Irradiate the flip-flop transitions

$W_{EPR}$

$W_{nmr}$

$|\pm\rangle + q^* |\mp\rangle$

Equilibrium

Positive Enhancement

No Enhancement

Negative Enhancement

$V_e - V_n$

$V_e$

$V_e + V_n$
Solid Effect with Trityl Radical

- Soluble in aqueous media
- Frequency dependence shows a *well resolved* solid effect
- Peaks in the enhancement curves at $\omega_c \pm \omega_n$

$$\delta_e < \omega_n$$

90 MHz < 211 MHz

- Enhancements are significant but modest only $\pm 15$!
Paramagnetic Centers for DNP

- EPR lineshapes are dominated by $g$-anisotropy
- BDPA linewidth $\sim 21$ MHz
  ---- *Solid effect*
- TEMPO powder pattern $\sim 600$ MHz
  ---- *Thermal mixing or cross effect*
Three-Spin Process in Thermal Mixing/Cross Effect DNP

If \( \omega_{e2} - \omega_{e1} \approx \omega_n \)

If \( \omega_{e2} - \omega_{e1} \approx \omega_n \)
degenerate

Kan Hu
PhD Thesis
2006

Equilibrium
\( \Delta \omega_e < \omega_n \)

Equilibrium
\( \Delta \omega_e > \omega_n \)

Equilibrium
\( \Delta \omega_e = \omega_n \)
1. **μwave irradiation** and e-e cross-relaxation burn a hole in the EPR line.

2. **Two electrons** separated by $\omega_n$ flip-flop and the difference in energy is used to flip a nucleus.

3. $\omega_{2e} - \omega_{1e} = \omega_n$

4. **Enhancement**

$$\varepsilon \sim \frac{\gamma e}{\gamma N}$$

- 657 for $^1H$
- 2615 for $^{13}C$

$$\varepsilon^{TM} \propto \left( \frac{B_1^2}{B_0} \right) T_{1e} T_{1n}$$

$$\left( \tau_{een} \right)^{-1} = 4 \int \frac{1}{T_{2e}^{SA}} \frac{\int_{\infty}^{\omega_n} g(\omega) g(\omega - \omega_n)}{g(0)}$$
DNP Enhancement at 250 GHz

$^{13}$C-Urea/Glycerol/D$_2$O

16 scans
RD = 12 s
$T=98K^*$

$\mu$wave off
$x \sim 100$

$\mu$wave on

$^{13}$C Chemical Shift (ppm)

- Cross Effect DNP enhancement scales as $\omega_0^{-1}$

Expected enhancement

$\mathcal{E}_{250} = \mathcal{E}_{140} \left( \frac{140}{250} \right)$

$170 \left( \frac{140}{250} \right) \approx 95$

$\mathcal{E} \sim 100$
DNP Enhancements from Biradicals

Rigid biradicals are constrained in unfavorable conformations -- EPR frequency separation!

BTOXA yields the smallest $\varepsilon$ -30!

Coaxial $g$-tensors!

- An optimal $^1$H $\varepsilon$ requires a strong $e^- - e^-$ dipolar coupling.
  - BTurea has shorter $R_{e-e}$, but no greater $\varepsilon$?

Incorrect tensor orientation?

- A flexible tether is important.
  - BT2E yields the largest $\varepsilon$–175!!

Short distance and correct $g$-tensor orientation!
• Rigidity of the tether of TOTAPOL improves DNP performance.
• Narrower distribution of $e^- - e^-$ distance, but no constraint of relative g-tensor orientation.
A longer tether reduces constraints on relative g-tensor orientation.
Rigid bis-TEMPO (Tordo/ Prisner) Biradical

$^{13}$C-Urea/DMSO/D$_2$O/H$_2$O

- Rigid biradical satisfying $\omega_{1e}-\omega_{2e} \sim \omega_n$ ! $e^-e^- \sim 30$ MHz !
- Preliminary result -- errors large !
- $\varepsilon \sim 340/660 \rightarrow 51\%$ efficient !

