Dynamic Nuclear Polarization with Millimeter Waves and Biradicals

(Why two electrons are better than one !)

Solid State NMR Winter School

Stowe®
MOUNTAIN RESORT

January 20-25, 2008

**Francis Bitter Magnet Laboratory
and
Department of Chemistry
Massachusetts Institute of Technology**

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
  - *DNP functions quite effectively in broad class of systems*
- Polarizing Agents and DNP Mechanisms
  - Biradical polarizing agents $\Rightarrow \varepsilon = 170\text{-}340$
  - Solid effect and cross effect DNP mechanisms
- DNP in Solution (and for Metabonomics)
  - Solid state polarization and laser T-jump
  - $\varepsilon^{\dagger} = \varepsilon (T_{obs}/T_{polar}) = 130\text{-}330$ for ^{13}C

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
  - *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

  $\varepsilon^{\dagger} = \varepsilon(T_{obs}/T_{polar})$=*130-330* for ^{13}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

A.W. Overhauser *Phys. Rev.* **92**, 411 (1953).

T.R. Carver, and C.P. Slichter, "Polarization of Nuclei Spins in Metals" *Phys. Rev.* **92**, 212 (1953).

Reviews of DNP

Abragam, A.; Goldman, M., *Nuclear magnetism: order and disorder*. Claredon Press: Oxford, 1982.

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"
Progress in Nuclear Magnetic Resonance Spectroscopy **17**, 33-67 (1985).

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
Magnetic Fields" J. Chem. Physics (in press) (2008)

A.B. Barnes, G. De Paëpe, P.C.A. van der Wel, K-N. Hu, C-G. Joo, V. S. Bajaj, M. L. Mak-Jurkauskas, J. R. Sirigiri, J. Herzfeld, R. J. Temkin, and R. G. Griffin,” High Field Dynamic Nuclear Polarization for Solid and Solution Biological NMR” Applied Magnetic Resonance (in press) (2008)

Recent Applications to Polymers

M. Afeworki, R.A. McKay, J. Schaefer
“Selective Observation of the Interface of Heterogeneous Polycarbonate Polystyrene Blends by Dynamic Nuclear-Polarization C-13 NMR-Spectroscopy”. *Macromolecules* **25**, 4084-4091 (1992).

Afeworki, M.; Vega, S.; Schaefer, J.
“ Direct Electron-to-Carbon Polarization Transfer in Homogeneously Doped Polycarbonates”
Macromolecules 25, 4100-4105 (1992).

Publications from the Griffin Group @ MIT

L. R. Becerra, G. J. Gerfen, R. J. Temkin, D. J. Singel, and R. G. Griffin,
"Dynamic Nuclear Polarization with a Cyclotron Resonance Maser at 5 T", Phys. Rev. Lett., 22, 3561 (1993)

G. J. Gerfen, L.R. Becerra, D.A. Hall, D. J. Singel, and R. G. Griffin,
"High Frequency (140 GHz) Dynamic Nuclear Polarization: Polarization Transfer to a Solute in a Frozen Aqueous Solution," >. Chem. Phys. ,102 (24), 9494-9497 (1995).

D. Hall, D. C. Maus, G. Gerfen, S. Inati, L. R. Becerra, F.W. Dahlquist, and R.G. Griffin,
"Polarization Enhanced NMR Spectroscopy of Biomolecules in Frozen Solution," Science 276, 930-931 (1997)

V. Weis, M. Bennati, M. Rosay, J.A. Bryant, and R.G. Griffin, ”
High Field DNP and ENDOR with a Novel Multiple-Frequency Resonance Structure,” J. Magn. Res. 140, 293-299 (1999)

C.T. Farrar, D.A. Hall, G.J. Gerfen, M. Rosay, J.-H. Ardenjaer-Larsen, and R.G. Griffin,
“High Frequency Dynamic Nuclear Polarization in the Nuclear Rotating Frame,” J. Magn. Res. **144,** 134-141(2000)

C.T. Farrar, D.A. Hall, G.J. Gerfen, S.J. Inati and R.G. Griffin,
“Mechanism of Dynamic Nuclear Polarization in High Magnetic Fields,” J. Chem. Physics **114**, 4922 – 4933 (2001)

M. Rosay, A. Zeri, N.S. Astrof, S.J. Opella, J. Herzfeld, and R.G. Griffin,
“ Sensitivity Enhanced NMR of Biological Solids: Dynamic Nuclear Polarization of Y21M fd Bacteriophage and Purple Membrane,” J. Am. Chem. Soc. **123,** 1010-1011 (2001)

References -continued

V.S. Bajaj, C.T. Farrar, M.K. Hornstein, I. Mastovsky, J. Bryant, K.E. Kreischer, B. Elena, J. Vieregg, R.J. Temkin, and R.G. Griffin, ”Dynamic Nuclear Polarization at 9 Tesla Using a Novel 250 GHz Gyrotron Microwave Source,” J. Magn. Resonance **160**, 85-90 (2003)

M. Rosay, J. Lansing, K. C. Haddad, W. W. Bachovchin, J. Herzfeld, R.J. Temkin, and R. G. Griffin “High Frequency Dynamic Nuclear Polarization in MAS Spectra of Membrane and Soluble Proteins,” J. Amer. Chem. Soc. **125**, 13626-27 (2003)

K.-N Hu, H.-h Yu, T. M Swager, R.G. Griffin, "Dynamic Nuclear Polarization with Biradicals" *J. Am. Chem. Soc. 126*, 10844-10845 (2004)

C. Song, K-N. Hu, T. M. Swager, R.G. Griffin, “TOTAPOL – A Biradical Polarizing Agent for Dynamic Nuclear Polarization Experiments in Aqueous Media” J. Am. Chem Soc. **128**, 11385-11390 (2006)

P. C.A. van der Wel, K-N. Hu, J. Lewandowski, R.G. Griffin “High Frequency Dynamic Nuclear Polarization in MAS Spectra of Amyloid Peptides - GNNQQNY Nanocrystals” J. Am. Chem. Soc. **128**, 10840-10846 (2006)

K-N. Hu, V. S. Bajaj, M. M. Rosay and R.G. Griffin ”High Frequency Dynamic Nuclear Polarization Using Mixtures of TEMPO and Trityl Radicals” J. Chem Phys. **126**, 044512 (2007)

K-N. Hu, C. Song, H-h.Yu, T. M. Swager, R. G. Griffin “High-Frequency Dynamic Nuclear Polarization Using Biradicals: Multi-Frequency EPR Lineshape Analysis J. Chem. Physics (in press) (2008)

K-N. Hu and R.G. Griffin ” Quantum Mechanical Theory of Dynamic Nuclear Polarization in Solid Dielectrics” J. Chem Phys. **(to be submitted)** (2008)

K-N. Hu and R.G. Griffin ” Quantum Mechanical Theory of Dynamic Nuclear Polarization in Solid Dielectrics Simulations with Relaxation ” J. Chem Phys. **(to be submitted)** (2008)

References -continued

M. L. Mak, V. S. Bajaj, M. K Hornstein, M. Belenky, R. G. Griffin, and J. Herzfeld, “Active Site of Early I ntermediates in the Proton-motive Photocycle of Bacteriorhodopsin: a Dynamic Nuclear Polarization-Enhanced Solid State NMR Study” Proc. Nat’l. Acad. Sci. **105**, 883-888 (2008)

Instrumentation for DNP

D.J. Singel, D. J. H. Seidel, H.; R.D. Kendrick, C.S. Yannoni
” A Spectrometer for EPR, DNP, and Multinuclear High-Resolution NMR”
Journal of Magnetic Resonance **81**, 145-161 (1989).

L.R. Becerra, G.J. Gerfen, B.F. Bellew, J.A. Bryant, D.A. Hall, S.J. Inati, R.T. Weber, S. Un, T.F. Prisner, A.E. McDermott, K.W. Fishbein, K.E. Kreischer, R.J. Temkin, D.J. Singel, and R.G. Griffin,
"A Spectrometer for Dynamic Nuclear Polarization and Electron Paramagnetic Resonance at High Frequencies",
Jour. Magn. Reson.A **11**7, 28-40 (1995).

M.K. Hornstein, V.S. Bajaj, R.G. Griffin, K.E. Kreischer, I. Mastovsky, M.A. Shapiro, J.R. Sirigiri, and R.J. Temkin,
Second Harmonic Operation at 460 GHz and Broadband Continuous Frequency Tuning of a Gyrotron Oscillator
, IEEE Transactions on Electron Devices, **52**:798-807 (2005).

P.W. Woskov, V.S. Bajaj, M.K. Hornstein, R.J. Temkin, and R.G. Griffin
“Corrugated Waveguide and Directional coupler for CW 250 GHz Gyrotron DNP Experiments,”
IEEE Transactions on Microwave Theory and Techniques, **53,** 1863-1869 (2005)

C. D. Joye, R. G. Griffin, M. K. Hornstein, K.-N. Hu, K. E. Kreischer,, M. Rosay, M. A. Shapiro, J. R. Sirigiri, R. J. Temkin, and P. P. Woskov,
“Operational Characteristics of a 14 Watt, 140 GHz Gyrotron for Dynamic Nuclear Polarization,
IEEE Transactions on Plasma Science **34**, 518-523 (2006)

V. S. Bajaj, M. K. Hornstein, K. E. Kreischer, P. P. Woskov, J. R. Sirigiri, , P. P. Woskov, M. L. Mak, J. Herzfeld, R. J. Temkin, and R.G. Griffin, “250 GHz CW gyrotron oscillator for dynamic nuclear polarization in biological solid state NMR,” Jour. Magnetic Resonance **189** 251–279 (2007)

References (continued)

Pulsed DNP

Integrated Solid effect

NOVEL

RF-DNP

S. Un, T. Prisner, R.T. Weber, M.J. Seaman, K.W. Fishbein, A.E. McDermott, D.J. Singel, and R.G. Griffin,
"Pulsed Dynamic Nuclear Polarization at 5T", Chem. Phys. Lett., 189, 54 (1992)

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,”
J. Chem. Physics **113,** 6759-6802 (2000)

V. Weis and R.G. Griffin
“Electron-nuclear cross polarization”
Solid State Nuclear Magnetic Resonance, **29**, 105-117 (2006)

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. Am. Chem. Soc. **124,** 8808-8809 (2002)

References -continued

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
Proc. Natl. Acad. Sci. U. S. A. **2003**, *100*, 10158-10163.

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”
J. Am. Chem Soc. J. Am. Chem. Soc. **128**, 9428-9432 (2006)

DNP - brief history


Overhauser


Slichter

*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**)*

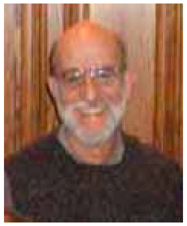
Liquids -- Hauser, Mueller-Warmuth, Richards, and others

Solids -- Abragam, Goldman, Provotorov, and others

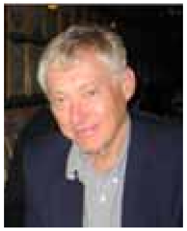
Nuclear magnetic ordering, polarized targets for particle physics, liquids at low fields


Abragam


Goldman


Yannoni


Wind


Schaefer

*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

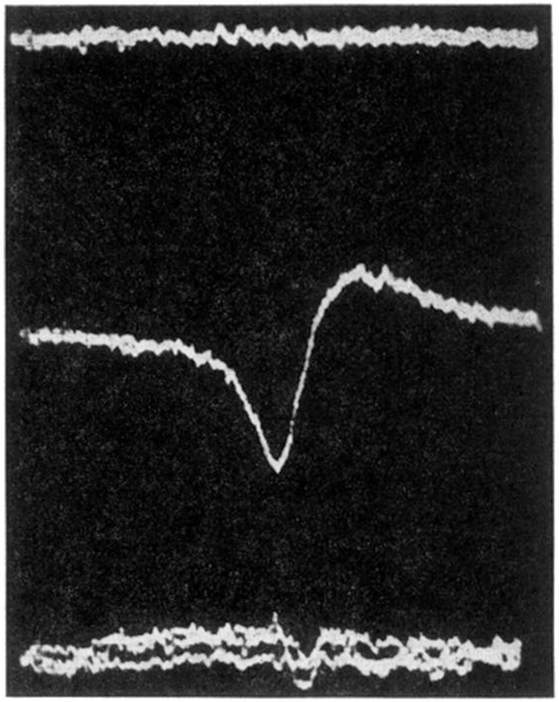
Melissa Hornstein
and her
460 GHz gyrotron
oscillator

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}Li w/o DNP

^{7}Li w/ DNP @ 84 MHz

^{1}H glycerol

FIG. 1. Oscilloscope pictures of 50-kc/sec nuclear resonance absorption *vs* static magnetic field. Field excursion 0.2 gauss. Top line: Li^7 resonance (lost in noise). Middle line: Li^7 resonance enhanced by electron saturation. Bottom line: Proton resonance in glycerin sample.

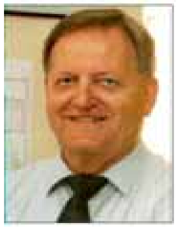
- *^{7}Li NMR @ $\omega_0/2\pi$= 50 kHz (30.3 Gauss)*
- *EPR @ $\omega_0/2\pi$= 84 MHz*

$\varepsilon \sim 100$

- 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)

DNP - brief history


Overhauser


Slichter

*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**)*

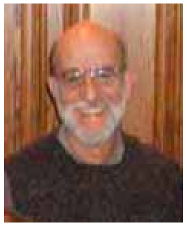
Liquids -- Hauser, Mueller-Warmuth, Richards, and others

Solids -- Abragam, Goldman, Atsarkin, Provotorov, and others

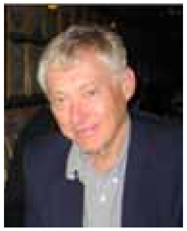
Nuclear magnetic ordering, polarized targets for particle physics, liquids at low fields


Abragam


Goldman


Yannoni


Wind


Schaefer

*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

Technique	***Authors***	***Enhancement (for ^{15}N)***
Fourier Transform NMR	Ernst and Anderson	~10-100
Polarization Transfer CP and INEPT	Hartmann & Hahn; Pines, Gibby & Waugh; Morris & Freeman	10
Indirect Detection (HSQC)	Bodenhausen and Ruben	~30
B_0 --200 to 800 MHz	Oxford, Bruker, Magnex	8
Cryoprobes	Peter Styles	2-4
TROSY	Pervushin, et.al.	2-5
High Frequency **Dynamic Nuclear Polarization**	Becerra,Gerfen,Prisner, McDermott, Un,Hall,Farrar, Rosay, Weis, Bennati, Hu, Bajaj, and RG^2	**~ 50-400**

Nobel Prize

Wolf Prize x 2

Nobel Prize

$$\varepsilon = 50\text{-}400$$

Significant consequences for NMR -- savings of ~ 2500 - 160,000 in time -- NEW SCIENCE !

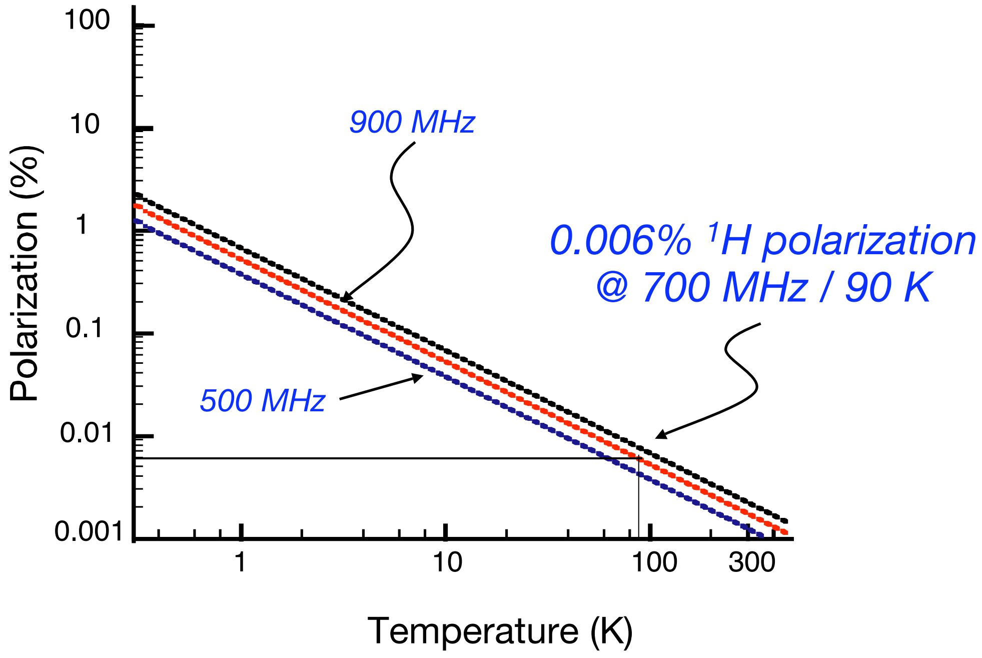
What are the THREE most important parameters in magnetic resonance

- Signal-to-noise

- Signal-to-noise

- Signal-to-noise

Nuclear Spin Polarization
Temperature and Field Dependence



POLARIZATION

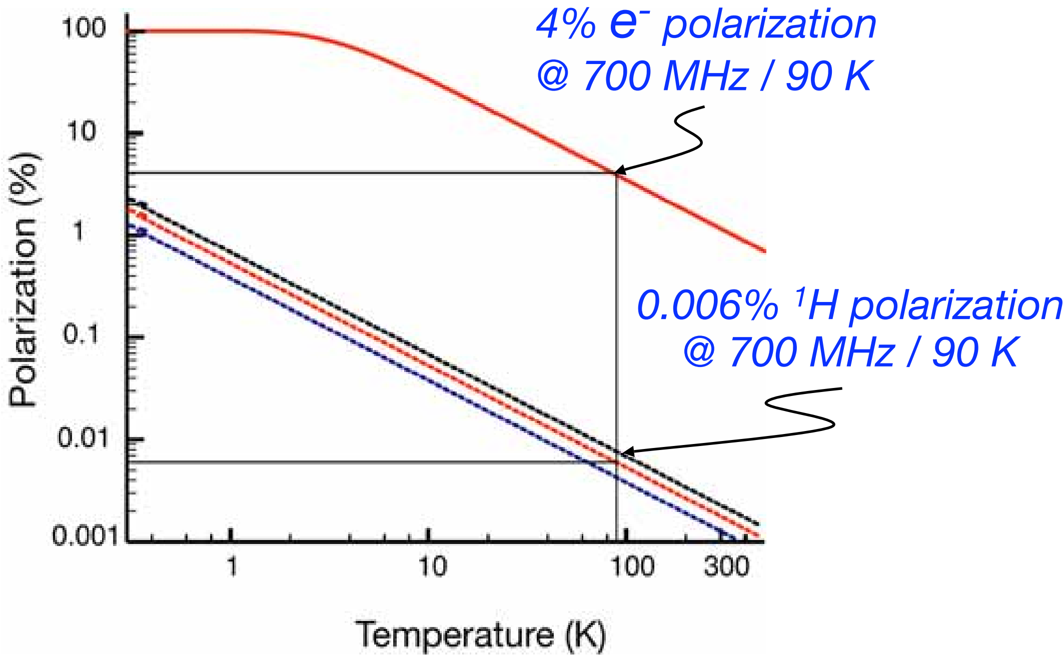
$$P = \frac{n^+ - n^-}{n^+ + n^-} = \tanh\left(\frac{\gamma\hbar B_0}{2kT}\right)$$

$$P = \frac{\gamma\hbar B_0}{2kT}$$

- **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% ^{1}H polarization @ 700 MHz / 90 K

