Winter School
Pulsed DNP and TSAR
• **Background and Rationale**
  DNP, EPR, Signal to Noise and bR
  DNP Enhancements of 100-400 in MAS Spectra @ 90 K
  *DNP functions quite effectively in multiple classes of systems*

• **CW DNP Mechanisms and Polarizing Agents**
  Solid Effect — \( \delta \sim \Delta << \omega_{0l} \)
  *two spins, without e⁻ - ¹H hyperfine coupling*
  Overhauser Effect — \( \delta \sim \Delta << \omega_{0l} \)
  *two spins, with e⁻ - ¹H hyperfine coupling*
  Cross Effect — \( \delta < \omega_{0l} < \Delta \)
  *three spins, with e⁻ - e⁻ - ¹H dipole coupling*

• **Time Domain DNP — NOVEL**
  NOVEL — lab frame-rotating frame cross-polarization

• **Instrumentation for DNP**
  Quadruple Resonance, LT MAS Probes
  Superconducting Sweep Coils
  Gyrotron Microwave Oscillators and Amplifiers

• Resolution and
Overhauser Effects in NMR

- **Overhauser effects** require mobile electrons or nuclei...
  Metals, 1D conductors, Na in NH₃, solution NOE’s

- *Overhauser DNP in insulators — new mechanism!*

- **Heteronuclear Overhauser effects** scale $\sim B_0^{-n}$ ....
  Translational and rotational spectral densities
  Heteronuclear ($^1H-^{13}C$) NOE’s are attenuated $>2.3$ T
  *Should not do $^{13}C$ protein NMR above $>60-100$ MHz*

  *Overhauser DNP scales as $B_0^{+n}!$

- **Time Domain Experiments** are not field dependent ....
  INEPT for $^1H-^{13}C/^{15}N$ polarization transfers

  *Pulsed DNP experiments are not field dependent!*
**Time Domain DNP**

**NOVEL -** $\omega_{0I} = \omega_{1S}$

- **NOVEL matching condition --** $\omega_{0I} = \omega_{1S}$
  - $n=4096$
  - $\tau_{90x}=15\ \text{ns}$, $\tau_{\text{match}}=100\ \text{ns}$, $\tau_1=20\ \mu\text{s}$
- **Lab frame/rotating frame matching --** Z-polarization
  - Should **not** manifest a $B_0$ dependence!

- Use pulses on the e- to build up polarization

*Henstra and Wenckebach, Mol. Physics (2008)*

$^1$H-$^{13}$C/$^{15}$N Cross Polarization

Hartmann-Hahn - $\omega_{1I} = \omega_{1S}$

- # $^1$H spins $\geq$ # $^{13}$C spins
- Generates transverse magnetization
Time Domain DNP

NOVEL - $\omega_{0l} = \omega_{1S}$

- # e- spins < # $^1H$ spins
- Substitute the $B_0$ field for $B_{1S}$
- Generates Z-polarization!

Davies ENDOR Spectrum
Benzophenone-Diphenylnitroxide

- Crystal structure and molecular structure
- 140 GHz Davies ENDOR spectrum

Time Domain DNP

NOVEL - $\omega_0 I = \omega_{1S}$

- Saturate $^1H$ signals with a train of “m” pulses
- Spin lock the electrons “n” times using $\omega_0 I = \omega_{1S}$
- Detect the signal with a solid echo

Microwave Field Profiles

**NOVEL** - $\omega_0 I = \omega_{1S}$

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NOVEL - $\omega_{0I} = \omega_{1S}$

Diphenyl-NO in Benzophenone

- NOVEL matching condition -

- Lab frame/rotating frame matching -- Z-polarization

- Should not manifest a $B_0$ dependence!

NOVEL at 80 K

- A factor of ~3 improvement by deuteration
- Also works well at low temperature

NOVEL - $\omega_{0I} = \omega_{1S}$

Trityl DNP Juice

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Jennifer Mathies (2014)
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• Resolution and
140 GHz Pulsed DNP Spectrometer
Gyro-Amplifier

- Gyroamplifier currently generating ~800 watts
- Quasioptical bridge for detection
- Time domain DNP -- no field dependence
- Experimental flexibility

Andy Smith and Bjoern Corzilius
Gyroamplifier, corrugated waveguide, NMR and EPR consoles

Quasi-optic network -- \( \lambda \sim 2.14 \text{ mm} \) (140 GHz) to 0.57 mm (527 GHz)

Ernst and coworkers -- time domain NMR!
Gyroamplifier generating ~400-1000 watts
Quasioptical bridge for detection
Time domain DNP -- no field dependence
ω₁S/2π~350-500 MHz
250 GHz Amplifier for Pulsed DNP

