Dynamic Nuclear Polarization (DNP) at 9.4 Tesla with a 30mW microwave source

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Why Dynamic Nuclear Polarization (DNP)?

1.10 to >100x signal increase

Big benefit to often signal to noise limited NMR experiments
Why Dynamical Nuclear Polarization now?

Since DNP tends to work best at low temperatures, the ability to do MAS at 25 K leads to the idea of adding DNP to CryoMAS.

2D $^{13}$C-$^{13}$C spectrum
with 500 ms $^{13}$C spin diffusion mixing period

Aβ 14-23
amino acid sequence: HQKLVFFAED
2.7 mg of sample, uniform 13C and 15N labeled at V18, A21

25 K, 6.7 kHz spin, 2048 total scans for 2D,
$^1$H $T_1 = 4$ sec, repeat rate 6 sec,
time required = 3.5 hours each

Cross peaks between A21 $\alpha$ and V18 $\alpha$ and $\beta$ indicate close contact of A21 and V18.
Dynamic Nuclear Polarization:
Transfer Electron spin polarization to Nuclear spin
to increase NMR signal.

Electrons have 600 times larger magnetic moment, hence larger polarization than nuclei.
In a 9.4 Tesla magnetic field at 25 K,

<table>
<thead>
<tr>
<th></th>
<th>Frequency</th>
<th>Polarization</th>
</tr>
</thead>
<tbody>
<tr>
<td>1H nuclei</td>
<td>400 MHz</td>
<td>$4 \times 10^{-4}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>= 0.02 Kelvin</td>
</tr>
<tr>
<td>Electrons</td>
<td>264 GHz</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>= 13 Kelvin</td>
</tr>
</tbody>
</table>

Electrons have significant polarization in this temperature/field range.
Need to apply magnetic field at electron spin resonance (264 GHz)
264 GHz (1mm wavelength) is an awkward frequency – high for microwaves, low for optical.
DNP  Mechanism at high field

Some possible methods for DNP:

1. Apply microwaves at sum or difference of electron and nuclear frequency
   [Solid state effect] Forbidden transition: one photon to change two spin states, only possible because the electron-nuclear interaction perturbs the eigenstates.
   This perturbation weakens at high field because the energy difference of the single spin states gets larger relative to the electron-nuclear interaction.

2. Cross polarization, or adiabatic passage effects.

3. Use multiple electron spins close together so electron-electron interactions can be significant.
   Conserve energy with nuclear spin flip by having electron-electron dipolar coupling [Thermal mixing] or electron-electron g-factor difference [Cross effect] = NMR frequency.
Design of DNP/ESR spectrometer for 9.4 Tesla:

DNP with a frequency-tunable CW microwave source.
Based on solid-state microwave source: 264 GHz, ~10 GHz tunable range
Small, simple, and relatively inexpensive
No requirement for swept field magnet
Lower power (30mW) than gyrotron or vacuum electronics

Detect ESR with no change to equipment setup.

Quasi-optical transmission: (quasi- because 1mm wavelength is finite relative to beam cross-section size.)
Metal waveguides at the fundamental mode are too lossy at this frequency.
Instead, use mirrors, wire grids (as polarizers), and oversize (multi-mode) corrugated waveguides.

Polarization control to allow all of the power to be transmitted in the circular polarization that can be absorbed by the electron spins.
Spectrometer setup: Optical plate
(shown not under NMR magnet)

1. Microwave source with horn.
2. Polarizer (wire grid)
3. Interferometer for polarization control.
4. Mirror to reflect microwaves into vertical corrugated waveguide.
5. Detector for ESR with horn.
4. Mirror to reflect microwaves into vertical corrugated waveguide.

6. Corrugated waveguide

Corrugated waveguide leads to bottom window of vacuum cryostat
**He-cooled NMR probe (non-spinning)**

NMR probe without radiation shield, next to its vacuum jacket

Vacuum window for entry of microwaves on bottom of cryostat (Plastic, HDPE)
7. Brass tube to convey microwaves to sample.

8. Modulation coil for AC magnetic field in z-axis for ESR detection.

9. Copper parts hold sample container.

10. RF coil lead. RF coil itself is barely visible at center of modulation coil.

Sample is at center of RF coil. Sample is sealed in a Teflon cup with a sapphire rod attached to Cu for thermal conductivity.
Electron spin dopants based on TEMPO nitrooxide

4-amino-TEMPO

DOTOPA-TEMPO

“Triple-Tempo”

TOTAPOL

“Double-Tempo”


Kan Hu

Wai-Ming Yau
ESR detection by microwave power absorption

ESR signal is detected as the derivative of the microwave absorption.

The microwaves pass through the sample, are reflected back through the sample, and down to the microwave optics. When the microwaves are circularly polarized, the returning microwaves end up at the detector.

The modulation coil provides an AC (6100 Hz) z-axis magnetic field (2 Gauss = 6 MHz).