POLARIZATION

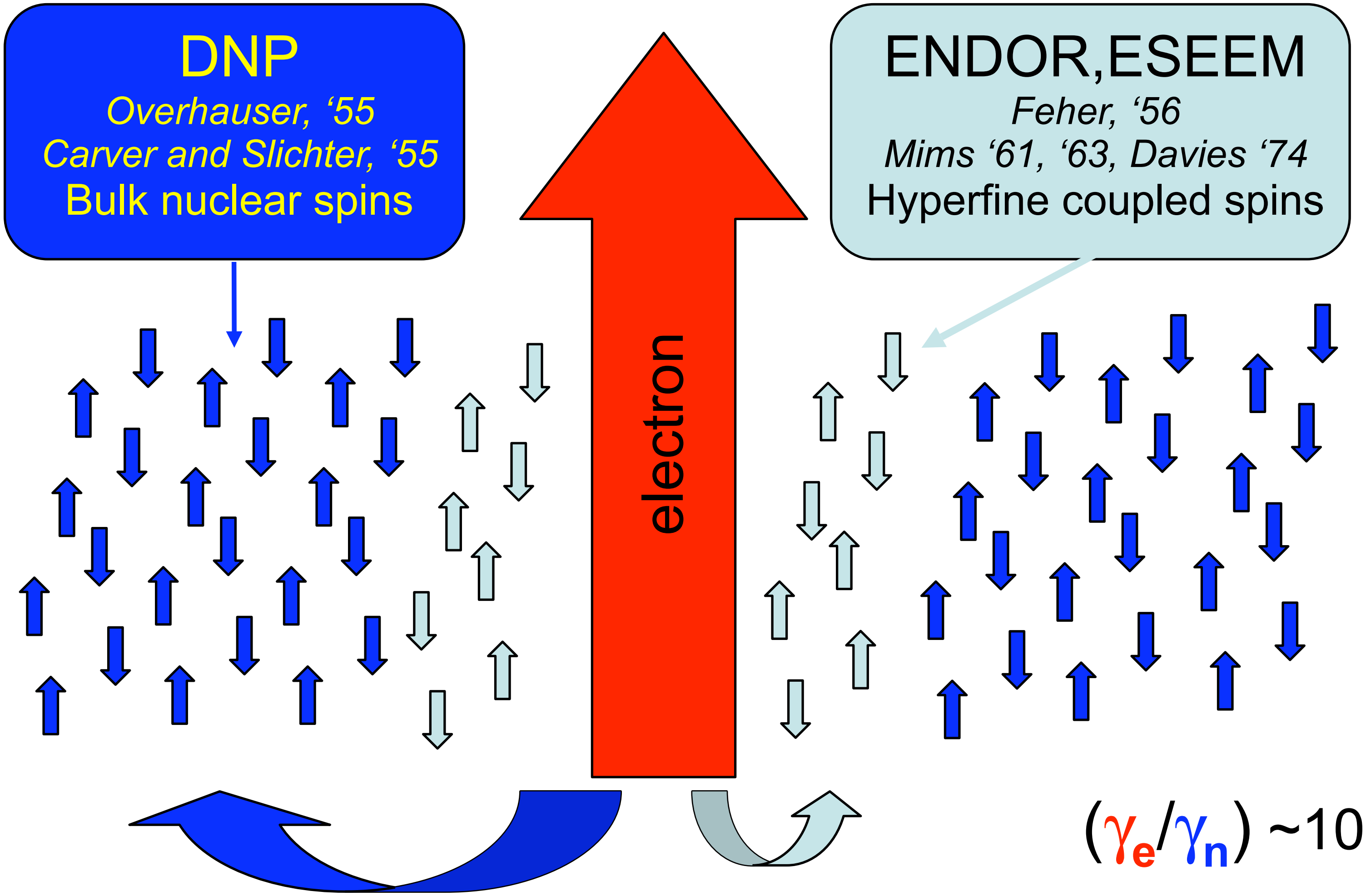
$$P = \frac{n^+ - n^-}{n^+ + n^-} = \tanh\left(\frac{\gamma\hbar B_0}{2kT}\right)$$

$$P = \frac{\gamma\hbar B_0}{2kT}$$

$$(\gamma_e/\gamma_{1H}) \sim 660$$

- 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



DNP

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

ENDOR,ESEEM

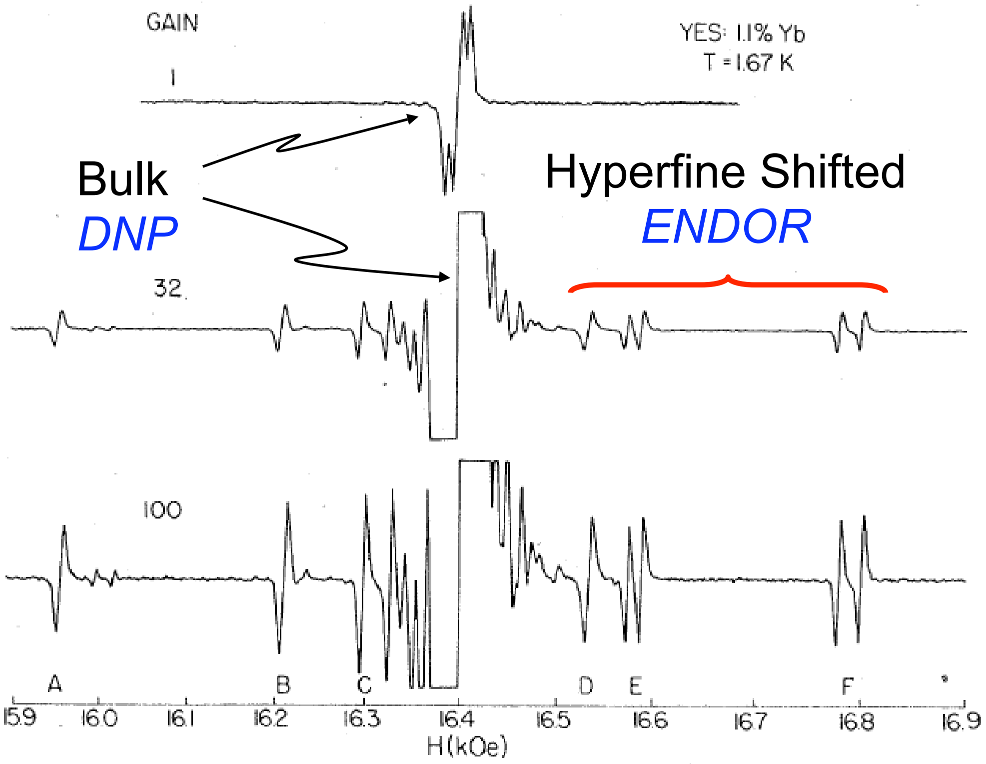
Feher, '56
Mims '61, '63, Davies '74
Hyperfine coupled spins

$(\gamma_e/\gamma_n) \sim 1000$

- How do we make electrons talk to nuclear spins ?
  With ENDOR, ESSEM and DNP

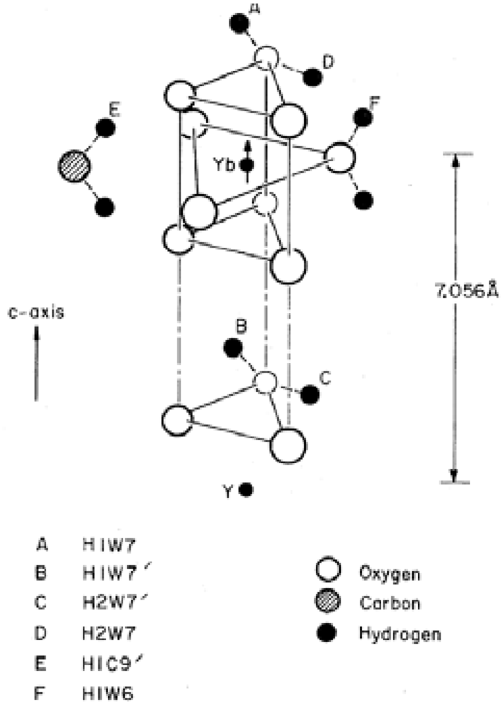
NMR Spectroscopist's View of ENDOR

Yttrium Ethyl Sulfate (YES) $9H_2O$:1.1% Yb^{3+} @ 1.67 K



Bulk *DNP*

Hyperfine Shifted *ENDOR*

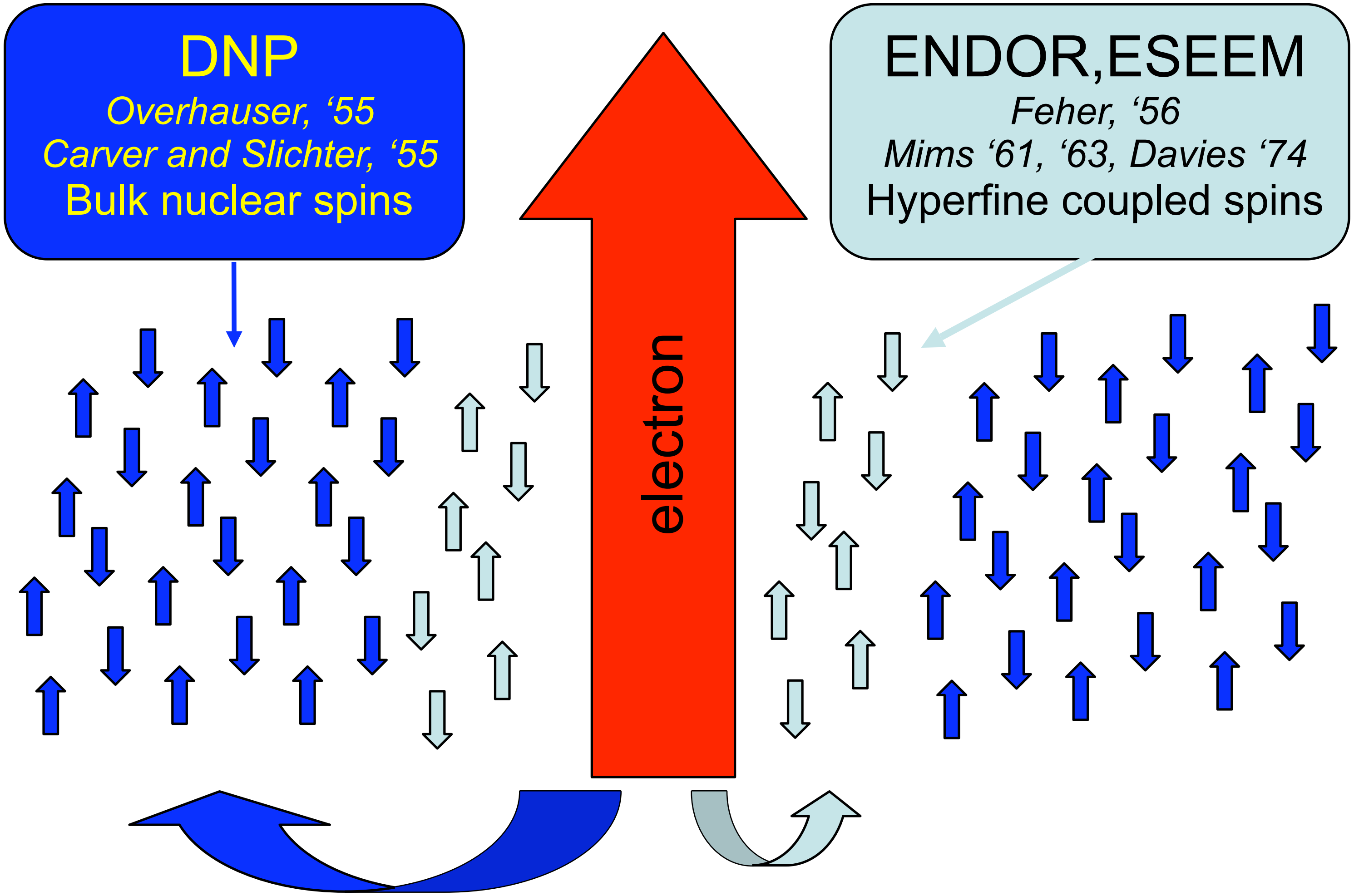


J.P. Wolfe, Phys. Rev. 16, 128(1977)

- ^1H'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 *Hyperfine shifted $\Rightarrow$ ENDOR*

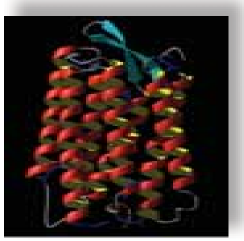
Transfer the Electron Polarization to



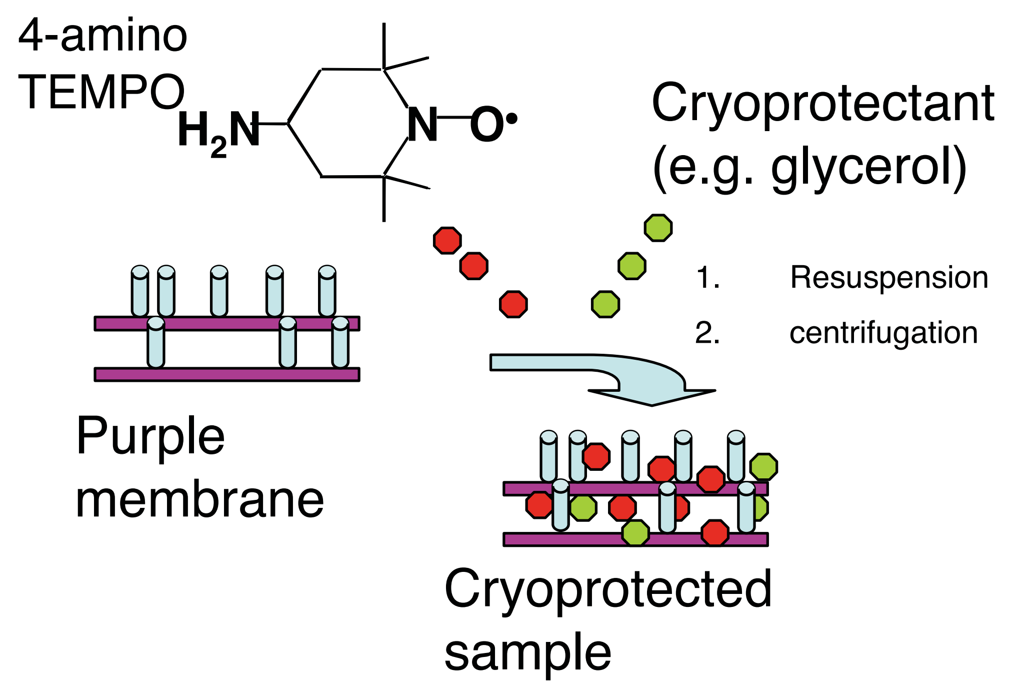
DNP
Overhauser, '55
Carver and Slichter, '55
Bulk nuclear spins

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

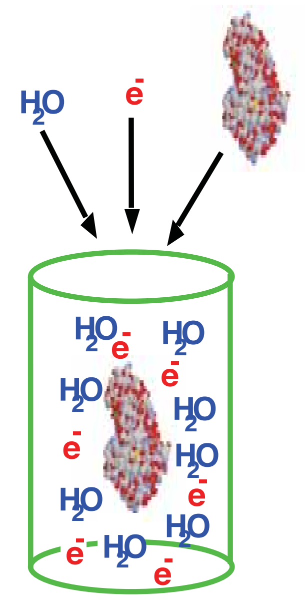
Cryoprotectant (e.g. glycerol)

1. Resuspension
2. centrifugation

Purple membrane

Cryoprotected sample

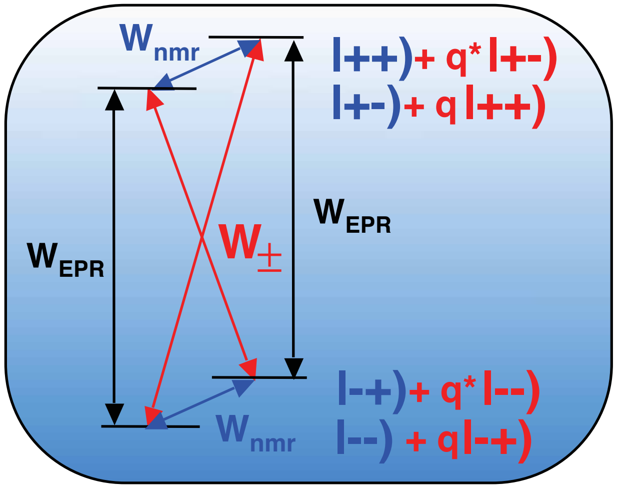
bacteriorhodopsin



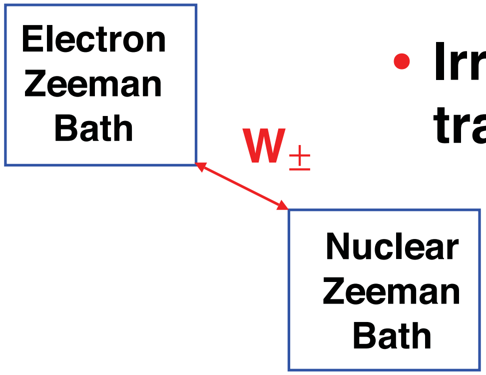
α-lytic protease

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

Dynamic Nuclear Polarization
Solid State Effect



$$|q| \propto \frac{D^{n.s.}_{e-n}}{\omega_n}$$

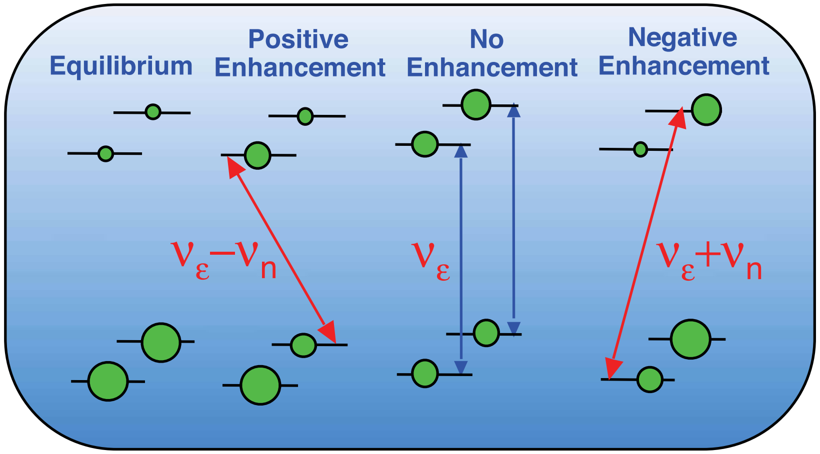


Electron Zeeman Bath

$W_{\pm}$

Nuclear Zeeman Bath

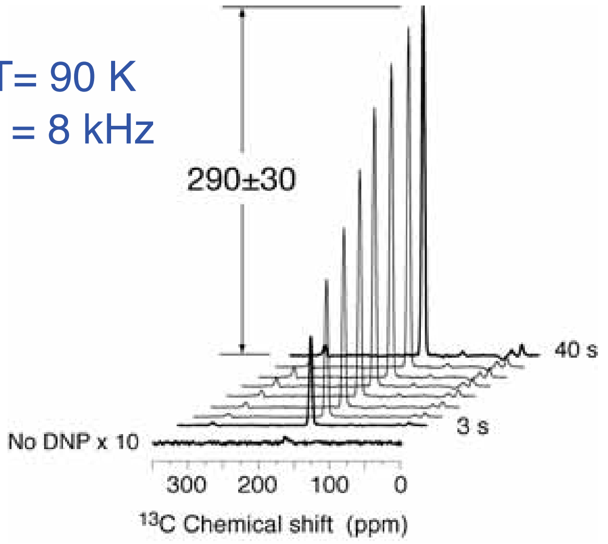
- Irradiate the flip-flop transitions

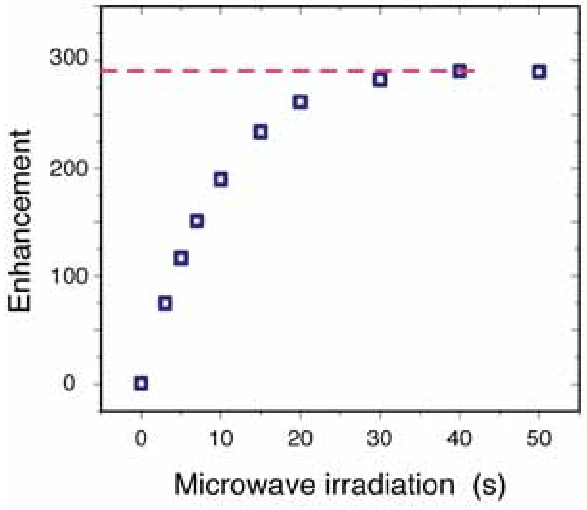


- **Enhancement** $\sim (\gamma_\varepsilon / \gamma_n)(\omega_1 / \omega_0)^2 (N_e / \delta) T_{1n}$

DNP Enhanced ^{13}C MAS Spectra
TOTAPOL/^{13}C-Urea

T= 90 K
ν_r = 8 kHz

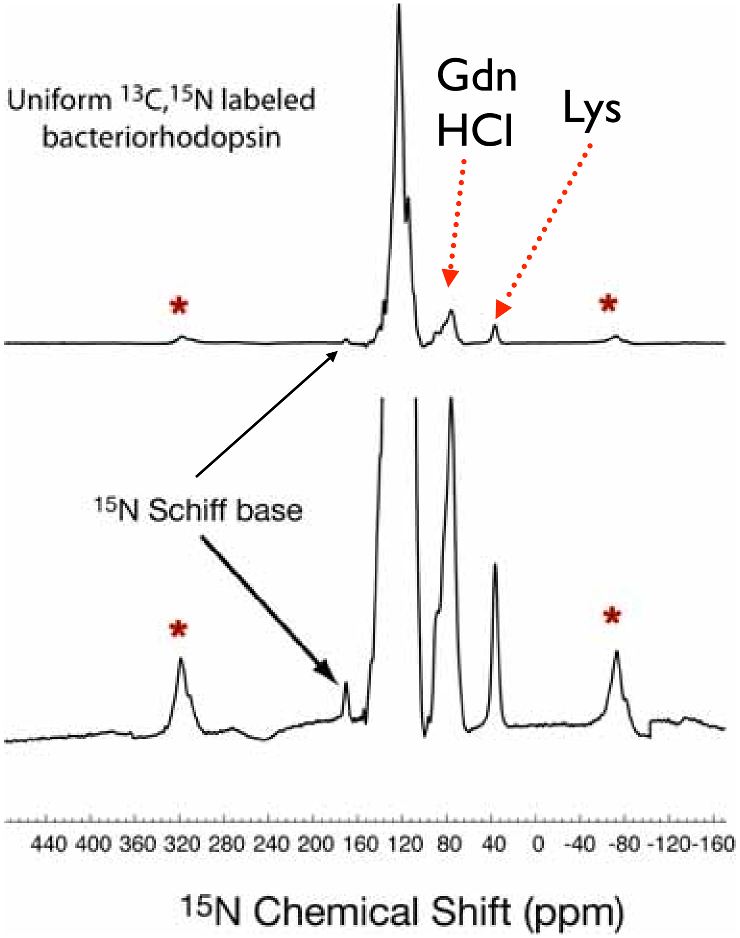




- ***Distance*** between the two TEMPO radicals in TOTAPOL yields an e^--e^- coupling of ~25 MHz
- Electron concentration ~ *10 mM* !
- Enhancements build up over ~40 seconds to a maximum of **290 ± 30 !**

Joo, Hu, Bryant and Griffin (2006)

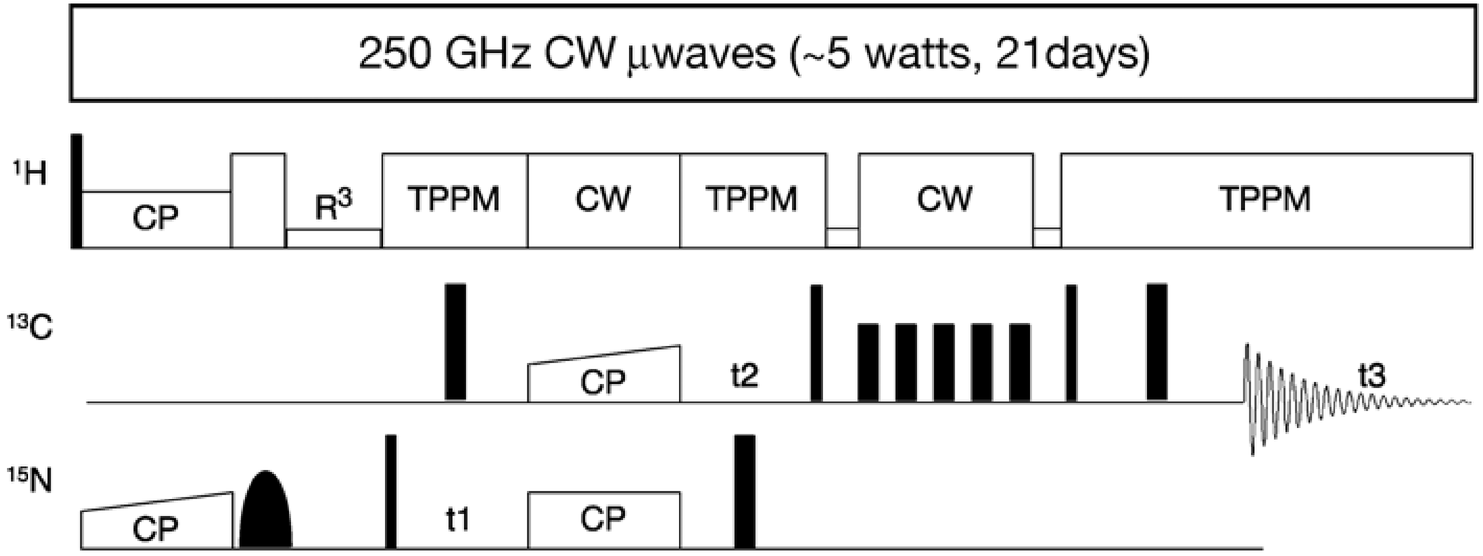
DNP Enhanced ^{15}N MAS Spectra of U-^{13}C,^{15}N-bR



