Introduction to Dynamic Nuclear Polarization

Solid State NMR Winter School

January 24-29, 2010

Francis Bitter Magnet Laboratory and Department of Chemistry Massachusetts Institute of Technology
• 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 ⇒ $\varepsilon = 170-340$
  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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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)


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

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

Publications from the Griffin Group @ MIT


References -continued


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 (future research)**

**Integrated Solid effect**

**NOVEL**

**RF-DNP**


V. Weis, M. Bennati, M. Rosay, and R.G. Griffin,

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"

J.H. Ardenkjaer-Larsen, B. Fridlund, A. Gram, G. Hansson, L. Hansson, M.H. Lerche, R. Servin,
M. Thaning, K. Golman, "Increase in signal-to-noise ratio of >10,000 times in liquid-state NMR

C-G. Joo , K-N. Hu , J. A. Bryant, and R. G. Griffin
"In situ Temperature Jump-High Frequency DNP Experiments: Enhanced Sensitivity in Liquid State NMR"

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$_3$

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. Wenkebach, 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)

Fig. 1. Oscilloscope pictures of 50-kc/sec nuclear resonance absorption of static magnetic field. Field excursion 0.3 gauss. Top line: Li resonance (lost in noise). Middle line: Li resonance enhanced by electron saturation. Bottom line: Proton resonance in glycerin sample.

- 7Li NMR @ $\omega_0/2\pi = 50$ kHz (30.3 Gauss)
- EPR @ $\omega_0/2\pi = 84$ MHz

\[ \varepsilon \sim 100 \]

• Initial demonstration of the Overhauser effect -- DNP
• Nuclear Overhauser effect is important in solution NMR!

Carver and Slichter, Phys. Rev. 92, 212-213 (1953)
Phys. Rev. 102, 975-980 (1956)
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$_3$

1960-1980 -- *Liquids and Solids* (0.3300 T, ~10 GHz)
Liquids -- Hauser, Mueller-Warmuth, Richards, and others
Solids -- Abragam, Goldman, Atsarkin, 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
*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>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fourier Transform NMR</td>
<td>Ernst and Anderson</td>
<td>~10-100</td>
<td></td>
</tr>
<tr>
<td>Polarization Transfer</td>
<td>Hartmann &amp; Hahn; Pines, Gibby &amp; Waugh; Morris &amp; Freeman</td>
<td>10</td>
<td>Nobel Prize</td>
</tr>
<tr>
<td>CP and INEPT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indirect Detection (HSQC)</td>
<td>Bodenhausen and Ruben</td>
<td>~30</td>
<td>Wolf Prize x 2</td>
</tr>
<tr>
<td>$B_0$ --200 to 800 MHz</td>
<td>Oxford, Bruker, Magnex</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Cryoprobes</td>
<td>Peter Styles</td>
<td>2-4</td>
<td></td>
</tr>
<tr>
<td>TROSY</td>
<td>Pervushin, et.al.</td>
<td>2-5</td>
<td></td>
</tr>
<tr>
<td>High Frequency Dynamic Nuclear Polarization</td>
<td>Becerra, Gerfen, Prisner, McDermott, Un, Hall, Farrar, Rosay, Weis, Bennati, Hu, Bajaj, Maly, Joo, Barnes, Mak and RG2</td>
<td>~ 50-400</td>
<td>Nobel Prize</td>
</tr>
</tbody>
</table>

$\varepsilon = 50-400$

**Significant consequences for NMR -- savings of ~ 2500 - 160,000 in time -- NEW SCIENCE !**
Nuclear Spin Polarization
Temperature and Field Dependence

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

Result -- “modest” increases in sensitivity and resolution!
Increases in magnet cost are non-linear!

\[ P = \frac{n^+ - n^-}{n^+ + n^-} = \tanh \left( \frac{\gamma \hbar B_0}{2kT} \right) \]
Electron and Nuclear Polarization
Temperature and Field Dependence

- 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!

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

\[ P = \frac{\gamma \hbar B_0}{2kT} \left( \frac{\gamma_e}{\gamma_{1\text{H}}} \right) \sim 660 \]
How do we make electrons talk to nuclear spins?