Solid State Source
30mW 248 GHz – 258 GHz

HV Modulator
Transmission Line

Gyrotron Amplifier

9.6 T Magnet
Electron Gun

Heterodyne Frequency Detector

Control System

Nanni, PRL 111,235101 (2013)
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2nd Order Recoupling TSAR
Third Spin Assisted Recoupling Mechanism (TSAR)
Pulse sequences

PAINCP pulse sequence

\[
\begin{array}{c}
\pi/2 \\
^1H & CP & C.W. & TPPM \\
^13C & C.W. \\
^15N & CP & C.W.
\end{array}
\]

Lewandowski, De Paëpe, Griffin JACS (2007)

PAR pulse sequence

\[
\begin{array}{c}
\pi/2 \\
^1H & CP & C.W. & TPPM \\
^13C & C.W.
\end{array}
\]
PAR Experiment
SpinEv Optimization Map

- Appropriate choice of $^{13}$C and $^1$H rf leads to PAR recoupling!

$\rho_C = \omega_{1C}/\omega_r$
$\rho_H = \omega_{1H}/\omega_r$

rotary resonance
Hartmann-Hahn

$\omega_r/2\pi = 20 \text{ kHz}$
$\omega_0/2\pi = 750 \text{ MHz}$
Homonuclear TSAR Mechanism
Second order average Hamiltonian theory

ZQ flip-flop cross terms (2 x 3) lead to PAR transfer!
Auto cross terms arise from 2 x 2 and 3 x 3 and attenuate PAR

De Paëpe, Lewandowski, Loquet, Bockmann, Griffin JCP 129, 245101 (2008)
Proton Assisted Recoupling (PAR) [a.k.a. Third Spin Assisted Recoupling (TSAR)]

- PAR functions via second order cross terms -- not direct $^{13}\text{C}-^{13}\text{C}$ terms

\[
H_{\text{int}} = \omega_{N1N2}(3N_{1z}N_{2z} - N_{1*}N_{2}) + \omega_{N1H2}N_{1z}^2H_{z} + \omega_{HN2}2H_{2}N_{2z}
\]

- Polarization transfer driven by...

- One bond and sequential cross peaks observed in $20 \text{ ms}$ of mixing

- PDSD requires $4 \text{ sec}$ of mixing for an equivalent spectrum!

(Reif, et. al. 2000)

Lewandowski, De Paepe and Griffin JACS 129, 728-29 (2007); JACS 131, 5769–5776 (2009)
• Low power decoupling -- $\omega_1/2\pi=16$ kHz
• Two regions that satisfy the matching conditions
Proton Assisted Recoupling @ $\omega_r/2\pi=65$ kHz

$^{13}\text{C} - ^{13}\text{C}$ PAR -- $U(^{13}\text{C}, ^{15}\text{N})-G_{B1}$

- Long distance transfer in uniformly labeled protein
- Resolved $^{13}\text{C} - ^{13}\text{C}$ J-couplings -- $^{13}\text{C}=\text{O}$ to aliphatic
- Long distance contacts are observed in the aliphatic region
Methods yielding long distance restraints

$^{13}$C–$^{13}$C correlation spectra of [U–$^{13}$C, $^{15}$N]–Crh protein

**DARR / RAD**

20 mg, 21 hours


**CHHC**

20 mg, 46 hours


**$^{13}$C–$^{13}$C PAR**

6 mg, 21.4 hours

De Paëpe et al. (2008)
Lewandowski et al. (2008)

DARR/RAD and CHHC spectra courtesy of Carole Gardiennet
High field (750/900 MHz) Protein Structure Determination

- Long distance transfer in **uniformly labeled protein**
- Superior to DARR, PDSD, etc.

**Crh, 2 x 85 residues**

- $\omega_r / 2\pi = 20$ kHz
- $\omega_{1c} \sim 50$ kHz
- $\omega_{1h} \sim 50$ kHz

Examples of interresidue contacts
Experimental: PAIN-CP vs. DCP at 750 MHz

[U-\textsuperscript{13}C,\textsuperscript{15}N]-f-MLF-OH, 20 kHz MAS

DCP:
3 ms
\textsuperscript{13}C rf - 45 kHz
\textsuperscript{15}N rf - 25 kHz
\textsuperscript{1}H rf – 112.5 kHz

PAIN-CP:
4 ms
\textsuperscript{13}C rf - 50 kHz
\textsuperscript{15}N rf - 50 kHz
\textsuperscript{1}H rf – 49 kHz

Relative 2D volumes for 1-bond cross-peaks

- PAIN-CP is more efficient!
Thank you for your attention!