- 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)

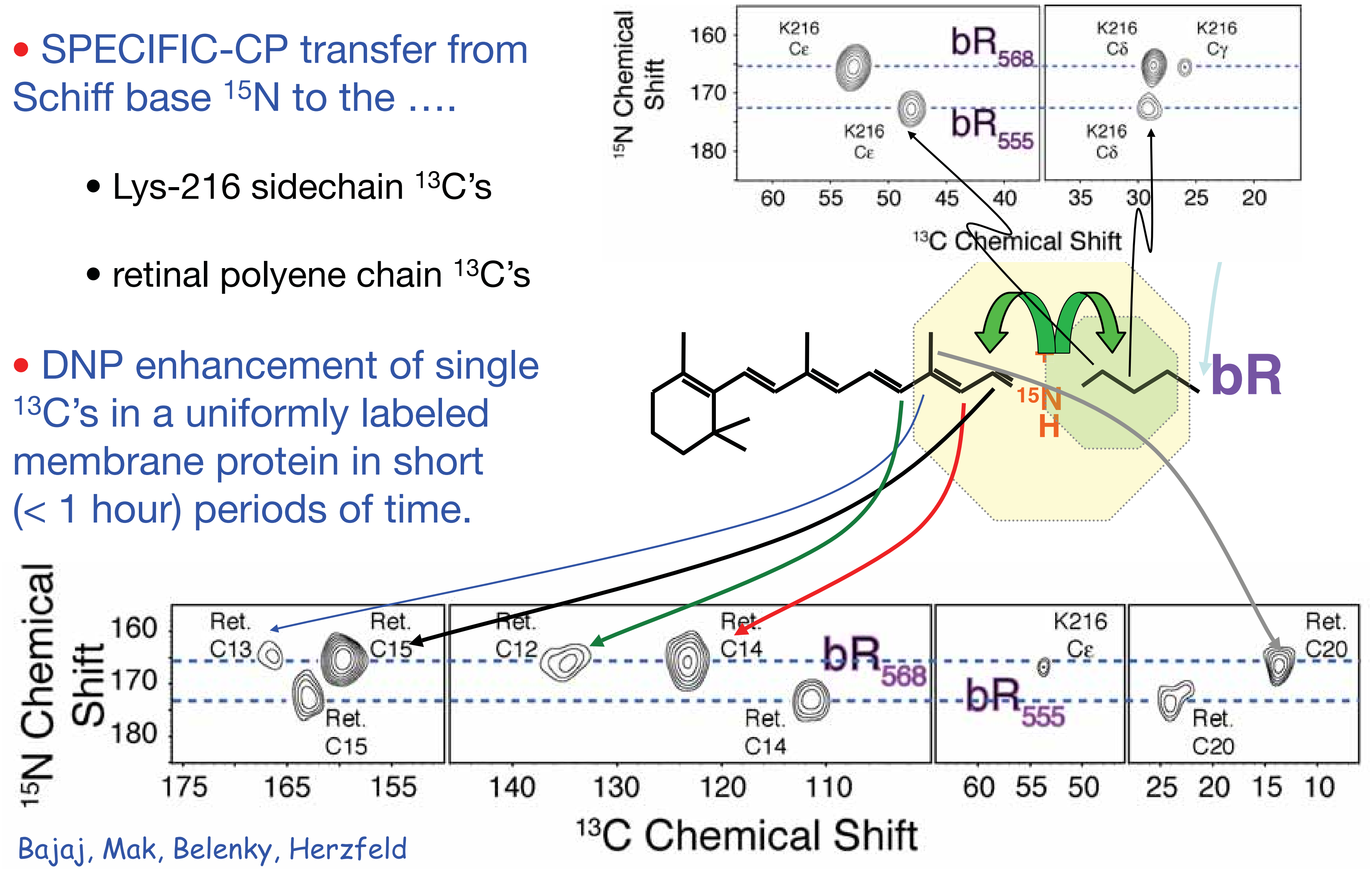
DNP Enhanced Selective 3D ^{15}N-^{13}C-^{13}C Experiment



- CW irradiation with 250 GHz microwaves (stability <1%)
- Select the Schiff base ^{15}N resonance with a Gaussian
- Transfer to the ^{13}C with SPECIFIC CP and RFDR

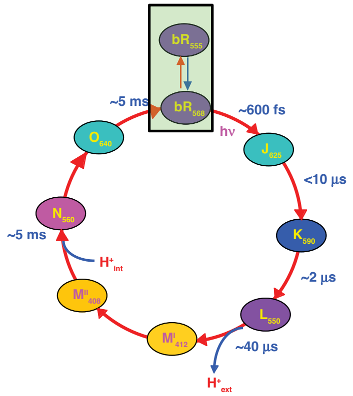
Assignment of Nζ-C15-Cx and Nζ-Cε-Cx

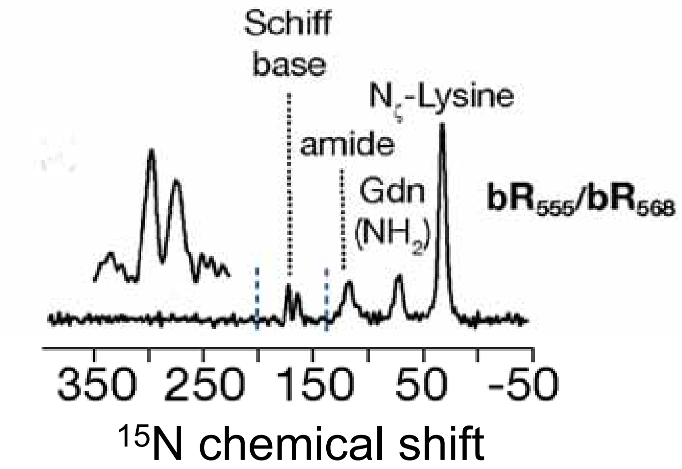
- SPECIFIC-CP transfer from Schiff base ^{15}N to the ….
  - Lys-216 sidechain ^{13}C's
  - retinal polyene chain ^{13}C's
- DNP enhancement of single ^{13}C's in a uniformly labeled membrane protein in short (< 1 hour) periods of time.



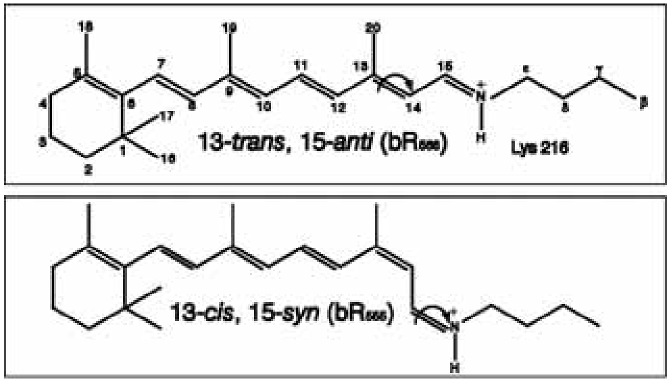
Bajaj, Mak, Belenky, Herzfeld (2007)

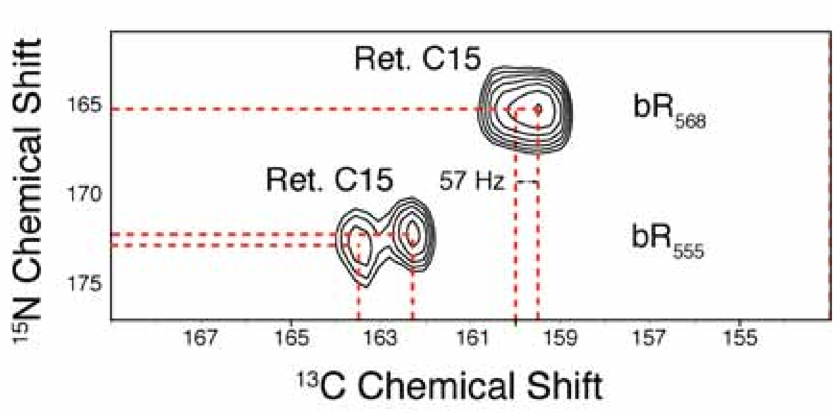
Correlation ^{15}N Schiff Base to ^{13}C-15





- bR_{568} has ^{13}C-^{13}C J-couplings
- bR_{555} has two confirmations

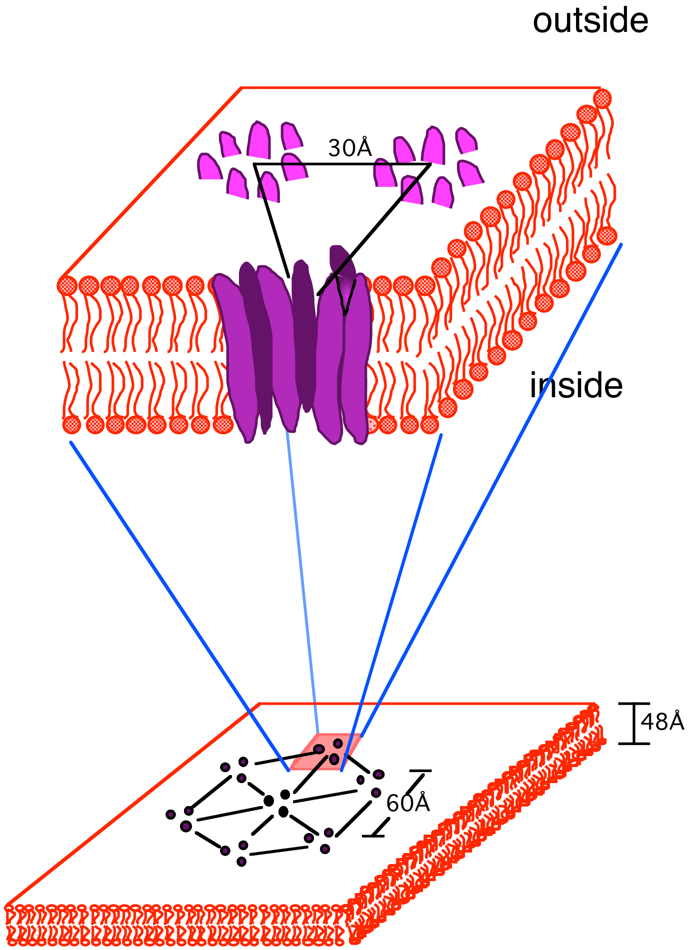




- Bacteriorhodopsin is a heterogeneous protein.
- Low temperatures quench dynamics

Bajaj, Mak, Herzfeld, Griffin (2007)

Bacteriorhodopsin Trimer

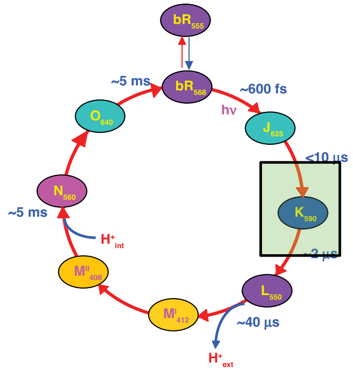


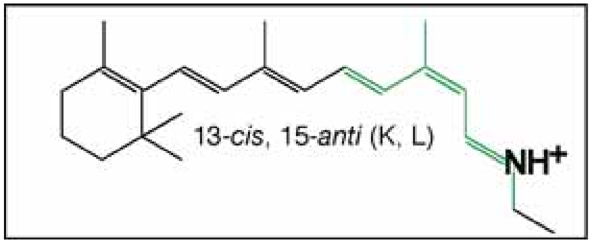
- Are the three members of the trimer equivalent ?

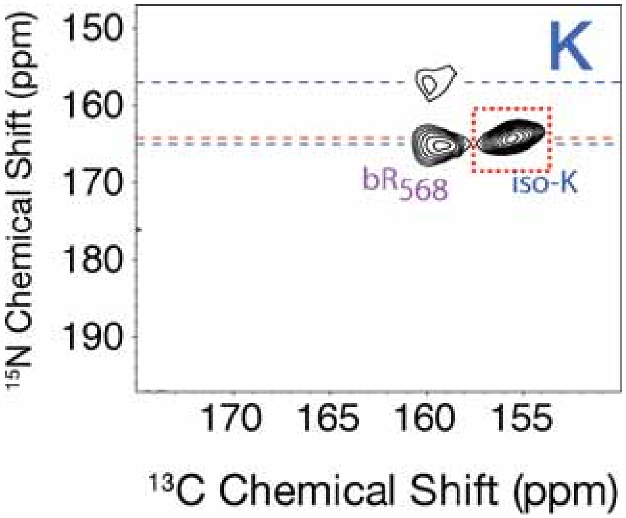
- Sheves et al Biochem **42**, 11281(2003) suggest they are not !

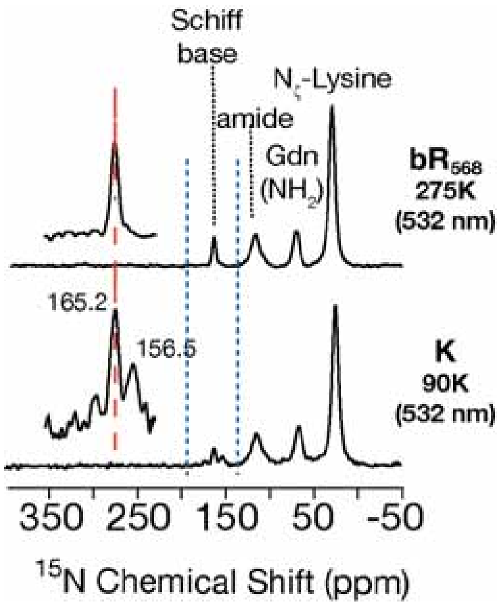
- ^{13}C-^{15}N spectra of bR_{555} indicates that the trimer is heterogeneous !

Heterogeneity in K
iso-K transient



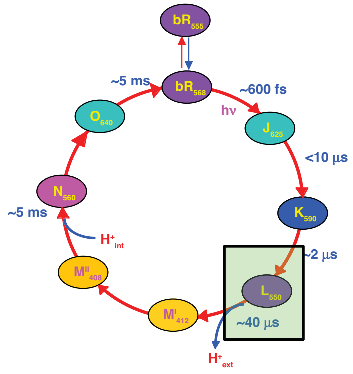


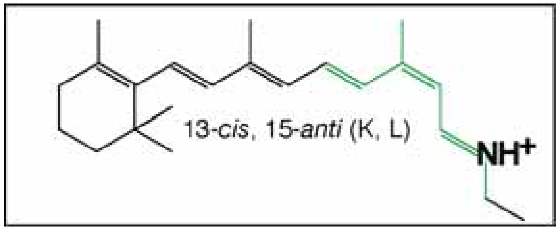


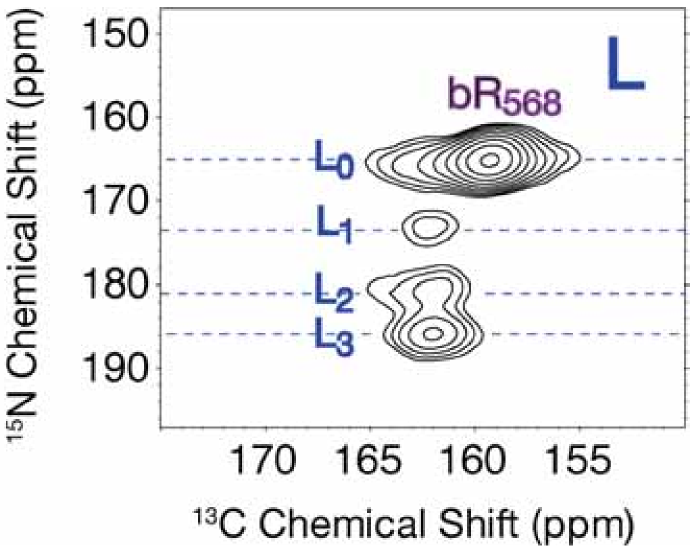


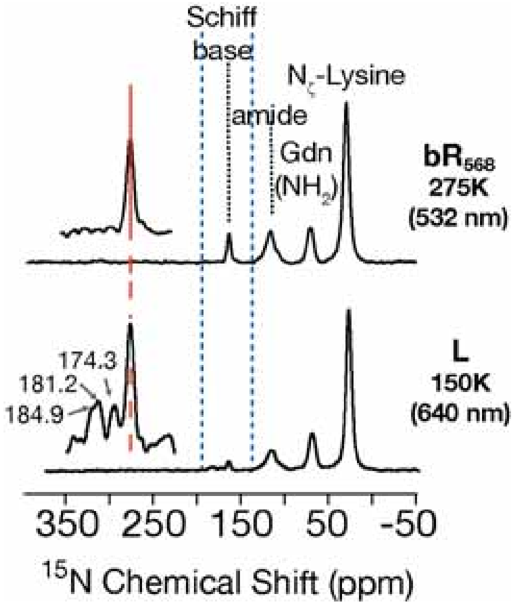
- Iso-K intermediate observed with non-linear sampling
  - decays over a period of hours
- K-shifted upfield from bR_{568}

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

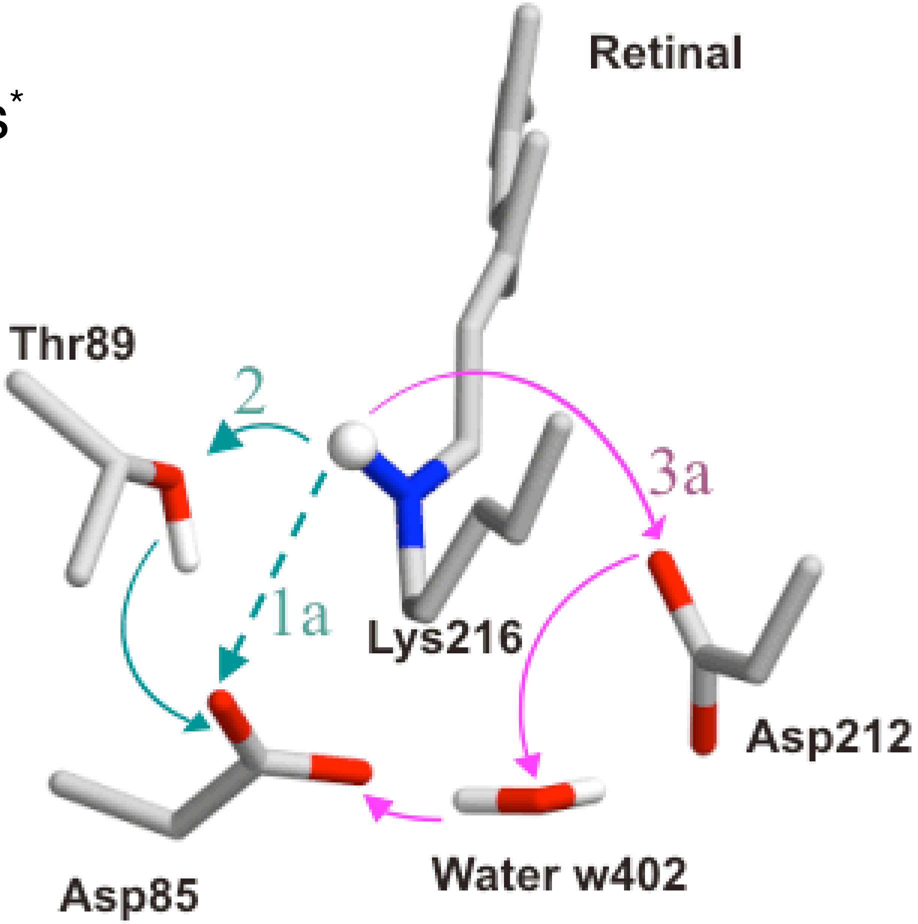
Proton Transfer Pathways in bR

Three QM/MM pathways all equally consistent with kinetics*

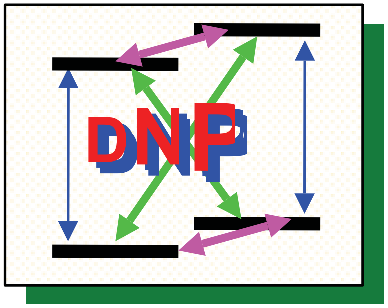
(1) Direct transfer:
SB-Ret → D85

(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

Outline

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

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

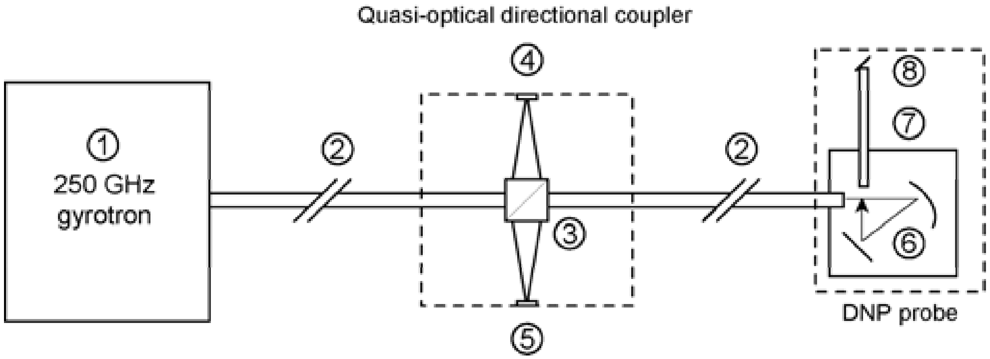
- DNP Enhanced MAS Spectra
  DNP Enhancements of *50-60* in MAS Spectra @ 90 K
  DNP functions quite effectively in broad class of systems

- Polarizing Agents and DNP Mechanisms

  Biradical polarizing agents ⇒ $\varepsilon = 175$
  Solid effect and cross effect DNP mechanisms