*With ENDOR, ESSEEM and DNP*
• $^1$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 $\omega_0 \pm \omega_{hf}$

$Bulk \ ^1H\ resonance \ \Rightarrow DNP \quad Hyperfine\ shifted \ \Rightarrow ENDOR$
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

4-amino-TEMPO

Cryoprotectant (e.g. glycerol)

Purple membrane

Cryoprotected sample

bacteriorhodopsin

α-lytic protease
Dynamic Nuclear Polarization
Solid State Effect

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}

\begin{align*}
|q| & \propto \frac{D_{e-n}^{n.s.}}{\omega_n} \\
\text{Electron Zeeman Bath} & \quad \text{Nuclear Zeeman Bath} \\
\text{Equilibrium} & \quad \text{Positive Enhancement} \\
\nu_e - \nu_n & \quad \nu_e \\
\text{No Enhancement} & \quad \text{Negative Enhancement} \\
\nu_e + \nu_n &
\end{align*}

• Irradiate the flip-flop transitions
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!
Distance between the two TEMPO radicals in TOTAPOL yields an e⁻–e⁻ coupling of ~25 MHz.

Electron concentration ~ 10 mM!

Enhancements build up over ~40 seconds to a maximum of 290 ± 30!

Joo, Hu, Bryant and Griffin (2006)
Are the three members of the trimer equivalent?

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

$^{13}\text{C}-^{15}\text{N}$ spectra of $\text{bR}_{555}$ indicates that the trimer is heterogeneous!
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)
DNP in Membrane Proteins
Distribution of Polarization via $^1$H Spin Diffusion

- Polarize the solvent $^1$H with biradicals and CW $\mu$waves
- $^1$H spin diffusion distributes the polarization to the protein

$\bullet$ biradical polarizing agent
$\bullet$ cryoprotectant

$\sim 50 \text{ Å}$
DNP Enhanced $^{15}$N MAS Spectra of U-$^{13}$C,$^{15}$N-bR
Are we polarizing the entire protein? YES!

Integrals of the resolved lines
(10-20% error)

<table>
<thead>
<tr>
<th></th>
<th>Theory</th>
<th>Expt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schiff base</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Amide</td>
<td>248</td>
<td>240±13</td>
</tr>
<tr>
<td>Arg</td>
<td>21</td>
<td>25±2</td>
</tr>
<tr>
<td>Lys</td>
<td>6</td>
<td>6±1</td>
</tr>
</tbody>
</table>

Three previous publications ....

van der Wel JACS 128, 10840-10846 (2006)
DNP in Membrane Proteins
Distribution of Polarization via $^1$H Spin Diffusion

- Polarize the solvent $^1$H with biradicals and CW $\mu$waves
- $^1$H spin diffusion distributes the polarization to the protein
The biradical (2 x TEMPO) is larger than the water pore (~7Å) in the GNNQQNY lattice.
**GNNQQQNY**$_{7-13}$ from Sup35 (Yeast Prion)

- Minimal unit to form prion-like fibrils
- Forms nanocrystals as well with a cross-β type structure!
- *Is it possible to polarize macroscopic nanocrystalline samples?*

Nanocrystals
100-200 nm width

X-ray structure
*Eisenberg et al (2005)*
Resolution is not compromised by the presence of 100 mM electrons

TOTAPOL does not penetrate the lattice !!!
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 ~$T_1$ of the crystals

$\epsilon \sim 120$
T$_1$ Values for GNNQQNY

- TOTAPOL is too bulky to penetrate the nanocrystals
- Solvent polarization diffuses from the surface to the interior of the nanocrystals
- Polarization builds up with $\sim T_1$ of the crystals

Peptide $\varepsilon = 120$

Glycerol $\varepsilon = 160$
Fick’s Law:

\[
\frac{\partial P}{\partial t} = D \frac{\partial^2 P}{\partial x^2} - \frac{P}{T_1 n}
\]