Thomas Prisner, Sevdalina Lyubenova - Frankfurt
Paul Tordo - Marseille

Yoh Matsuki, Thorsten Maly
Galia Deblouchina

MIT
Cross Effect in **TEMPO** / **Trityl** Mixtures
An Approximation to an Ideal Polarizing Agent

- Ideal polarizing agent --
  - tethered radicals
  - *small* *g*-anisotropies
  - $\omega_{1e} - \omega_{2e} \sim \omega_n$

• Notice that ....
  $g_{22(TEMPO)} - g(\text{trityl}) \approx 80 \text{ G} = 224 \text{ MHz}$ !

--- which is comparable to
  $\omega_{1H}/2\pi = 211 \text{ MHz}$ !

• **Demonstrates the importance of satisfying** $\omega_{1e} - \omega_{2e} \sim \omega_n$!
DNP in Trityl-TEMPO Mixtures
Approximation to an “Ideal Polarizing Agent”

- Notice that $g_{22}^{\text{TEMPO}} - g^{\text{trityl}} \approx 80 \text{ G} = 224 \text{ MHz}$!
- Demonstrates the importance of satisfying $\omega_{1e} - \omega_{2e} \sim \omega_n$!

Solid phase peptide synthesis
Two segmentally, overlapping U-\textsuperscript{13}C,\textsuperscript{15}N labeled peptides
GNNQQNY\textsubscript{(7-13)} from Sup35 (Yeast Prion)

- Minimal unit to form prion-like fibrils
- Forms nanocrystals as well with a cross-\(\beta\) type structure!
- Is it possible to polarize nanocrystalline samples?

Nanocrystals

X-ray structure

\textit{Eisenberg et al. (2005)}

Crystal width \(\sim 280\,\text{Å}\)
Monoclinic Crystals - X-ray Studies

How does this structure relate to the actual prion aggregates?

Biradical and the GNNQQQNY Lattice

• Biradical is larger than the water pore (~7Å) in the GNNQQQNY lattice.
Both the $^{13}\text{C}$ and $^{15}\text{N}$ spectra are enhanced by factors of $\sim120$.

$(U^{-13}\text{C},^{15}\text{N})$-GNNQqny in 60% glycerol/H$_2$O, $\omega_r/2\pi = 4.10$ kHz, $T=90$ K
140 GHz $^{13}\text{C}$ and $^{15}\text{N}$ DNP/MAS Spectra

GNNQqny Nanocrystals

- Biradical polarizing agent -- TOTAPOL -- employed in the experiment

- TOTAPOL is probably too bulky to penetrate the nanocrystals

- Solvent polarization likely diffuses from the surface to the interior of the nanocrystals

- Polarization builds up with $\sim T_1$ of the crystals

$\varepsilon \sim 120$
• TOTAPOL is probably too bulky to penetrate the nanocrystals

• Solvent polarization likely diffuses from the surface to the interior of the nanocrystals

• Polarization builds up with ~$T_1$ of the crystals
Fick’s Law: \[
\frac{\partial P}{\partial t} = D \frac{\partial^2 P}{\partial x^2} - \frac{P}{T_{1n}}
\]

Steady state and boundary conditions:

\[
\frac{1}{a} \int_{-w/2}^{w/2} P(x)dx = \frac{2 \sqrt{DT_{1n}}}{w} \varepsilon_0 \varepsilon_0 \tanh\left(\frac{w}{2 \sqrt{DT_{1n}}}\right)
\]

\[
\varepsilon = \varepsilon_0 \frac{2 \sqrt{DT_{1n}}}{w} \tanh\left(\frac{w}{2 \sqrt{DT_{1n}}}\right)
\]

TEM’s of GNNQQNY nanocrystallites

x 55,000 x 110,000

2000 Å.

van der Wel, at al JACS 2006
2D DNP Enhanced $^{13}$C and MAS Spectra

GNNQqny Nanocrystals

- Enhanced Polarization diffuses from the solvent into the crystals
- 2D spectral acquisition is accelerated -- ~20 minutes
- One of the initial examples of the multi-D spectra enhanced with DNP
• **Background and Rationale**
  DNP, EPR, Signal to Noise and bR

• **Instrumentation for DNP**
  Quadruple Resonance, LT MAS Probes
  Gyrotron Microwave Sources