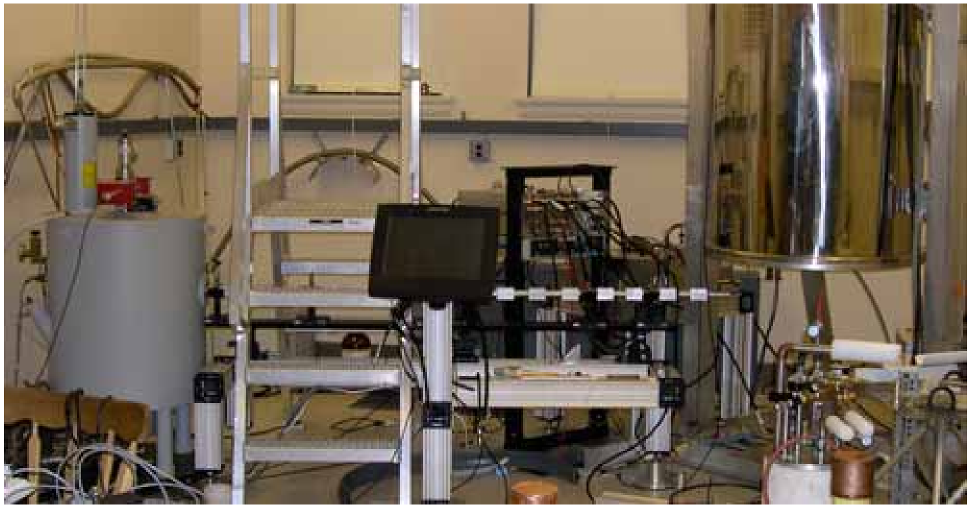
- DNP in Solution (and for Metabonomics)
  Solid state polarization and laser T-jump
  $\varepsilon^{\dagger} = \varepsilon(T_{obs}/T_{polar}) = 130\text{-}330$ for ^{13}C

High Frequency DNP Spectrometer



Three basic components

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



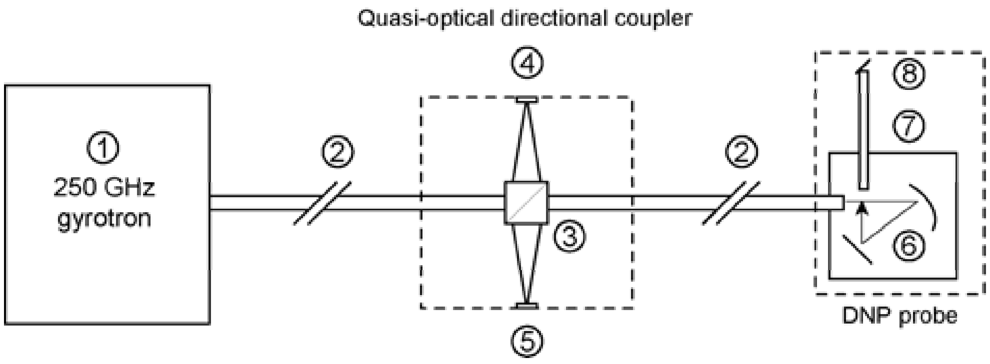
Bajaj, Kreischer, Woskow, Temkin

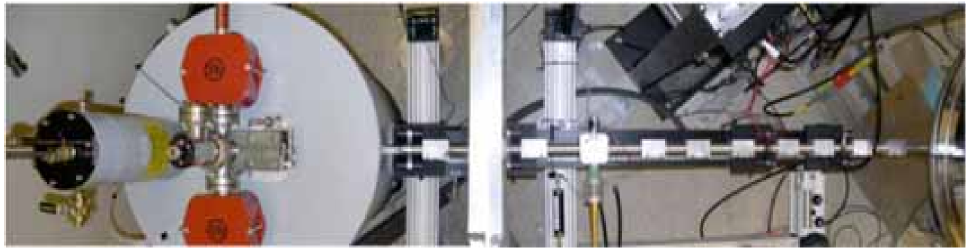
High Frequency DNP Spectrometer

Three basic components

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

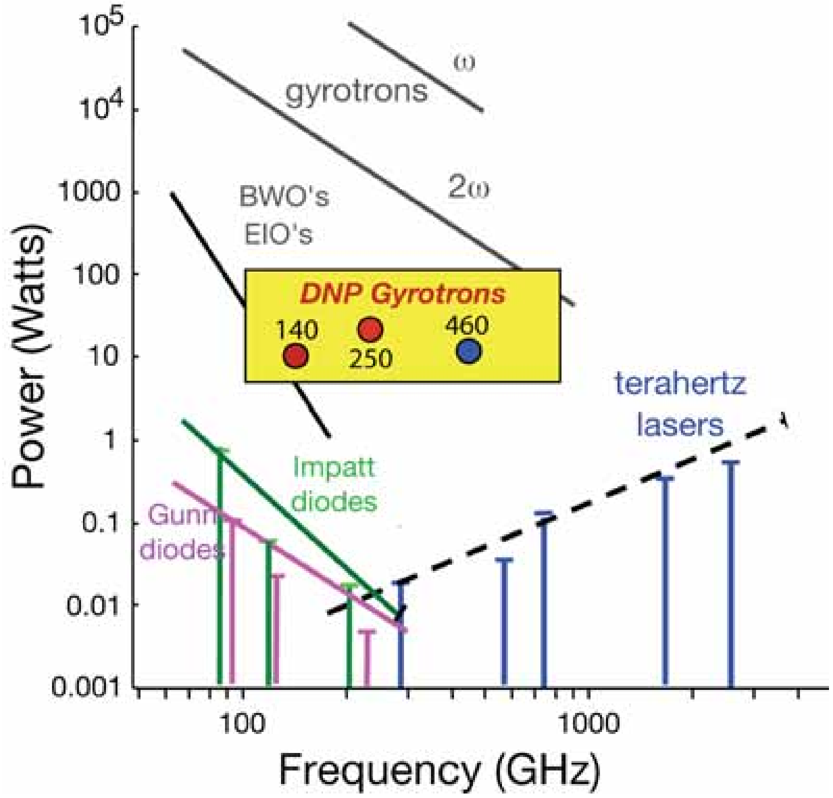
Bajaj, Kreischer, Woskow, Temkin





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



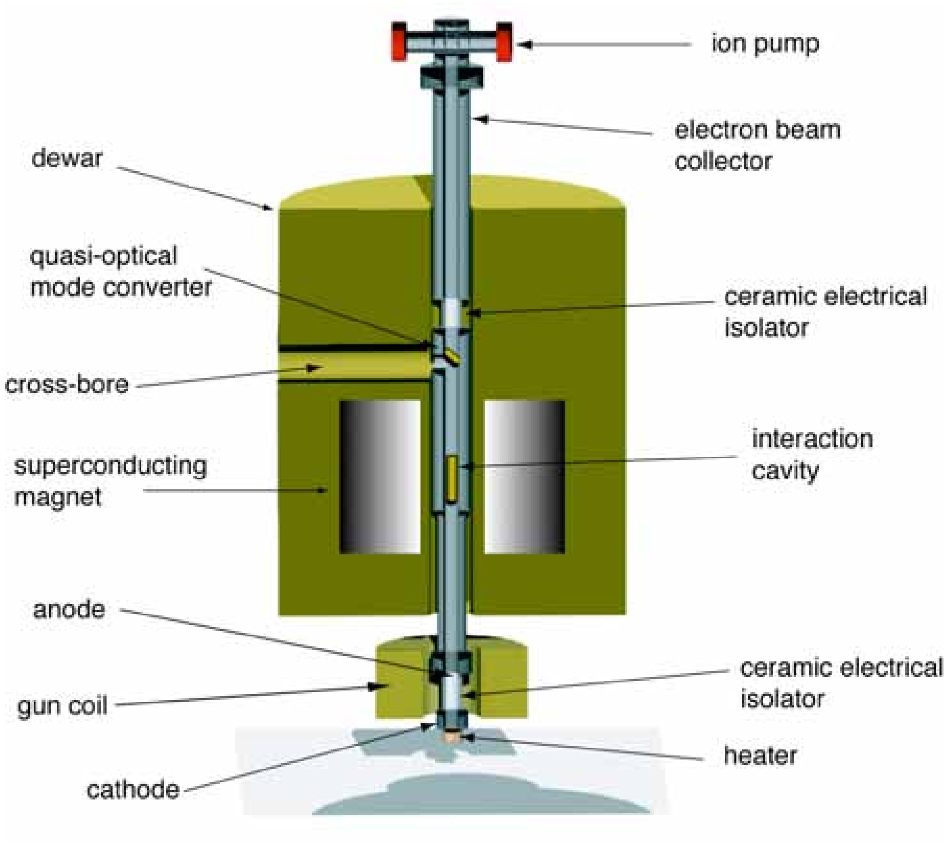
250/ 460 GHz Gyrotron

- 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



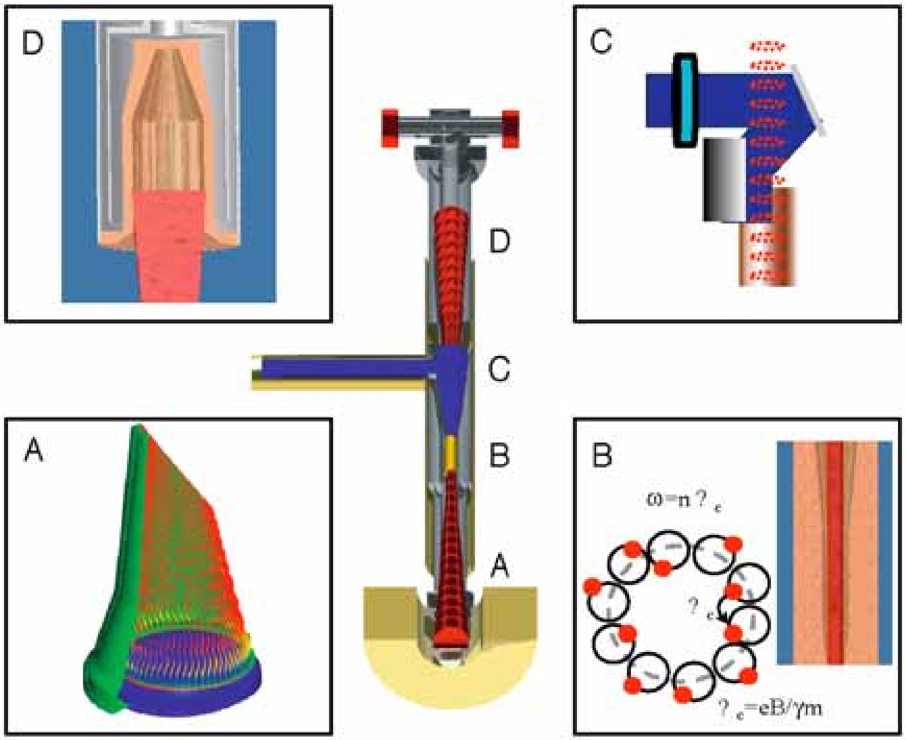
250/ 460 GHz Gyrotron

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



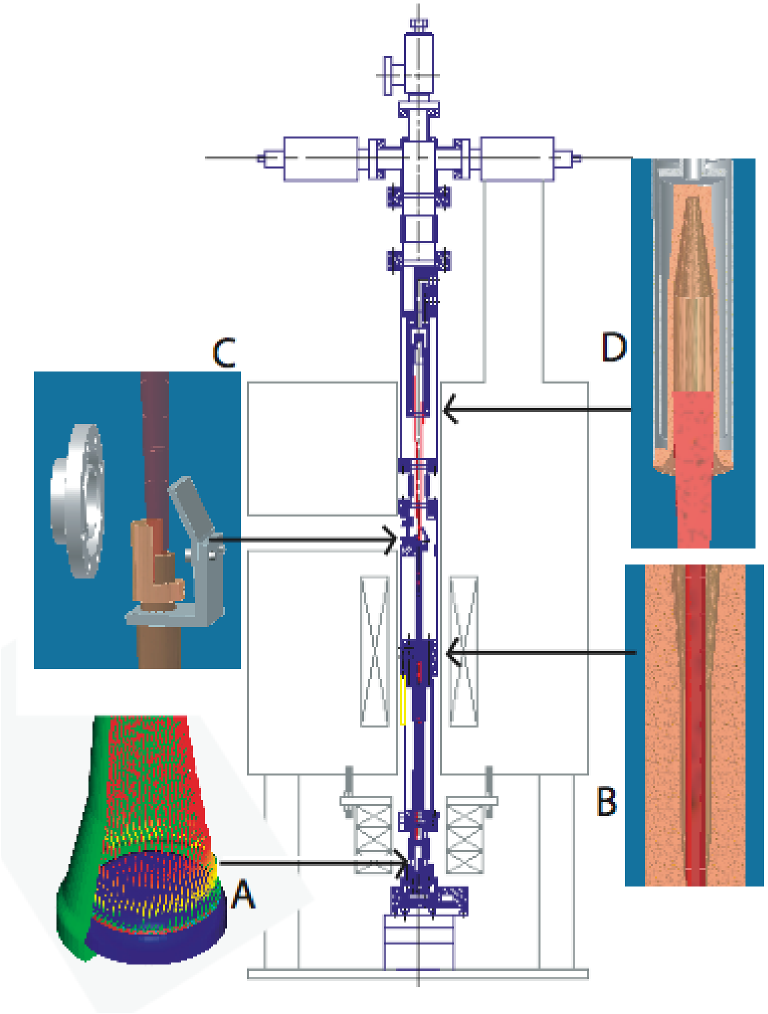
250/ 460 GHz Gyrotron

A: Electron emission from an annular ring

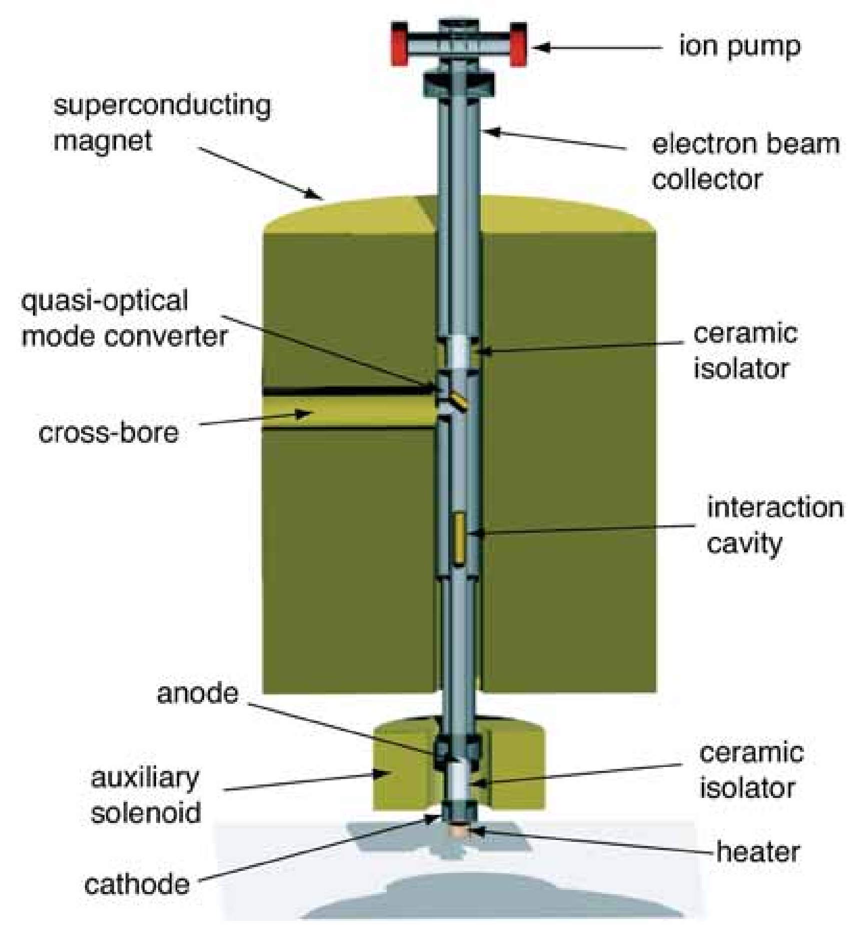
B: Bunching in the cavity and emission of microwaves

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

D: Electrons are collected in the collector

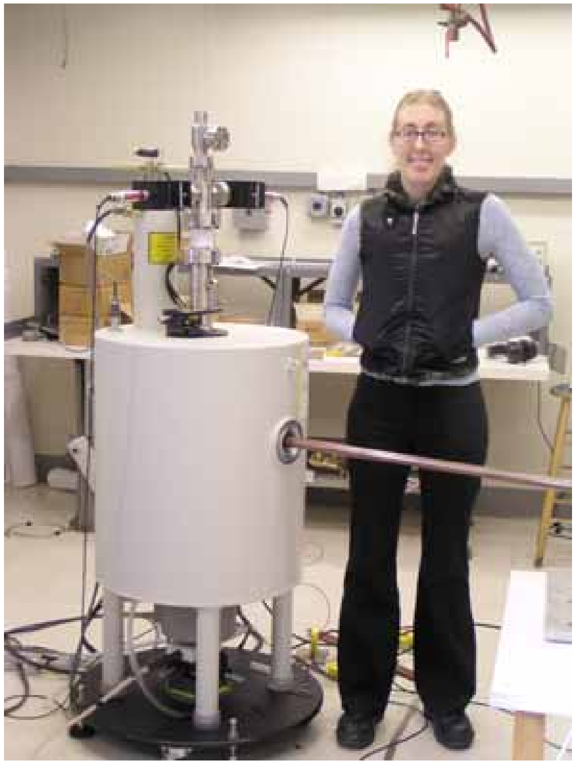


460 GHz/ 700 MHz Gyrotron Oscillator



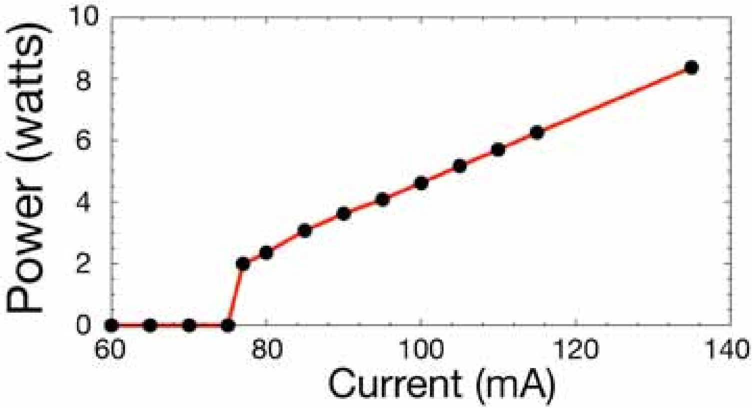
Hornstein,Kreischer, Temkin, et al (2004)

460 GHz Gyrotron Operation

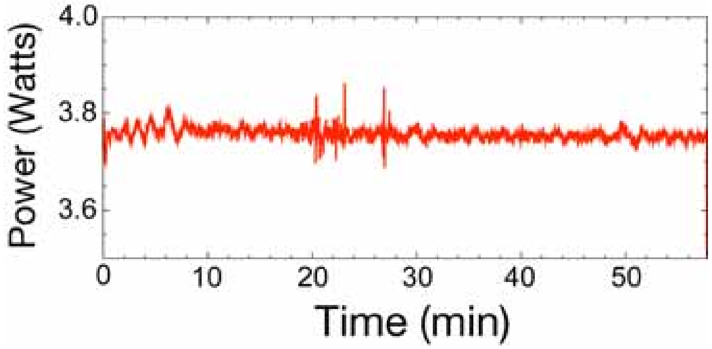


Melissa Hornstein and her
460 GHz gyrotron oscillator

8 watts CW power

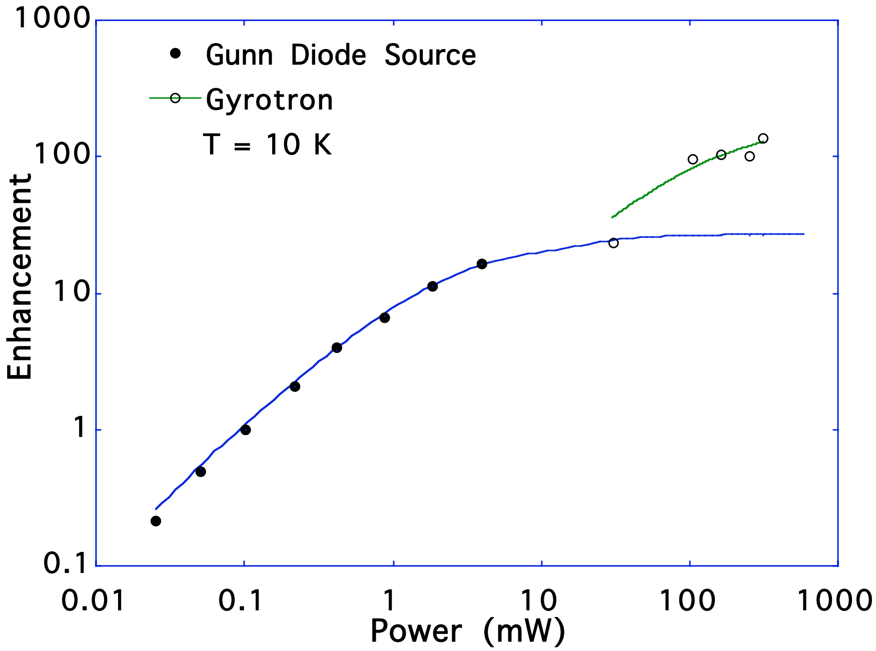


stable CW operation



- Time stability -- several hours of CW operation
- Record high powers -- ≤ 8 watts @ 460 GHz

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 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

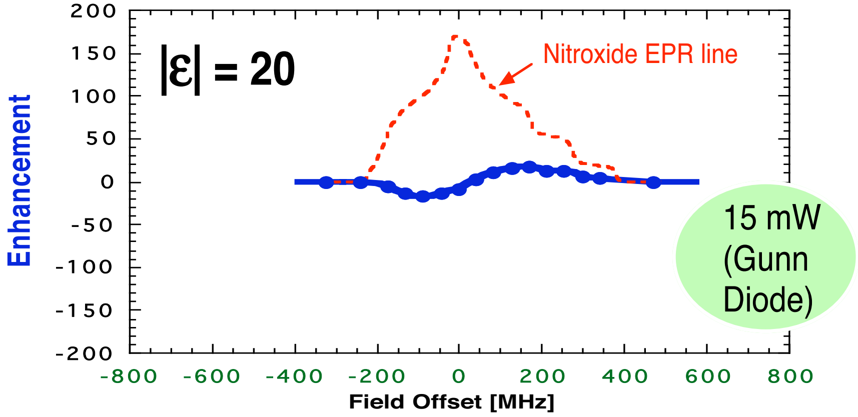
G. J. Gerfen, L. R. Becerra, D. A. Hall, and R. G. Griffin[a)]
Francis Bitter National Magnet Laboratory and Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