The signal from the microwave detector is fed into a lock-in amplifier, and the AC absorption signal (= the derivative of the absorption) is detected.
1H NMR signal enhancement by DNP

264 GHz CW microwave irradiation.
Field modulation used during DNP:
20 Gauss rms = 56 MHz for electron spins at 6100Hz

1H NMR echo using DNP with field modulation,
and without any DNP
16K, 10ul sample, 40mM 4-amino TEMPO
25/75 mol% glycerol/water

x10 expanded y scale
DNP enhancement of 1H NMR, and ESR as a function of microwave frequency

**Dynamical Nuclear Polarization (DNP) at 16 K**

10μl sample (glycerol/water 25/75 mol% doped with 40mM 4-amino TEMPO)

- Signal enhancement relative to thermal polarization at 16 K

**Electron Spin Resonance**

Same sample and conditions as for DNP

- ESR absorption lineshape (integral, arb. units)
DNP microwave frequency dependence

DNP lineshape is not strongly dependent on having multiple Tempo radicals linked together. Signal increase from DNP is strongly affected.

16K, 10ul samples of 25/75 mol% glycerol/water
Time dependence of DNP polarization and T1 (thermal polarization)

Polarization time for DNP is important, not just signal increase. Not good to have a big signal that takes forever to polarize. Our conditions show DNP polarization time comparable to T1 (thermal). Time constants vary strongly with dopant, its concentration, and temperature.

40mM 4-amino-Tempo, 16K, 10ul sample, 25/75 mol% glycerol/water

160mM 4-amino-Tempo, 35K, 10ul sample, 25/75 mol% glycerol/water

$T_{DNP} =$ DNP Polarization buildup (microwaves on)

$T_{1n} =$ recovery after saturation (microwaves off = no DNP)

~130 s

~3 s
## Multiple linked Tempo radicals improve DNP

<table>
<thead>
<tr>
<th>Temp.</th>
<th>Dopant</th>
<th>Signal Increase</th>
<th>Buildup Time, $T_{DNP}$ (s)</th>
<th>Signal/Noise in fixed time = Signal/Sqrt($T_{DNP}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>80 K</td>
<td>TripleTEMPO 30mM</td>
<td>10x</td>
<td>1.4</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Totapol 20mM</td>
<td>6x</td>
<td>8.3</td>
<td>4.1</td>
</tr>
<tr>
<td></td>
<td>TEMPO 40mM</td>
<td>11x</td>
<td>50</td>
<td>1.8</td>
</tr>
<tr>
<td>35 K</td>
<td>TripleTEMPO 30mM</td>
<td>26x</td>
<td>2.4</td>
<td>73</td>
</tr>
<tr>
<td></td>
<td>Totapol 20mM</td>
<td>11x</td>
<td>18.5</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>TEMPO 40mM</td>
<td>13x</td>
<td>90</td>
<td>3.6</td>
</tr>
<tr>
<td>16 K</td>
<td><strong>TripleTEMPO 30mM</strong></td>
<td><strong>59x</strong></td>
<td><strong>4.5</strong></td>
<td><strong>265</strong></td>
</tr>
<tr>
<td></td>
<td>Totapol 20mM</td>
<td>26x</td>
<td>41</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td>TEMPO 40mM</td>
<td>29x</td>
<td>126</td>
<td>15</td>
</tr>
</tbody>
</table>
Increase in DNP with kHz field modulation

Bz field modulation at kHz frequency improves DNP significantly (up to 2.3x signal)

Slow field modulation has no effect. Timescale for benefit does not depend on size of field modulation or the microwave power level.

Dependence of DNP on field modulation frequency

20mM Totapol “Double-Tempo”, 35K, 10ul sample, 25/75 mol% glycerol/water

10 Gauss field modulation is equivalent to 28 MHz for the electrons
Slow electron spectral diffusion = narrow hole saturated in electron line by microwaves

**Simulation:** Bloch equations with relaxation, sinusoidal modulation of magnetic field, and spectral diffusion. Electron polarization averaged over complete modulation.

![Graph showing electron spin saturation region](image)

**Parameters:** 10 kHz modulation frequency, T1e = 2 ms, T2e = 4 us, B1 = 0.03 G, spectral diffusion = $10^{17}$ Hz$^2$/s

![Graph showing total electron spin saturation as a function of modulation frequency](image)
Comparison between Experiment and Simulation

No adjustable parameters for normalized frequency dependence.
Parameters: some extrapolated from 140 GHz.

Simulation has a good fit considering extrapolation of parameters.
Experiment: 135% increase (DNP more than double) with field modulation
Simulation: 200% increase (DNP triples)

What does simulation indicate will help DNP?

Modulate field or frequency faster than $1/T_{1e}$
Large $B_1$, modulation amplitude.
Large spectral diffusion, long $T_{1e}$
$T_{2e}$ does not matter in this range

What about MAS? It involves $g$-anisotropy modulation at kHz frequencies.

Parameters:
$T_{1e} = 2$ ms, $T_{2e} = 4$ us, $B_1 = 0.03$ G,
spectral diffusion = $10^{17}$ Hz$^2$/s
Microwave power dependence of DNP signal

35K, 10ul sample, 25/75 mol% glycerol/water

Temperature dependence of DNP signal

30mM “Triple-Tempo”, 10ul sample, 25/75 mol% glycerol/water, pH 3 acetate buffer

400x increase in signal/noise in fixed time at 7K with DNP compared to 80K without DNP

100x increase in signal/noise in fixed time at 25K where we can definitely do MAS
Improving Biomolecular Solids NMR by Increasing Signal to Noise:

With solid-state source at 264 GHz, Dynamic Nuclear Polarization (DNP) can provide at least 30x increased signal at 35 K, on top of the benefit of low temperature (~1/T)

Goal: combine DNP with low-temperature MAS at T ~ 25 K.


Thanks to:
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Kan Hu