• **DNP Enhanced MAS Spectra**
  DNP Enhancements of 50-60 in MAS Spectra @ 90 K
  *DNP functions quite effectively in broad class of systems*

• **Polarizing Agents and DNP Mechanisms**
  Biradical polarizing agents $\Rightarrow \varepsilon = 170-340$
  Solid effect and cross effect DNP mechanisms

• **DNP in Solution (and for Metabolomics)**
  Solid state polarization and laser T-jump
  $\varepsilon^* = \varepsilon \left( \frac{T_{\text{obs}}}{T_{\text{polar}}} \right) = 130-330$ for $^{13}$C
Dipolar relaxation is not useful for electron-nuclear polarization transfer -- no spectral density.

Scalar relaxation (with transient complex) is effective for producing DNP enhancements.

Loening, Rosay, Weis and Griffin (2002)
TJ-DNP (in brief)

**Principle:**
Efficient transfer of DNP polarization from solid state to liquid state

DNP enhancement in the solid state

$2\text{M}^{13}\text{C} \text{ urea, DMSO/water}$
$5.35\text{mM biradical}$
$90 \text{ K, } \omega/2\pi = 8 \text{ kHz}$

$\varepsilon_{H} = 290 \pm 30$

$\varepsilon^{\dagger}_{C} (\text{expt}) = 290 \times 4 \times 3.3 \sim 3800$
TJ-DNP Experimental scheme

Solid State

$\varepsilon \sim 290 \pm 30$

$\omega_r = 800 \text{ Hz}$

$\tau_{\text{DNP}} = 15-45 \text{ s}$

Liquid State

$\varepsilon^\dagger \sim 400 \pm 15$

$140 \text{ GHz}$

$1^H$

$13^C$

$\text{CO}_2 \text{ laser}$

$13^C$ Urea/DMSO/water

$\text{CO}_2 \text{ Laser}$

Melting

$\text{CO}_2$ laser

Detection

Recycle

Heating

CP

DNP
• Polarization is performed at 140 GHz and 90 K with slow rotation -- 300-800 Hz

• Melting is achieved with a 10 watt CO₂ laser
DNP in liquids -- urea

$\varepsilon^+ = 400 \pm 15$

2M $^{13}$C urea, DMSO/water
5.35mM biradical
90 K, $\omega_r/2\pi = 800$ Hz

Non-protonated carbons

$\text{H}_2\text{N} \quad \text{C} \quad \text{NH}_2$
Effect of deuteration: Glucose-$^{2}$H$_{7}$

$\varepsilon^+(C_{2-5}) = 150$

$\varepsilon^+(C_1) = 131$

$\varepsilon^+(C_6) = 146$

$^{2}$H labeling of the glucose molecule attenuates losses due to relaxation!

$^{13}$C$_{6}$, $^{2}$H$_{7}$ glucose/water 8/2
10 mM biradical
Enhancement in **liquids** ($\varepsilon^\dagger$) and **solids** ($\varepsilon$)

**DNP**

80-90 K

**T-Jump**

CO$_2$ laser

**NMR**

Liquid State

DNP in solid state:

$$\varepsilon_H = \frac{\gamma_e}{\gamma_H} = 660$$

Cross polarization:

$$\frac{\gamma_H}{\gamma_C} = 4$$

Boltzmann distribution:

$$\frac{T_{\text{final}}}{T_{\text{initial}}} = \frac{300\text{K}}{90\text{K}} = 3.3$$

$$\varepsilon^\dagger = \varepsilon \left( \frac{T_{\text{final}}}{T_{\text{initial}}} \right)$$

$$\varepsilon^\dagger_{(\text{max})} = 660 \times 4 \times 3.3 \sim 8700$$

*Hype your polarization* - multiply by the Boltzmann factor!
TJ-DNP: Recycling

TJ-DNP experiment can be recycled every 60-120s.

Applications:
Signal averaging and multidimensional NMR
2D spectrum: Glucose-$^{2}\text{H}_7$

$^{13}\text{C}_6,^{2}\text{H}_7$ glucose/water 8/2
10 mM biradical

$\varepsilon^\dagger \sim 120-170$
Thank you for your attention!