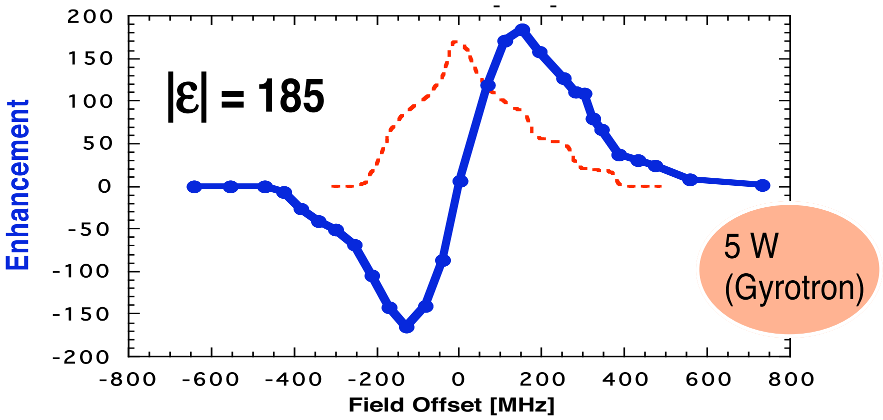
R. J. Temkin
Plasma Fusion Center, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

D. J. Singel
Department of Chemistry, Montana State University, Bozeman, Montana 59717

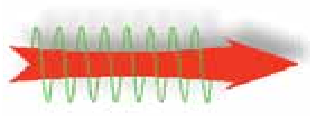
(Received 16 February 1995; accepted 20 March 1995)

J. Chem. Phys., **102** , 9494-9497 (1995).



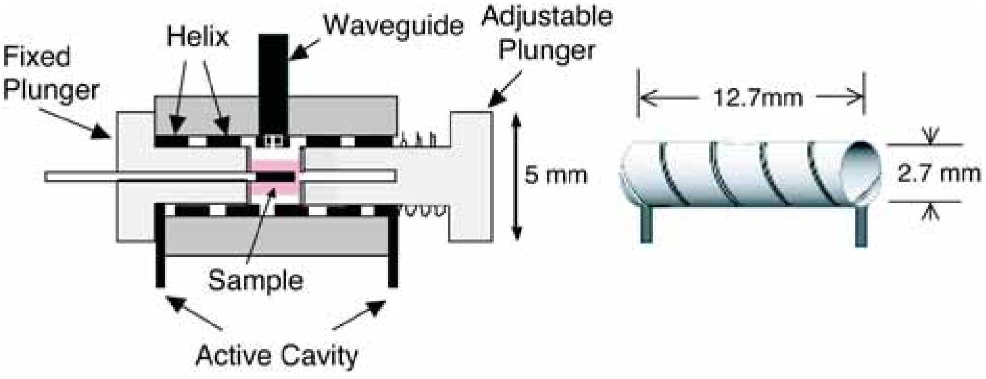


- DNP in aqueous media — biological samples
- Three spin mechanism applicable at high fields



Microwave Resonators

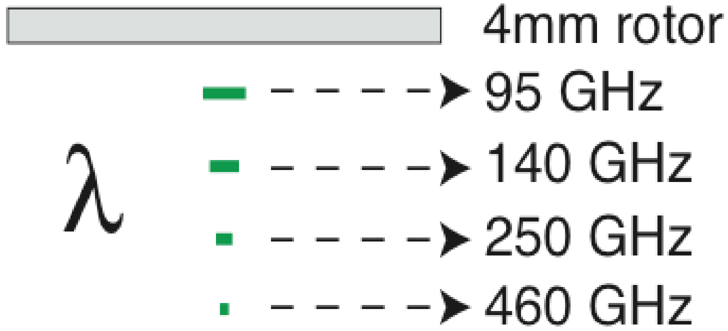
Solid state source with microwave resonant cavity



- TE_{011} resonator with 30 mW Impatt diode source

V=0.5 µl

Weis, et al JMR (1999)



4mm rotor

λ

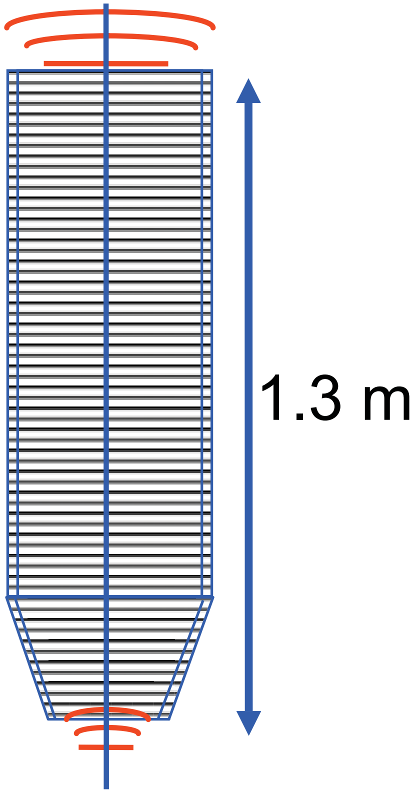
- - - - -➤ 95 GHz
- - - - -➤ 140 GHz
- - - - -➤ 250 GHz
- - - - -➤ 460 GHz

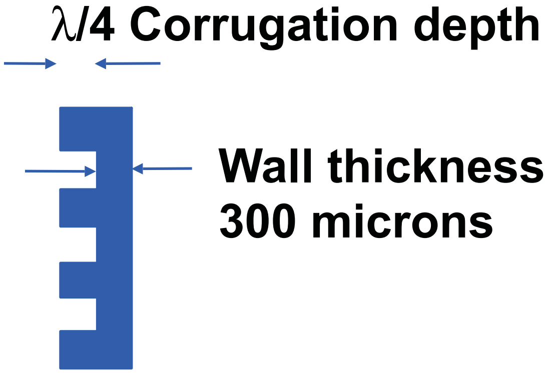
- Rotor dimensions are comparable to λ -

V~ 40-60 µl

- MAS sample size complicates design of high-Q cavity
- Smaller volume decreases S/N by ~200-300 !

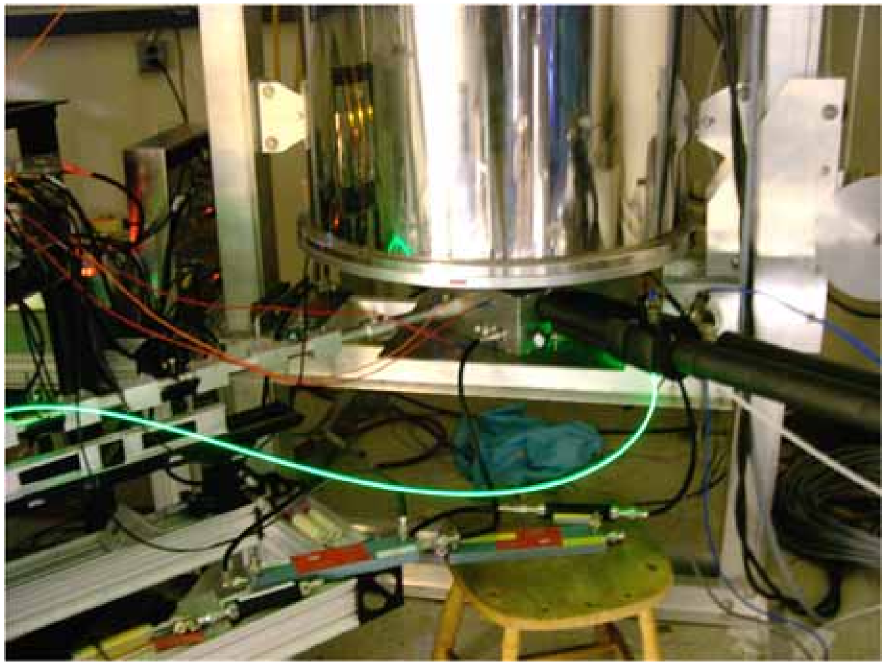
Corrugated Waveguide

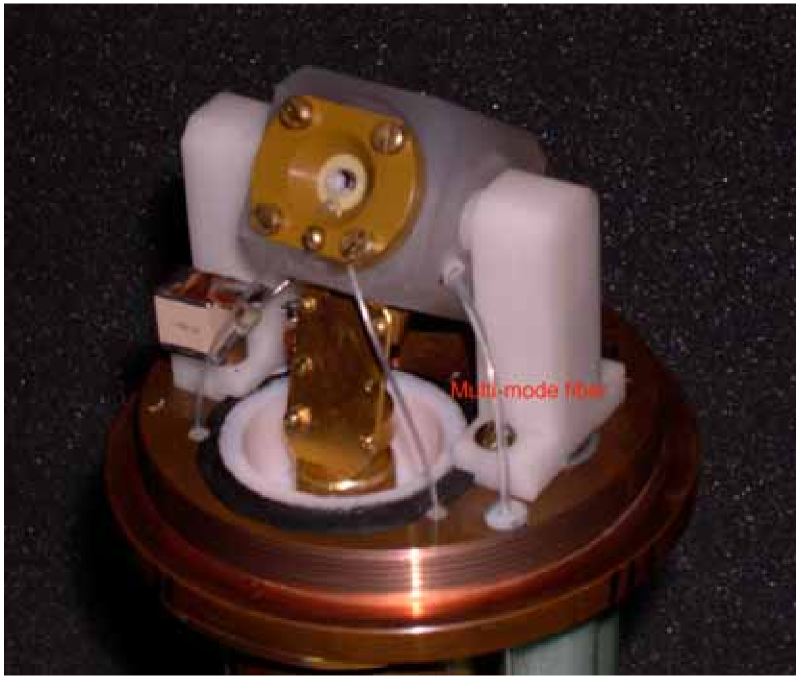




- Very low insertion loss (0.01dB/m)
- Cryogenic Operation
- Excellent mode and polarization characteristics

Quadruple Resonance DNP/MAS Probe w/ Optical Irradiation of the Sample





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

Vik Bajaj, Jeff Bryant

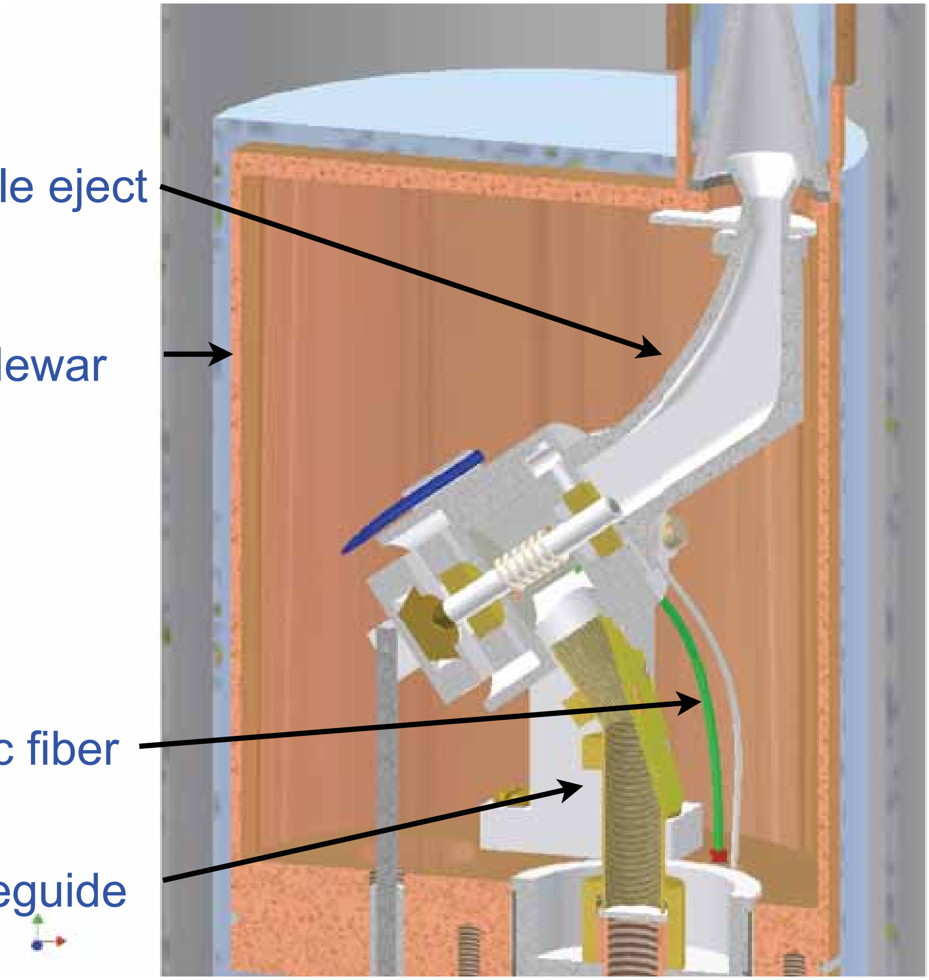
Cambridge Instruments

DNP Cryogenic MAS Probe

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

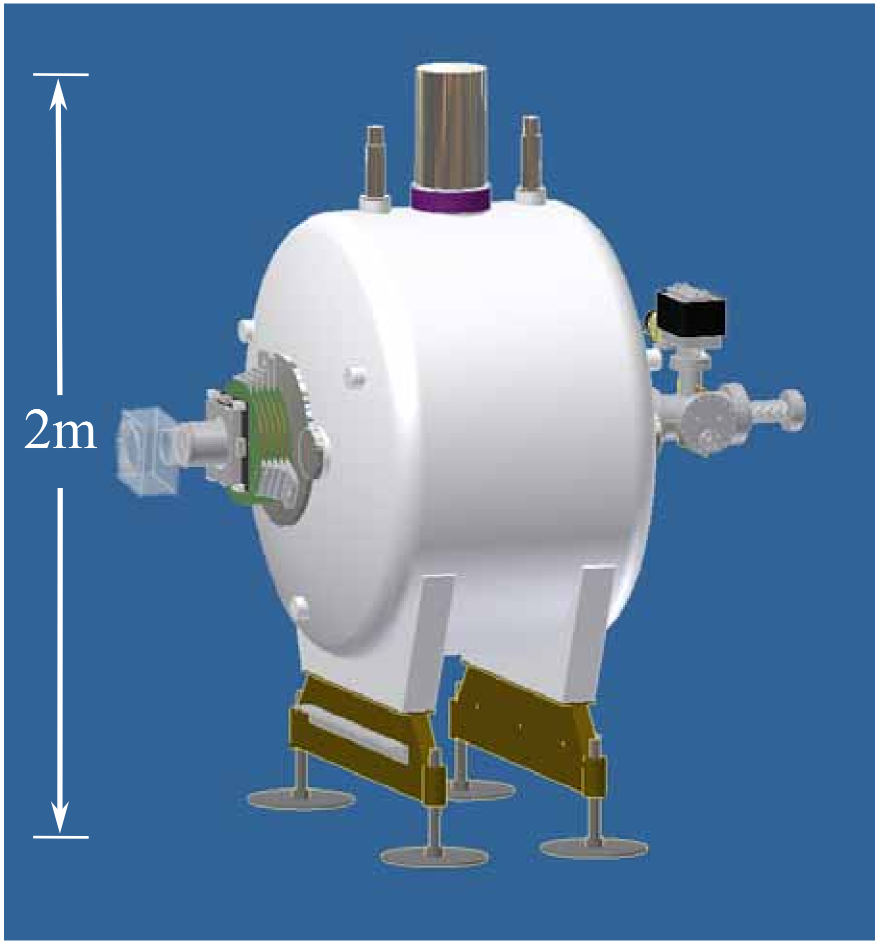


140 GHz Amplifier - Pulsed DNP & EPR

- Provides phase shifted pulses @ 140 GHz for

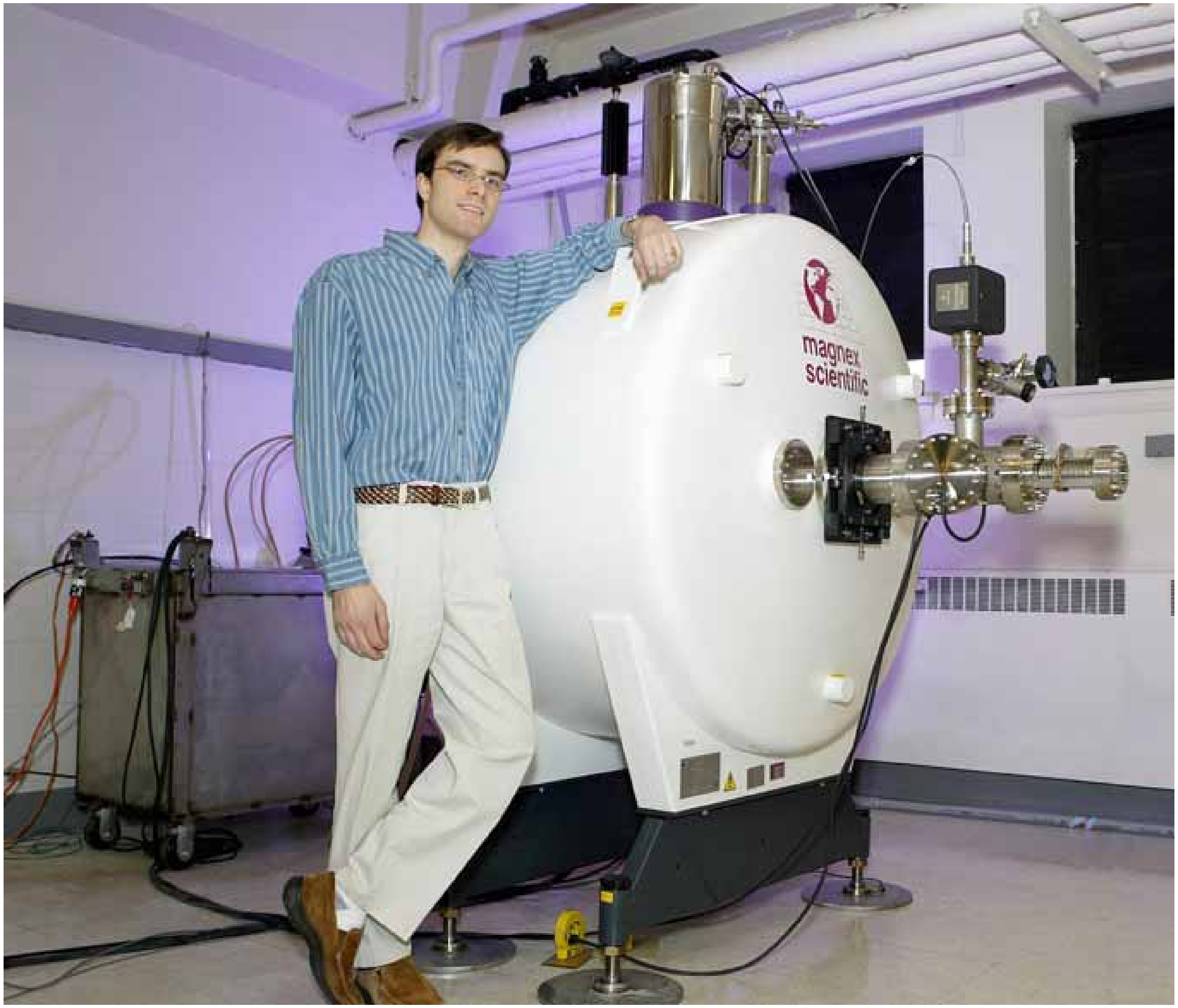
  pulsed DNP and EPR

  - 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

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
  - *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
  - $\varepsilon^{\dagger} = \varepsilon(T_{obs}/T_{polar}) =$ *130-330* for ^{13}C

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 ….

$$\delta \sim \Delta << \omega$$

δ=homogeneous linewidth of the EPR spectrum

Δ=breadth of the EPR spectrum

ω=nuclear Larmor frequency (^{1}H, ^{13}C, ^{15}N)

CW Dynamic Nuclear Polarization Mechanisms

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

$$\delta, \Delta >> \omega$$

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

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

$$\Delta > \omega > \delta$$

CE -- operative at high fields where $\Delta g >> \delta$, 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)
π -- 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

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 ….

$$\delta \sim \Delta << \omega$$

δ=homogeneous linewidth of the EPR spectrum

Δ=breadth of the EPR spectrum

ω=nuclear Larmor frequency (^{1}H, ^{13}C, ^{15}N)

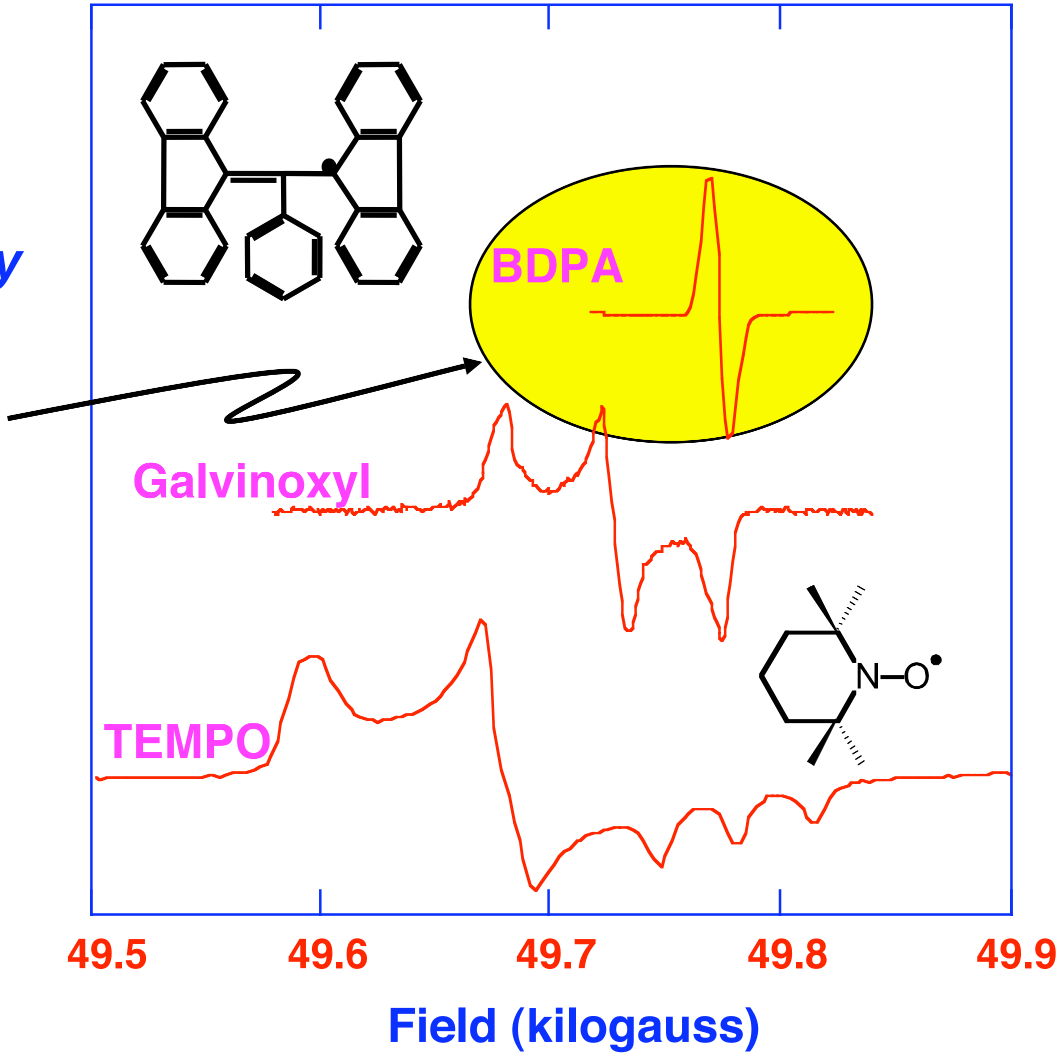
Paramagnetic Centers for DNP

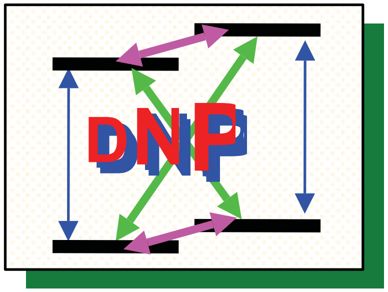
- **EPR lineshapes are Dominated by *g- anisotropy***