Steady state and boundary conditions:

\[
\frac{1}{a} \int_{-w/2}^{w/2} P(x) dx = \frac{2 \sqrt{DT_1 n}}{w} \varepsilon_0 P_0 \tanh \left( \frac{w}{2 \sqrt{DT_1 n}} \right)
\]

\[
\varepsilon = \varepsilon_0 \frac{2 \sqrt{DT_1 n}}{w} \tanh \left( \frac{w}{2 \sqrt{DT_1 n}} \right)
\]

TEM’s of GNNQQNY nanocrystallites

x 55,000  x 110,000

2000 Å.

van der Wel, at al JACS 2006
DNP in Amyloid Nanocrystals
Distribution of Polarization via $^1$H Spin Diffusion

- When the dimensions of the system are large a polarization gradient develops!
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
- Irradiate the SB with a Gaussian pulse

Bajaj, Mak, Belenky, Herzfeld, Griffin (2009)
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)
**Resolution of Nζ-C15-Cx and Nζ-Cε-Cx**

90 K Spectra of Bacteriorhodopsin

- DNP enhancement of single $^{13}$C’s in a U-$[^{13}$C,$^{15}$N] bR (< 1 hour)
- $^{13}$C linewidths 1.2-1.7 ppm
- $^{13}$C-14 2.2 ppm
- $^{13}$C-20 methyl group!

Mak, Barnes, Bajaj, Belenky, Herzfeld (2009)
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  Biradical polarizing agents ⇒ ε = 175
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- DNP in Solution (and for Metabonomics)
  Solid state polarization and laser T-jump
  \( \varepsilon^t = \varepsilon \left( \frac{T_{\text{obs}}}{T_{\text{polar}}} \right) = 130-330 \) for \(^{13}\text{C}\)
Components of a DNP System

- **Probe**
  - Barnes, et al. (2009) poster on display

- **Gyrotron**
  - Bajaj, et al. (2007)
  - Song, et al. (2006)

- **Transmission Line**
  - Woskow, et al. (2005)

- **Polarizing Agents**
  - Matsuki, et al. (2009)

T. Maly, 2010
Three basic components

- Microwave source — Gyrotron oscillator
- Transmission line — Corrugated waveguide
- NMR probe w/ waveguide
  Schaefer/McKay transmission line probe

Bajaj, Kreischer, Woskow, Temkin
**Three basic components**

- Microwave source — Gyrotron oscillator
- Transmission line — Corrugated waveguide
- NMR probe w/ waveguide
  Schaefer/McKay transmission line probe

*Bajaj, Kreischer, Woskow, Temkin*
Frequency Calibration

• NMR frequencies ---
  – generally refer to $^1$H frequencies
  – 42.577 MHz/Tesla

• EPR frequencies ---
  – dealing with $g=2$ electrons
  – 28.0 GHz/Tesla

$$\left( \frac{\gamma_e}{\gamma_H} \right) = \frac{2800}{42.577} \approx 657$$

<table>
<thead>
<tr>
<th>Magnetic Field (Tesla)</th>
<th>$^1$H NMR Frequency (MHz)</th>
<th>g=2 EPR Frequency (GHz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>211</td>
<td>140</td>
</tr>
<tr>
<td>8.93</td>
<td>380</td>
<td>250</td>
</tr>
<tr>
<td>14.09</td>
<td>600</td>
<td>395</td>
</tr>
<tr>
<td>16.44</td>
<td>700</td>
<td>460</td>
</tr>
<tr>
<td>18.79</td>
<td>800</td>
<td>527</td>
</tr>
</tbody>
</table>
High Frequency Microwave Sources for Dynamic Nuclear Polarization

- 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
250/ 460 GHz Gyrotron

• 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
460 GHz/ 700 MHz Gyrotron Oscillator

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.

High power millimeter wave source is clearly preferable for DNP!
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
Magnetic Tuning @ 460 GHz

- Generate > 4 W’s power over 0.8 GHz of bandwidth
- $I_{\text{beam}}$ = 60-105 mA, $V$ = 12.9 kV -- higher current will yield additional power
- $B_0$ field sweep is not necessary!