- **BDPA linewidth ~21 MHz**
  ---- Solid effect

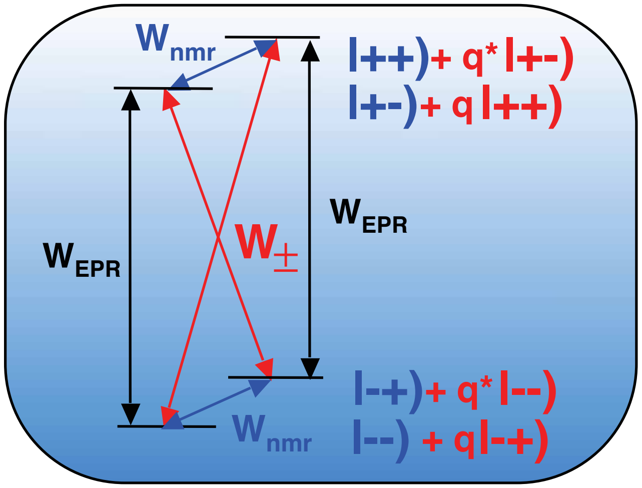
- **TEMPO powder pattern ~600 MHz**
  ---- Thermal mixing or cross effect

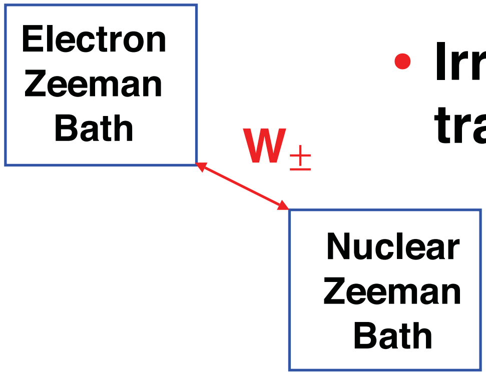
$\omega_e/2\pi = 28$ GHz/T





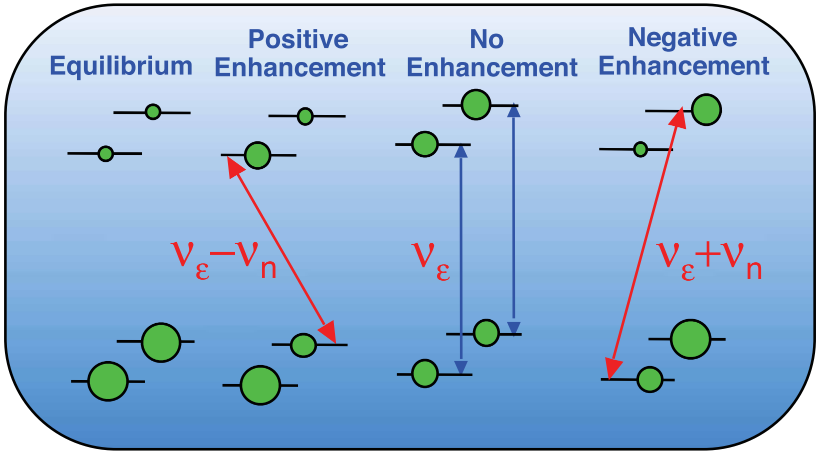
Dynamic Nuclear Polarization
Solid State Effect





- **Irradiate the flip-flop transitions**

$$|q| \propto \frac{D^{n.s.}_{e-n}}{\omega_n}$$



- **Enhancement** $\sim (\gamma_\varepsilon/\gamma_n)(\omega_1/\omega_0)^2(N_e/\delta)T_{1n}$

DNP with Gyrotrons

VOLUME 71, NUMBER 21 PHYSICAL REVIEW LETTERS 22 NOVEMBER 1993

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. Griffin[1]

[1] *Francis Bitter National Magnet Laboratory and Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139*

[2] *Plasma Fusion Center, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139*

[3] *Department of Chemistry, Harvard University, Cambridge, Massachusetts 02138*

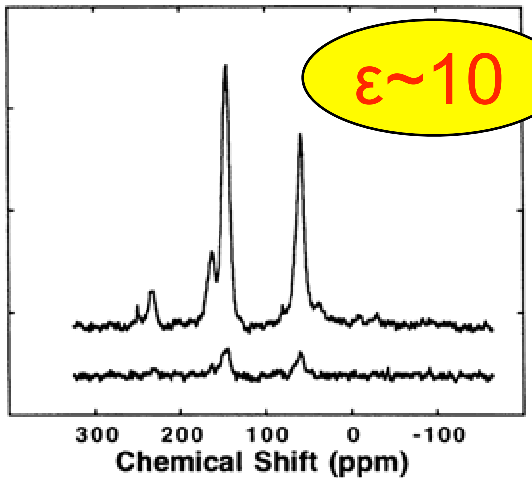
(Received 26 July 1993)

$(\gamma_e/\gamma_n) \sim 660$

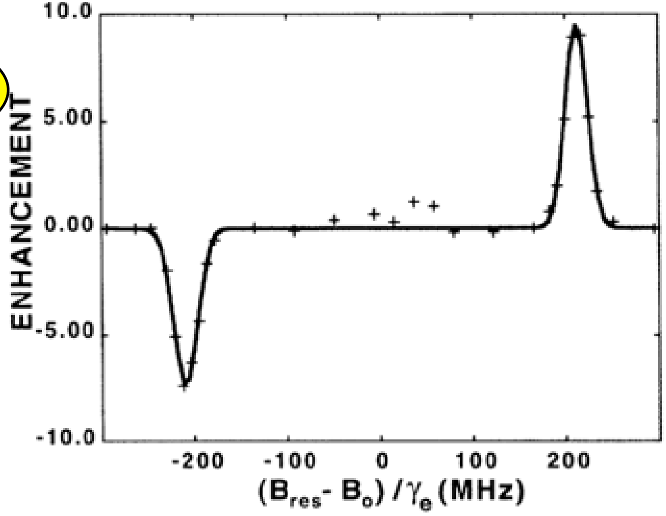
DNP (dynamic nuclear polarization) experiments at 5 T are reported, in which a cyclotron resonance maser (gyrotron) is utilized as a 20 W, 140 GHz microwave source to perform the polarization. MAS (magic angle spinning) NMR spectroscopy with DNP has been performed on samples of polystyrene doped with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

1.5% efficient

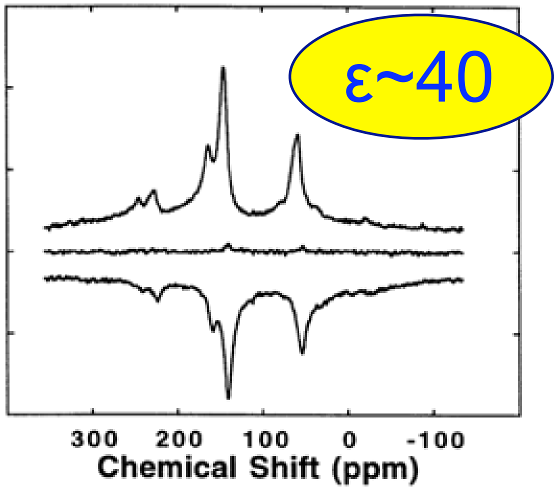
$e^- \rightarrow {}^1H \rightarrow {}^{13}C$



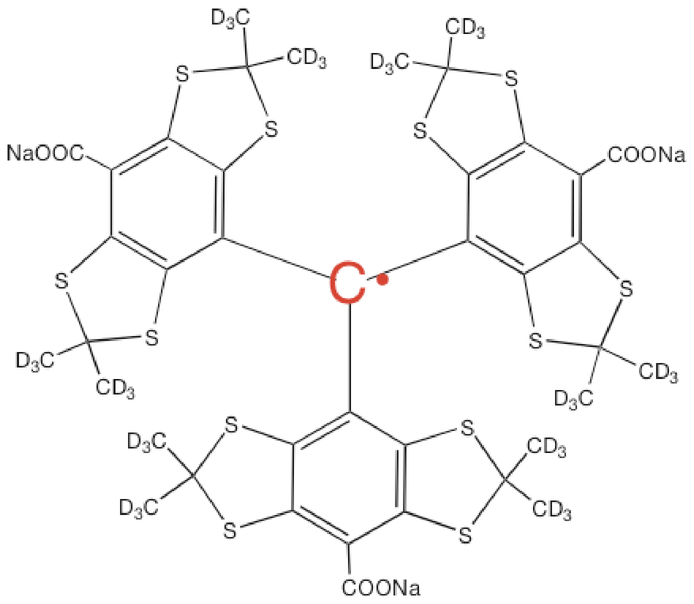
ε_{max} @ $\omega_e \pm \omega_n$

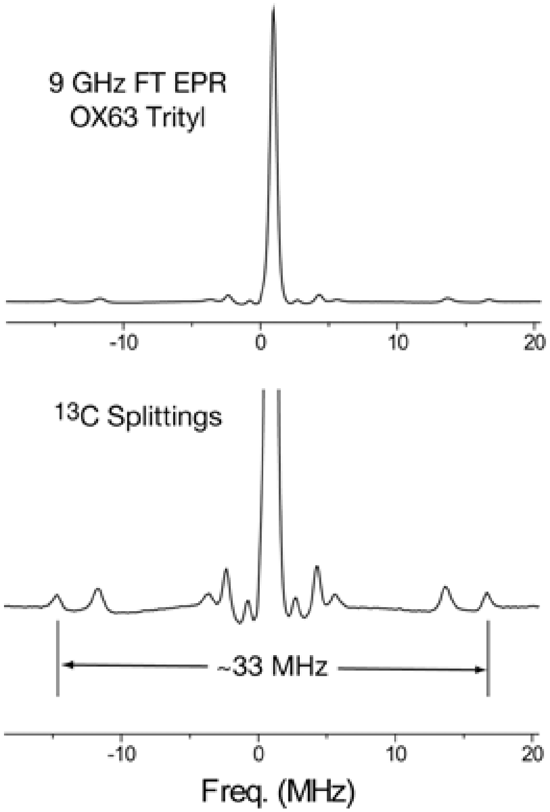


$e^- \rightarrow {}^{13}C$



Trityl Radical
Structure and FT EPR Spectrum

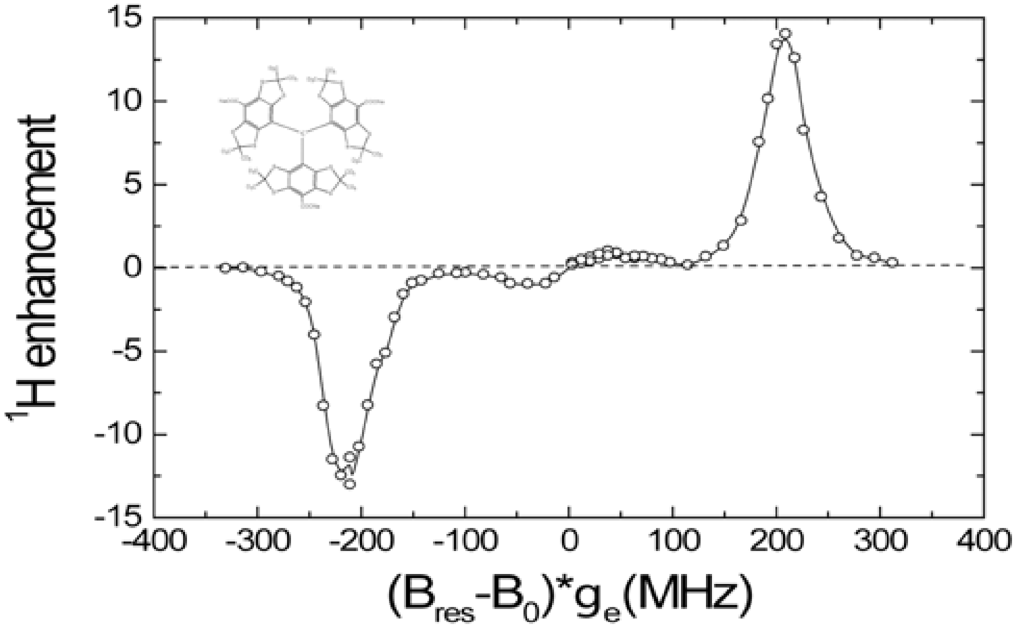




- **Small g-anisotropy yields a solid effect enhancement mechanism**

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

K. Hu et. al (2004)

- Enhancements are significant but modest only ±15 ! ☹ **!**

CW Dynamic Nuclear Polarization Mechanisms

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

$$\delta, \Delta >> \omega$$

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

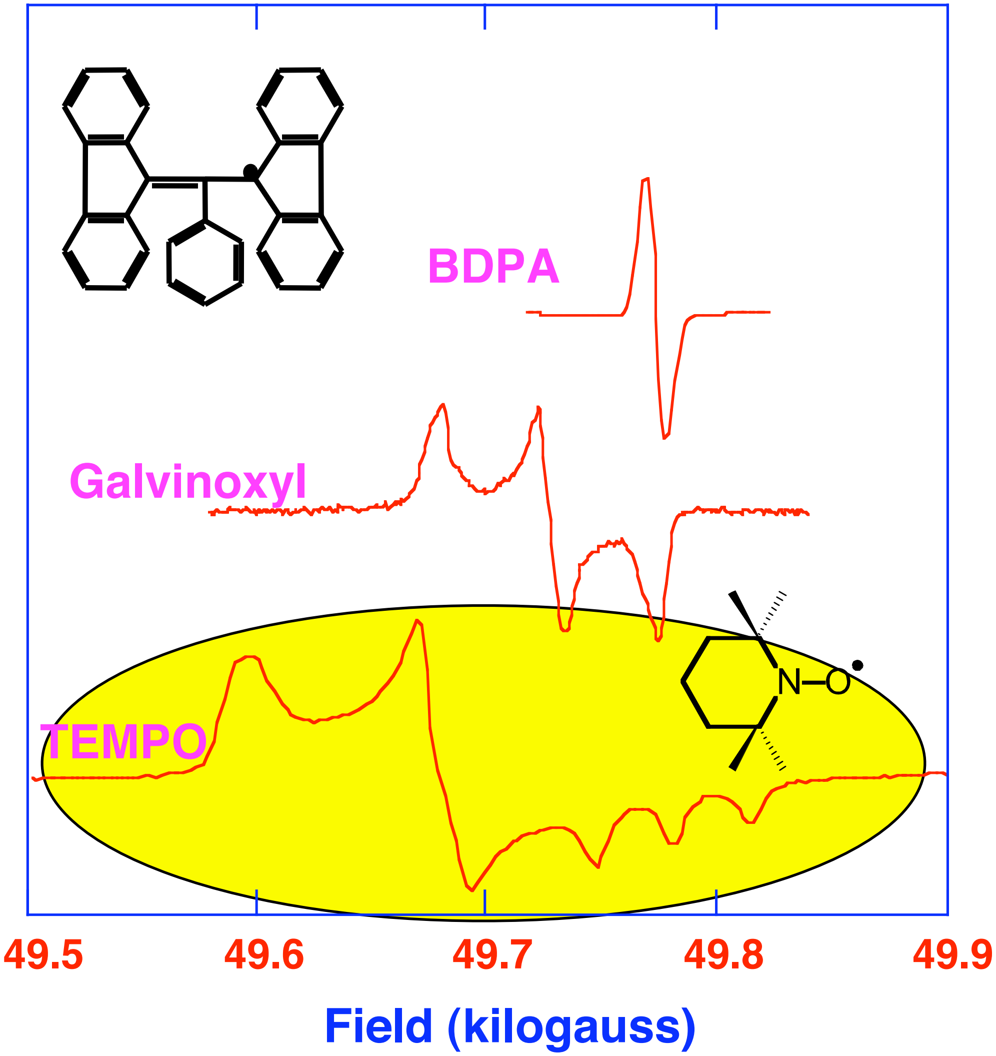
Cross Effect (CE) -- insulating solids, but ….

$$\Delta > \omega > \delta$$

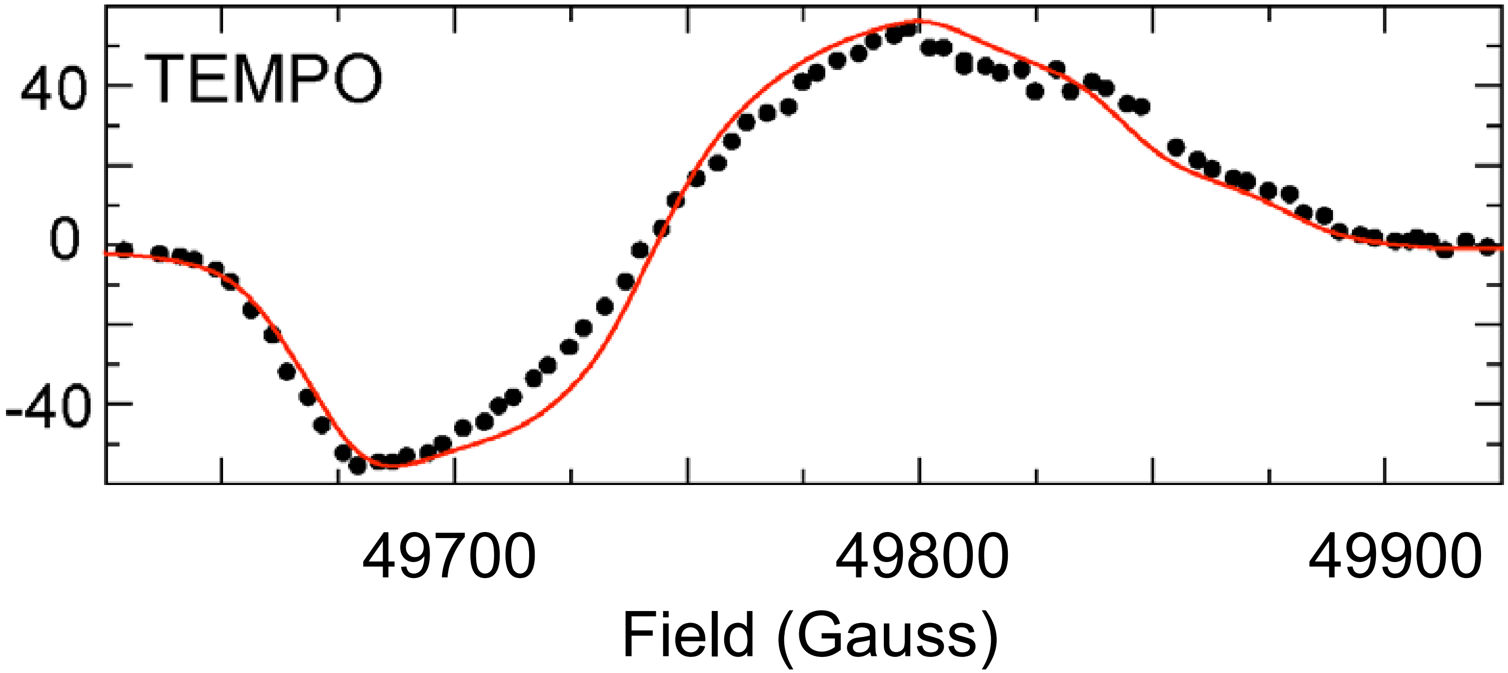
CE -- operative at high fields where $\Delta g >> \delta$, the line is *inhomogeneously* broadened.

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*



Cross Effect DNP
mit TEMPO

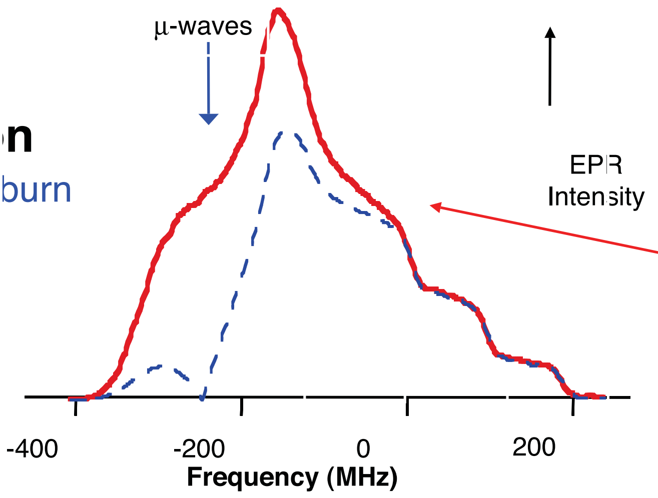


- Note *different* field profiles for the solid and cross effects
- Smaller enhancements (~± *15*) obtained with the solid effect
- Larger enhancements (~± *45*) observed with the cross effect and monomeric TEMPO

Thermal Mixing/ Cross Effect DNP

1. μwave irradiation

and e⁻-e⁻ cross-relaxation burn a hole in the EPR line.



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

TEMPO EPR Absorption Lineshape

4. Enhancement

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

657 for ^{1}H

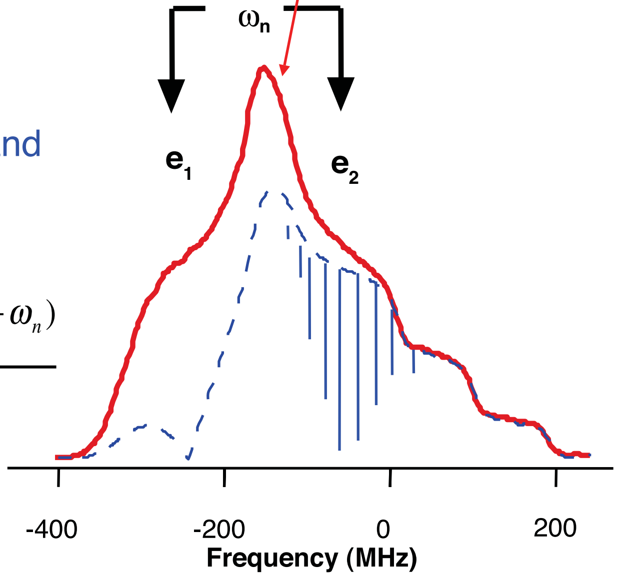
2615 for ^{13}C

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

2. Two electrons

separated by ω_n flip-flop and the difference in energy is used to flip a nucleus.

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



Thermal Mixing/ Cross Effect DNP

- NO• EPR spectrum is ~600 MHz wide

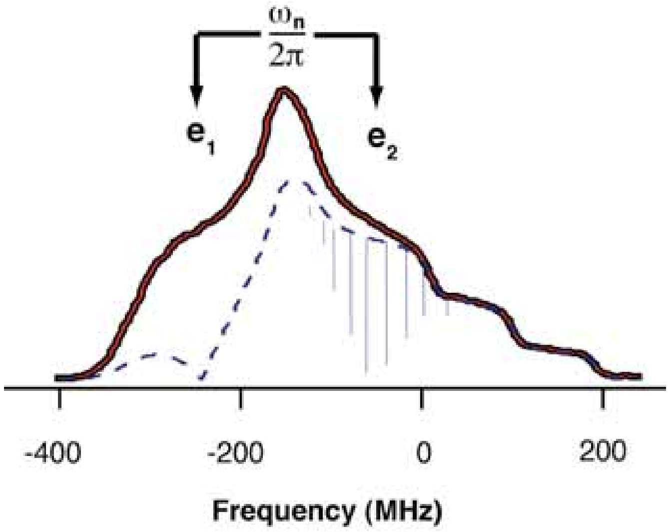
- Thermal mixing and the cross effect are …

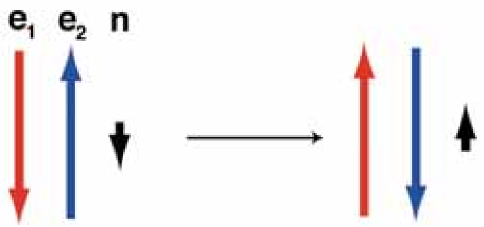
three spin process

involving the irradiation of a dipolar coupled electron spin system

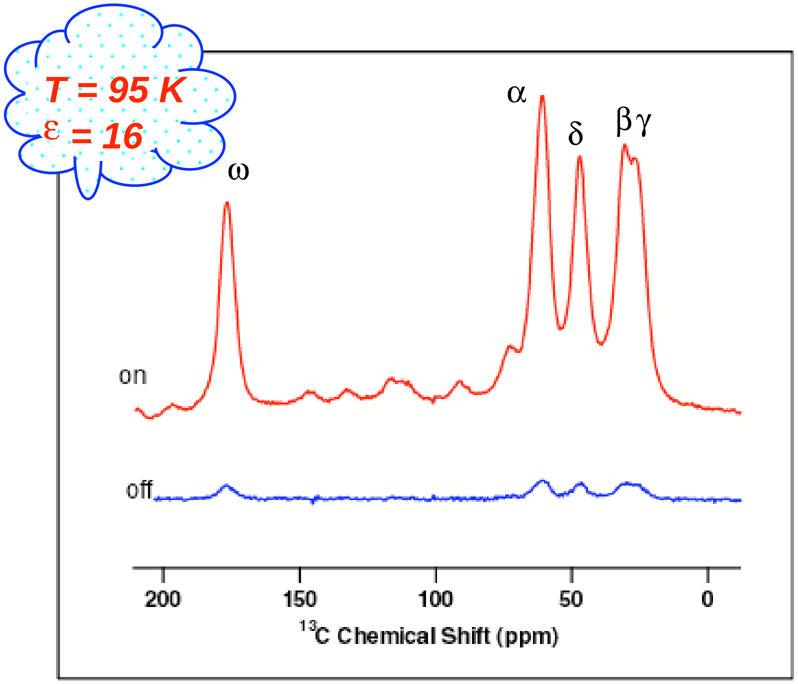
- Flip two electrons and then a nuclear spin.

C.F. Hwang and D.A. Hill, PRL18, 110-112 (1967)

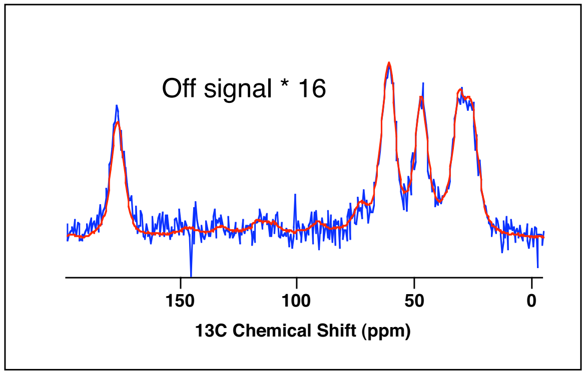




DNP Enhanced ^{13}C-MAS Spectra
U-^{13}C,^{15}N-Proline

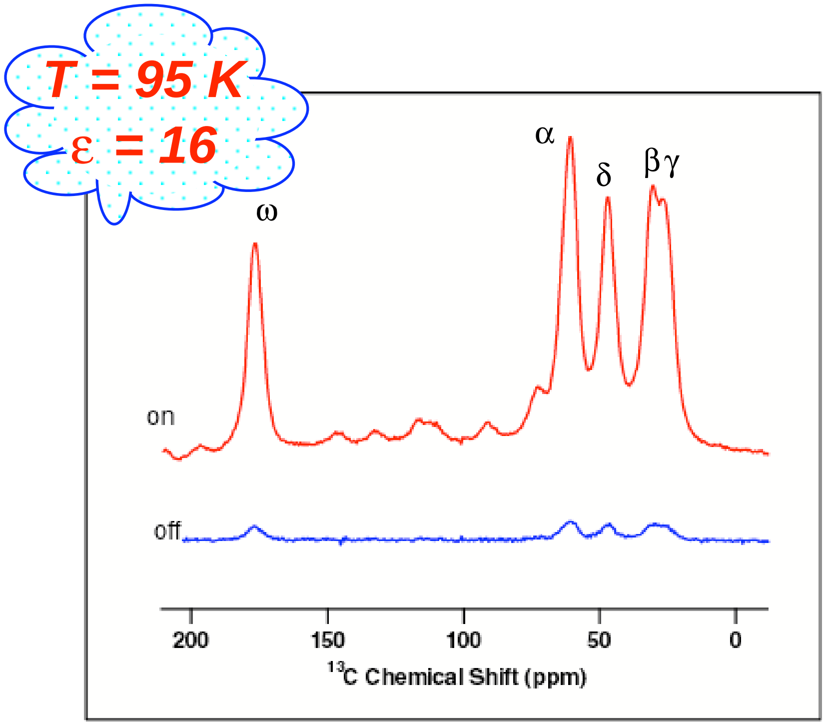


- $\omega_r/2\pi$ = = 4.6 kHz, RD = 4.8, 32 shots, 1 watt of 140 GHz at the top of the probe
- Significant enhancements are possible at LN_2 temperatures where MAS is relatively straightforward



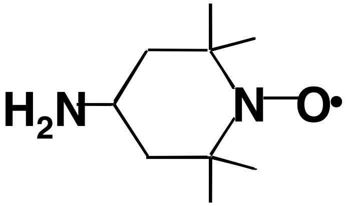
Rosay, Weis, Kreischer, Temkin and Griffin *JACS* (2002)

DNP Enhanced ^{13}C-MAS Spectra
U-^{13}C,^{15}N-Proline


T = 95 K
ε = 16

- $\omega_r/2\pi$ = = 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

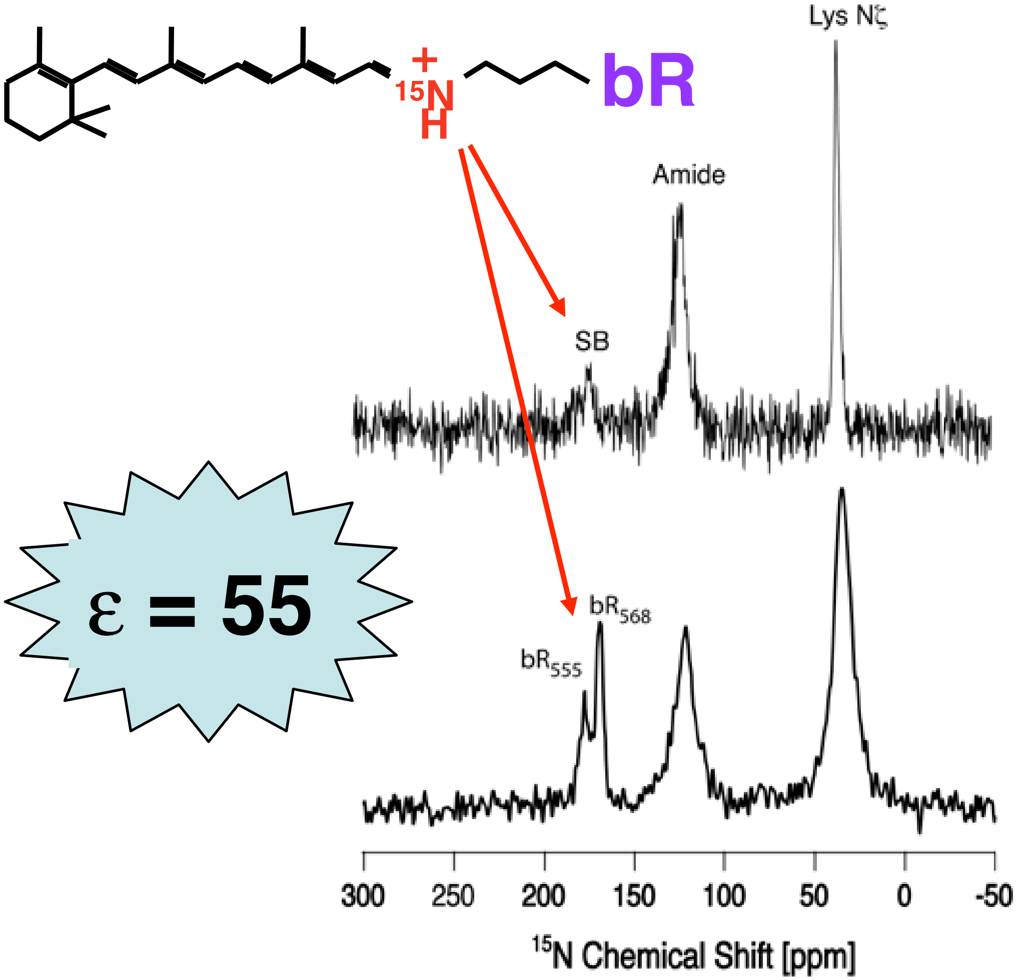
40 mM 4-amino TEMPO



2.4% efficient

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

DNP Enhanced ^{15}N MAS Spectra
ε-^{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

ε = 55

~8 % efficient

M. Rosay, et. al. JACS (2003)

CW Dynamic Nuclear Polarization Mechanisms

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

$$\delta, \Delta >> \omega$$

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

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

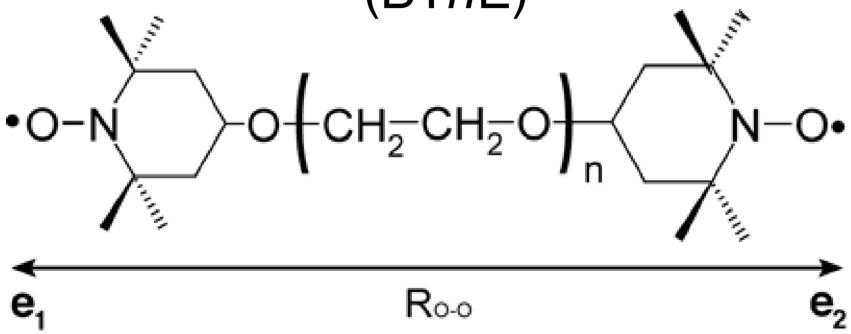
$$\Delta > \omega > \delta$$

CE -- operative at high fields where $\Delta g >> \delta$, the line is *inhomogeneously* broadened.

Biradical Polarizing Agents

Bis TEMPO *n*-ethylene glycol

(BT*n*E)



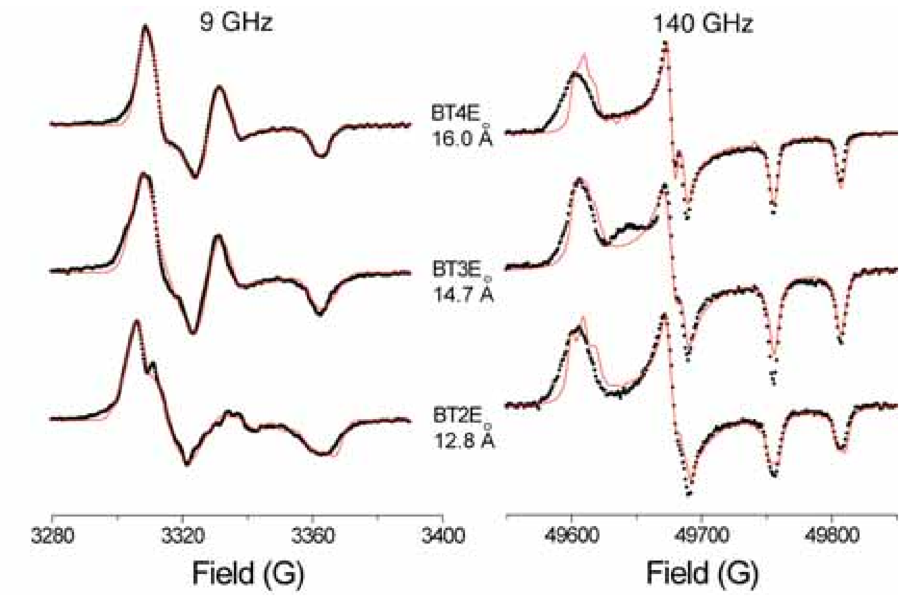
Kan Hu
Bruce Yu

- 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

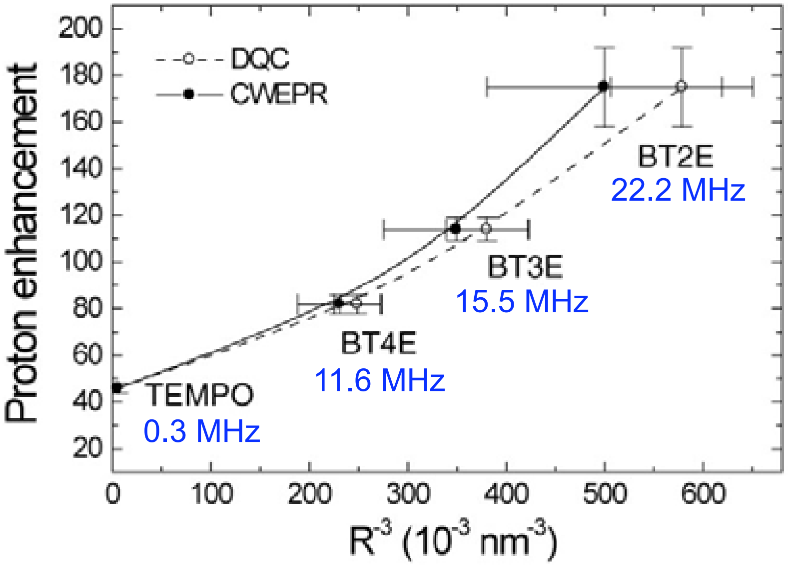
Simulation of 9 and 140 GHz **EPR** spectra

^{15}N, ^{2}H-labeled



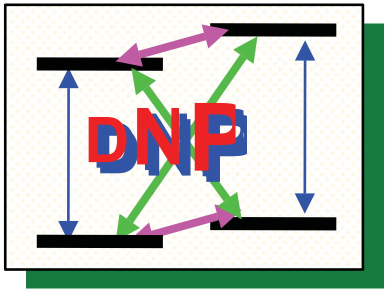
- Relative g-tensor orientations permitted to vary.
- $R_{e\text{-}e}$ determined by regression.
- Distance in 40 mM TEMPO ~35 Å

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



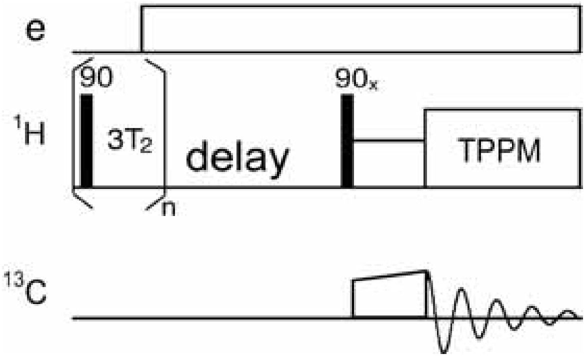
- Decreasing $R_{e\text{-}e}$ increases e^{-}-e^{-} dipole coupling & ^{1}H enhancement

45 ⇒ 175 !

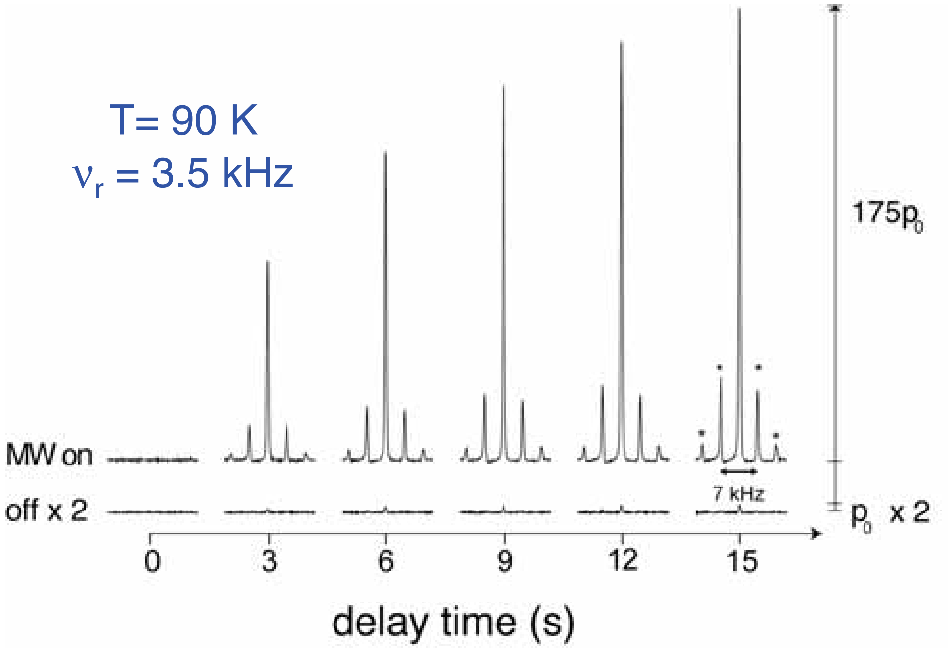


DNP Enhanced ^{13}C MAS Spectra
BT2E/^{13}C-Urea

Measurement
of DNP enhancements



K. Hu et. al (2004)

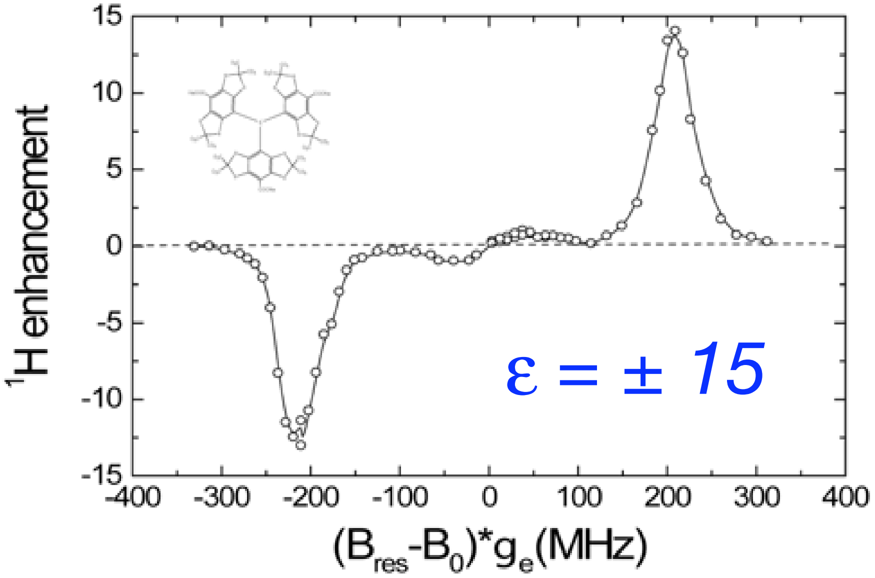

T= 90 K
ν_r = 3.5 kHz

- ***Distance*** between the two TEMPO radicals in BT2E yields a 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 !**

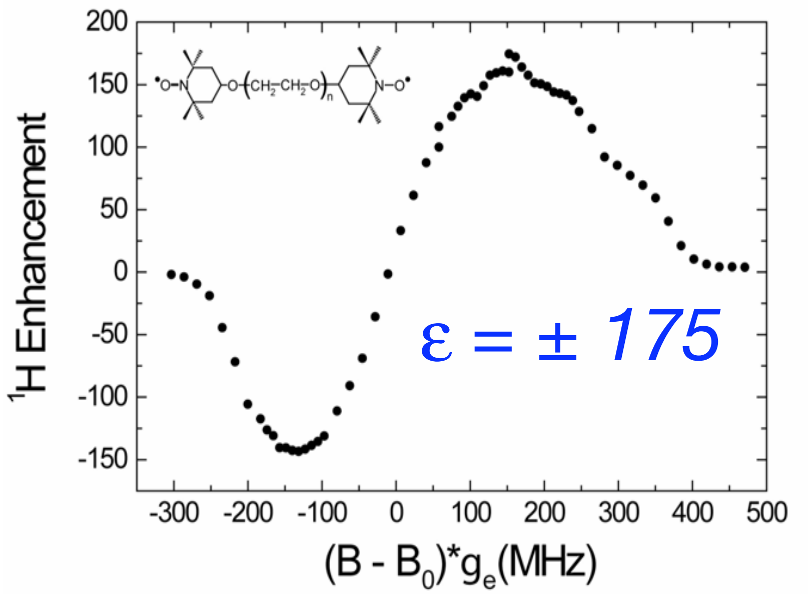
ε = 175

Solid and Cross Effect DNP

Solid Effect

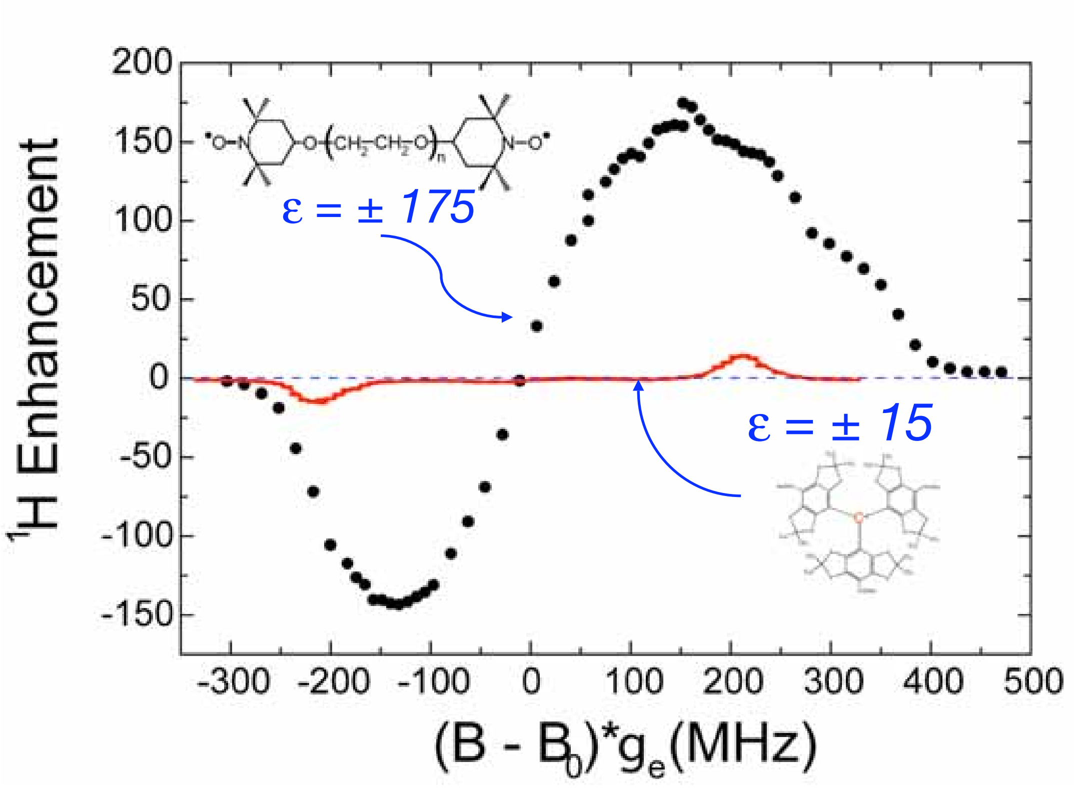


Cross Effect



- Optimal enhancements of ± *175* are observed with biradicals -- BT2E at a factor of four lower electron concentration !