A. Torrezan, S-T. Han, J. Sirigiri, and RJT (2008)
Microwave Resonators

Solid state source with microwave resonant cavity

- TE011 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 ~200-300!
Corrugated Waveguide

- Very low insertion loss (0.01dB/m)
- Cryogenic Operation
- Excellent mode and polarization characteristics

1.3 m

\( \lambda/4 \) Corrugation depth

Wall thickness 300 microns
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, $\omega_r/2\pi \sim 10$ kHz
- Optical irradiation (532/650 nm) of samples to generate photochemical intermediates

Barnes, et. al. JMR (2009)
Challenges for cryogenic sample exchange:

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

\[ \text{Cambridge Instruments} \]
\[ \text{DNP Cryogenic MAS Probe} \]

\[ \text{Alexander Barnes} \]
\[ \text{Instrument session} \]
263 GHz Gyrotron in Bruker-Billerica DNP Lab

Gyrotron tube
NMR magnet
Transmission line
Gyrotron magnet
Control system
Chillers

Melanie Rosay, 2009
263 GHz Gyrotron in Bruker-Billerica DNP Lab

- Cooling cabinet
- 400 WB
- Gyrotron tube
- Transfer line
- LT/DNP probe
- Transmission line
- Gyrotron magnet
- Control system
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  Solid state polarization and laser T-jump
  ε′ = ε(T_{obs}/T_{polar}) = 130-330 for 13C
Overhauser Effect (OE) -- applicable to systems with mobile electrons -- i.e., metals, liquids, 1D conductors (discussed later wrt liquid state DNP)

Solid Effect (SE) -- single electron, 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 (\(^{1}H\), \(^{13}C\), \(^{15}N\))
CW Dynamic Nuclear Polarization Mechanisms

Thermal Mixing (TM) -- multiple electrons, insulating solids, but ....

δ, Δ >> ω

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

Cross Effect (CE) -- two electrons, insulating solids, but ....

Δ > ω > δ

CE -- operative at high fields where Δg >> δ, the line is *inhomogeneously* broadened.
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)
\[ \pi -- CW \text{ on the electrons} \]
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.

****See Andy Smith’s poster****
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) -- single electron, insulating solids (organic, biological systems) when ....

\[ \delta \sim \Delta << \omega \]

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

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

Enhancement $\sim \left( \frac{\gamma_e}{\gamma_n} \right) \left( \frac{\omega_1}{\omega_0} \right)^2 \left( \frac{N_e}{\delta} \right) T_{1n}$
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. Griffin1

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
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(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-bisdi phenylene-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.

\[ \gamma_e / \gamma_n \sim 660 \]

\[ 1.5\% \text{ efficient} \]

\[ e^- \rightarrow ^1H \rightarrow ^13C \]

\[ \varepsilon \sim 10 \]

\[ \varepsilon \sim 40 \]
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 ±15!

**CW Dynamic Nuclear Polarization Mechanisms**

**Thermal Mixing (TM)** -- *multiple* electrons, 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)** -- *two* electrons, 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 $\sim 21$ MHz
  ---- *Solid effect*
- TEMPO powder pattern $\sim 600$ MHz
  ---- *Thermal mixing or cross effect*
• 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
Thermal Mixing
Homogeneous EPR

1. **μ-wave 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 $^1\text{H}$
   2615 for $^{13}\text{C}$

\[ \varepsilon^{TM} \propto \left( \frac{B_1^2}{B_0} \right) T_{1e} T_{1n} \]
DNP Enhanced $^{13}$C-MAS Spectra

$U^{13}$C,$^{15}$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

$T = 95$ K
$\varepsilon = 16$

40 mM 4-amino TEMPO

Rosay, Weis, Kreischer, Temkin and Griffin JACS (2002)

2.4% efficient
DNP Enhanced $^{15}$N MAS Spectra

$^{15}$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)** -- many electrons, 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)** -- two electrons, insulating solids, but ....

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

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

Inhomogeneous EPR

1. μ-wave 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.
   \[
   (\tau_{een})^{-1} = 4 |g|^2 \frac{1}{T_{2e}^5} \int_0^\infty \frac{g(\omega)g(\omega - \omega_n)}{g(0)} \, d\omega
   \]

3. \( \omega_{2e} - \omega_{1e} = \omega_n \)
   TEMPO EPR
   Absorption Lineshape

4. Enhancement
   \[ \varepsilon \sim \gamma_e \frac{\gamma_e}{\gamma_N} \]
   657 for \(^1\text{H}\)
   2615 for \(^{13}\text{C}\)
Monomeric Polarizing Agents

4-amino TEMPO

Cryoprotectant (e.g. glycerol)

1. Resuspension
2. Centrifugation

Randomly oriented and dispersed paramagnetic centers

Suboptimal utilization of e⁻-e⁻ dipolar coupling
• NO\• EPR spectrum is ~600 MHz wide

• Thermal mixing and the cross effect are …

**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)
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
\(^{15}\text{N}, \, ^{2}\text{H}\)-labeled

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

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

- Decreasing \(R_{\text{e-e}}\) 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 ~22 MHz

- Reduced electron concentration from 40 mM to 10 mM
- Enhancements build up over ~15 seconds and the maximum appears to be ~175!

\[ \varepsilon = 175 \]

*K. Hu et. al (2004)*
**Distance** between the two TEMPO radicals in TOTAPOL yields an $e^{-}-e^{-}$ coupling of $\sim 25$ MHz.

- Reduced electron concentration *from 40 mM to 10 mM*
- Enhancements build up over $\sim 15$ seconds and the maximum appears to be $\sim 160$!

*C. Song, T. Swager et. al., JACS (2006)*
• 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 $\sim$25 MHz
- Electron concentration $\sim 10 \text{ mM}$!
- Enhancements build up over $\sim$40 seconds to a maximum of $290 \pm 30$!

Joo, Hu, Bryant and Griffin (2006)
Paramagnetic Centers for DNP

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

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

Irradiate the flip-flop transitions
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 $\pm 15$!
• 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 \( \sim \omega_n/2\pi \).
• **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 ~21 MHz
  ---- Solid effect
- TEMPO powder pattern ~600 MHz
  ---- Thermal mixing or cross effect
Three-Spin Process in Thermal Mixing/Cross Effect DNP

If $\omega_{e1} \sim \omega_{e2}$

Equilibrium

$\Delta \omega_e < \omega_n$

$\Delta \omega_e > \omega_n$

$\Delta \omega_e = \omega_n$

Positive Enhancement

Negative Enhancement

Kan Hu
PhD Thesis 2006
1. \(\mu\)wave 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 \(^1\text{H}\)

\(2615\) for \(^{13}\text{C}\)

\[
\varepsilon^{TM} \propto \left( \frac{B_1^2}{B_0} \right) T_{1e} T_{1n}
\]
DNP Enhancement at 250 GHz
$^{13}$C-Urea/Glycerol/D$_2$O

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

$$\epsilon_{250} = \epsilon_{140} \left( \frac{140}{250} \right)$$

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

$$\epsilon \approx 100$$
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 $^1H \varepsilon$ 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
Biradical Models from EPR Analyses

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!

- $\varepsilon \sim 250/660 \rightarrow 38\%$ efficient!

62,500 in time!
Rigid *bis*-TEMPO (Tordo/Prisner) Biradical
$^{13}$C-Urea/DMSO/D$_2$O/H$_2$O

- Enhancement at infinite power: $b$Tbk > TOTAPOL
- Presently insoluble in glycerol/H$_2$O!

62,500 in time!
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 $\omega_{1e} - \omega_{2e} \sim \omega_n$!

Direct $^{13}$C Enhancements

Trityl/$^{13}$C-Urea

- Distance between the trityl radicals and $^{13}$C is large
- $^{13}$C spin diffusion is slow
- Build up time is 10 minutes!

$\varepsilon = 325$

$T = 90$ K
$\nu_r = 3.5$ kHz
• $^{13}\text{C}$ yields larger enhancements than $^1\text{H}$ -- $^{13}\text{C}$ $T_1$ is longer
• Trityl @ 1.2 K yields $\varepsilon \sim 300$
• $\omega_n/2\pi=51$ MHz fits the low frequency side of the spectrum
Thank you for your attention!