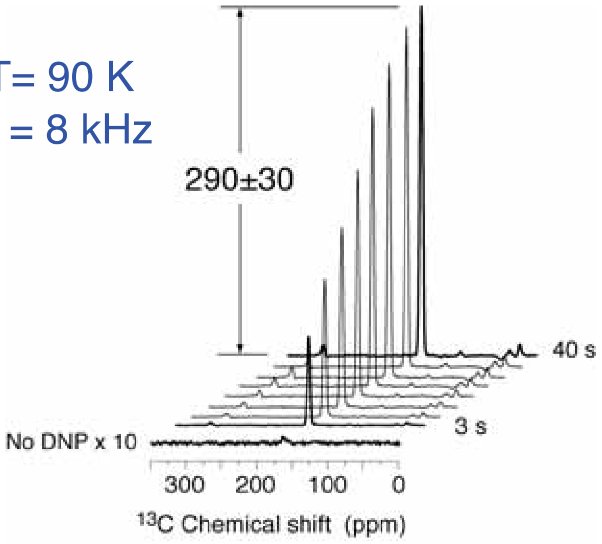
Solid and Cross Effect DNP

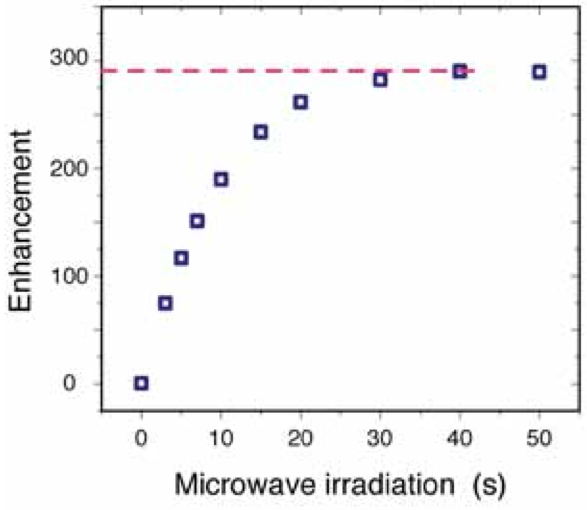


- Smallest enhancements (~± *15*) obtained with the solid effect
- Optimal enhancements of ± *175* are observed with biradicals
  -- BT2E at a factor of four lower electron concentration !

DNP Enhanced ^{13}C MAS Spectra
TOTAPOL/^{13}C-Urea

T= 90 K
ν_r = 8 kHz





- ***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 !**

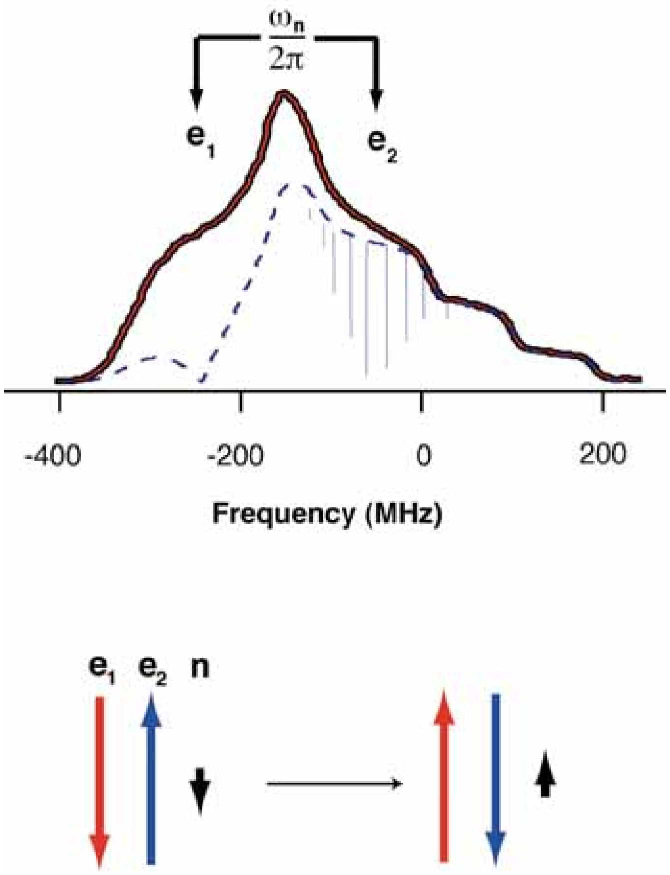
Joo, Hu, Bryant and Griffin (2006)

Thermal Mixing/ Cross Effect DNP

- 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)

Qualitative
Thermal Mixing/Cross Effect DNP
g-tensor Orientations

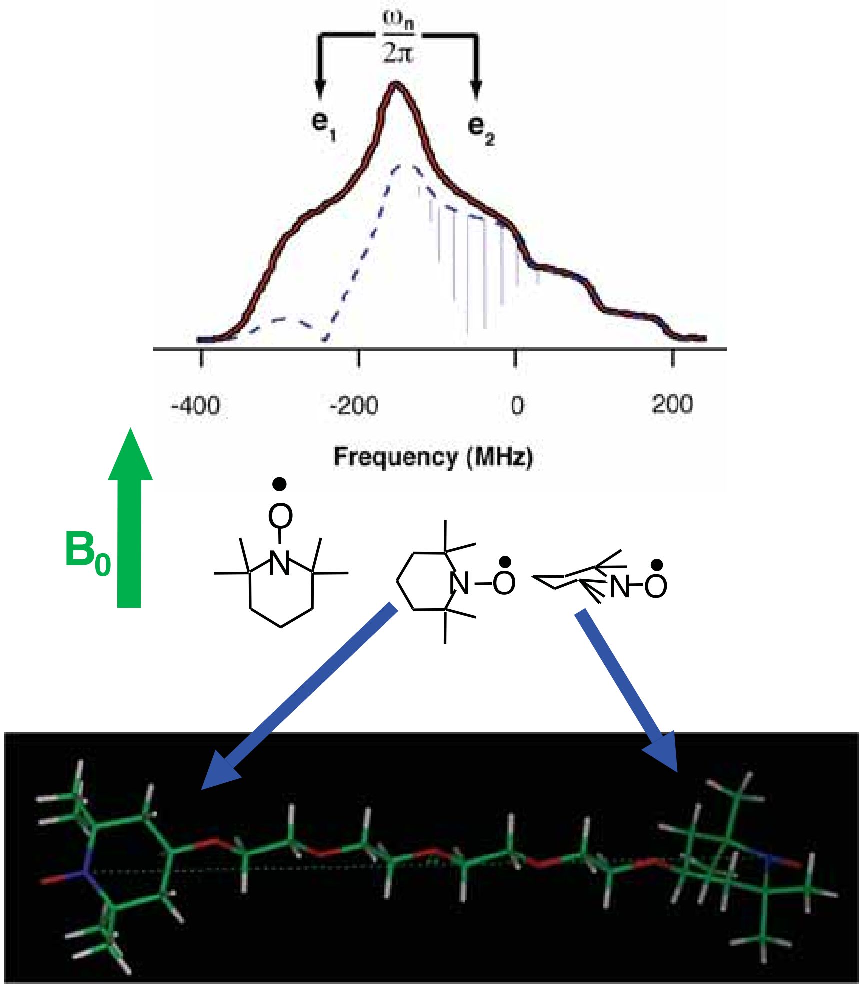
- Correct relative orientations of the TEMPO molecules are required to yield *two lines* separated by

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

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

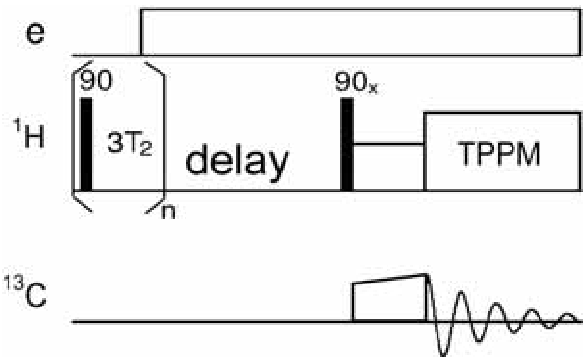
- **g-tensor orientations yield two lines separated by ~ $\omega_n/2\pi$?**



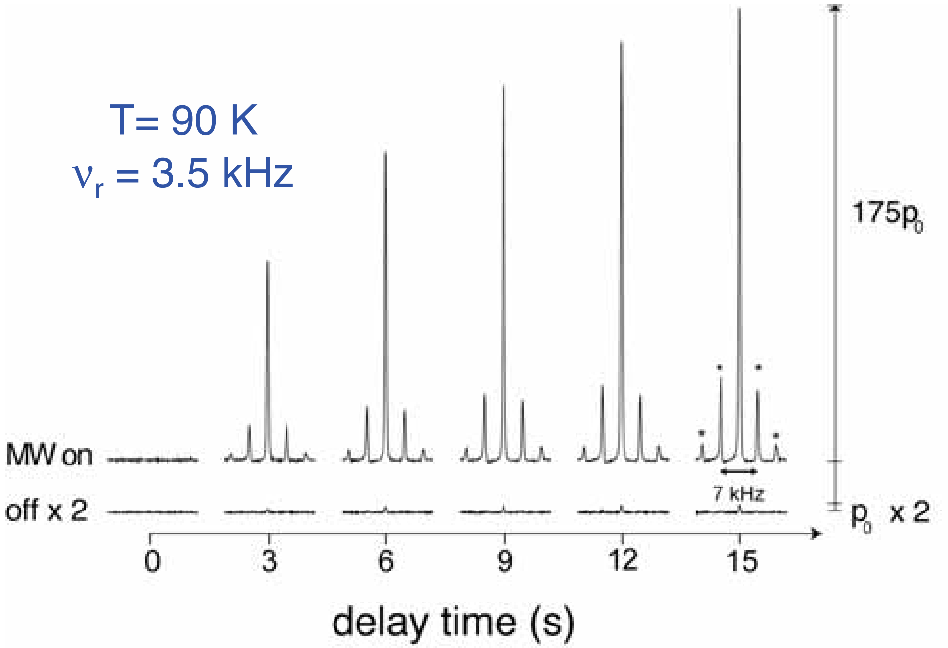
DNP Enhanced ^{13}C MAS Spectra

BT2E/^{13}C-Urea

Measurement
of DNP enhancements



K. Hu et. al (2004)



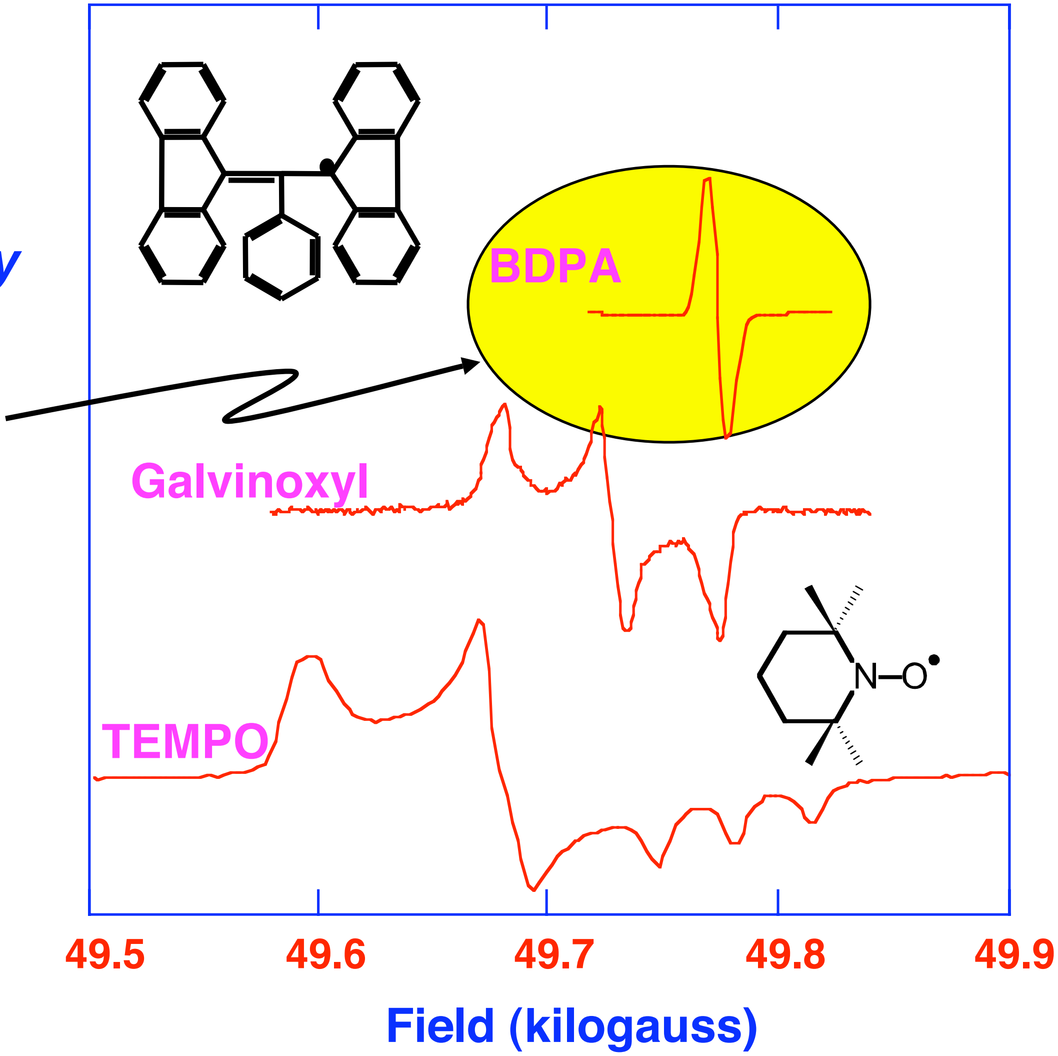
- ***Distance*** between the two TEMPO radicals in BT2E yields a e^--e^- coupling of ~25 MHz
- Electron concentration drops from *40 mM to 10 mM* !
- Enhancements build up over ~15 seconds and the maximum appears to be ~ **175 !**

Paramagnetic Centers for DNP

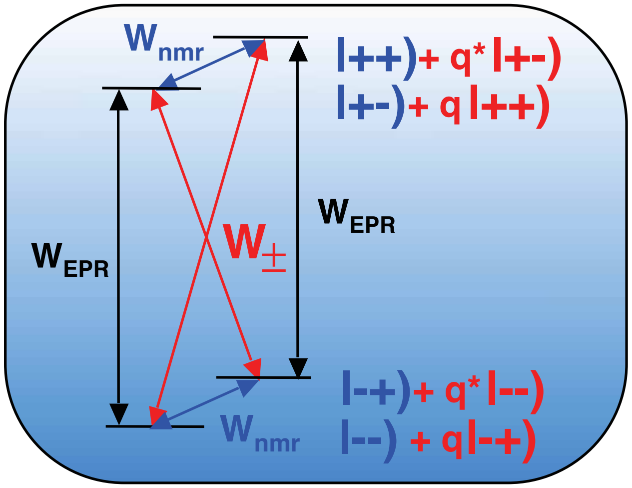
- **EPR lineshapes are Dominated by *g- anisotropy***

- **BDPA linewidth ~21 MHz**
  ---- *Solid effect*

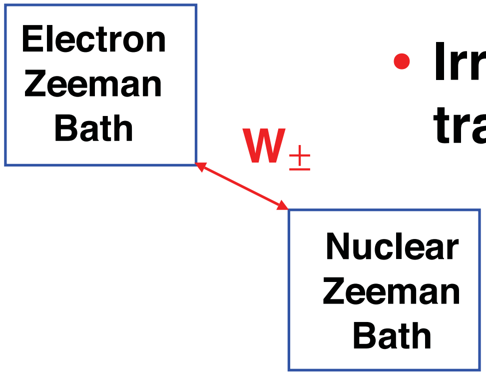
- **TEMPO powder pattern ~600 MHz**
  ---- *Thermal mixing or cross effect*



Dynamic Nuclear Polarization
Solid State Effect



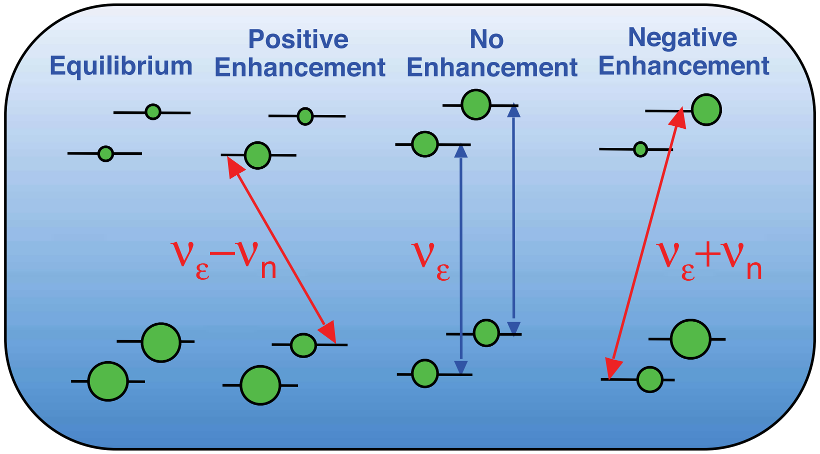
$$|q| \propto \frac{D^{n.s.}_{e-n}}{\omega_n}$$



Electron Zeeman Bath

Nuclear Zeeman Bath

- Irradiate the flip-flop transitions



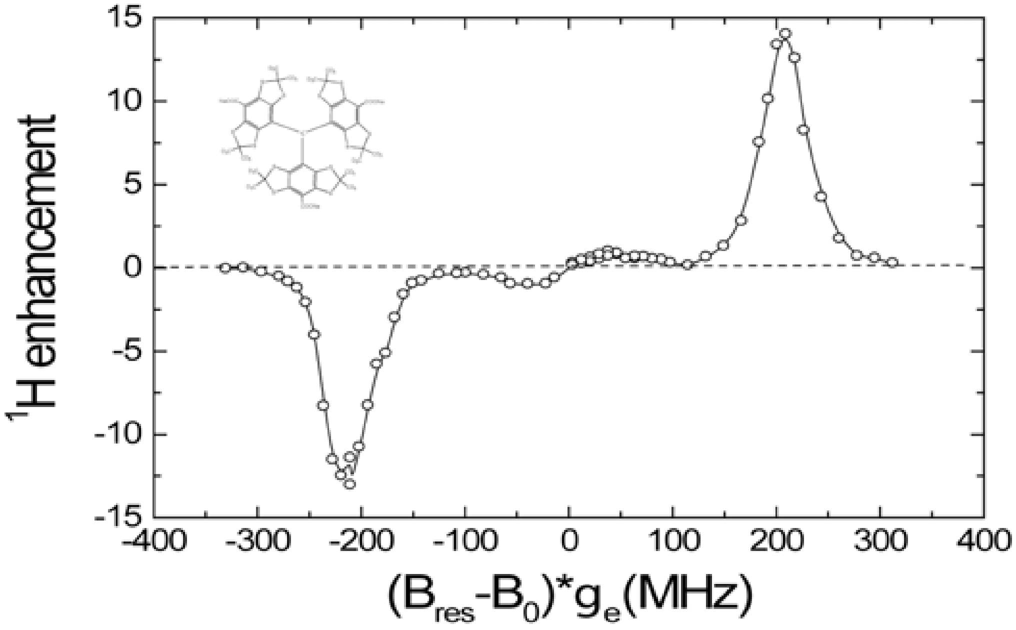
- Enhancement $\sim (\gamma_\varepsilon / \gamma_n)(\omega_1 / \omega_0)^2 (N_e / \delta) T_{1n}$

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

K. Hu et. al (2004)

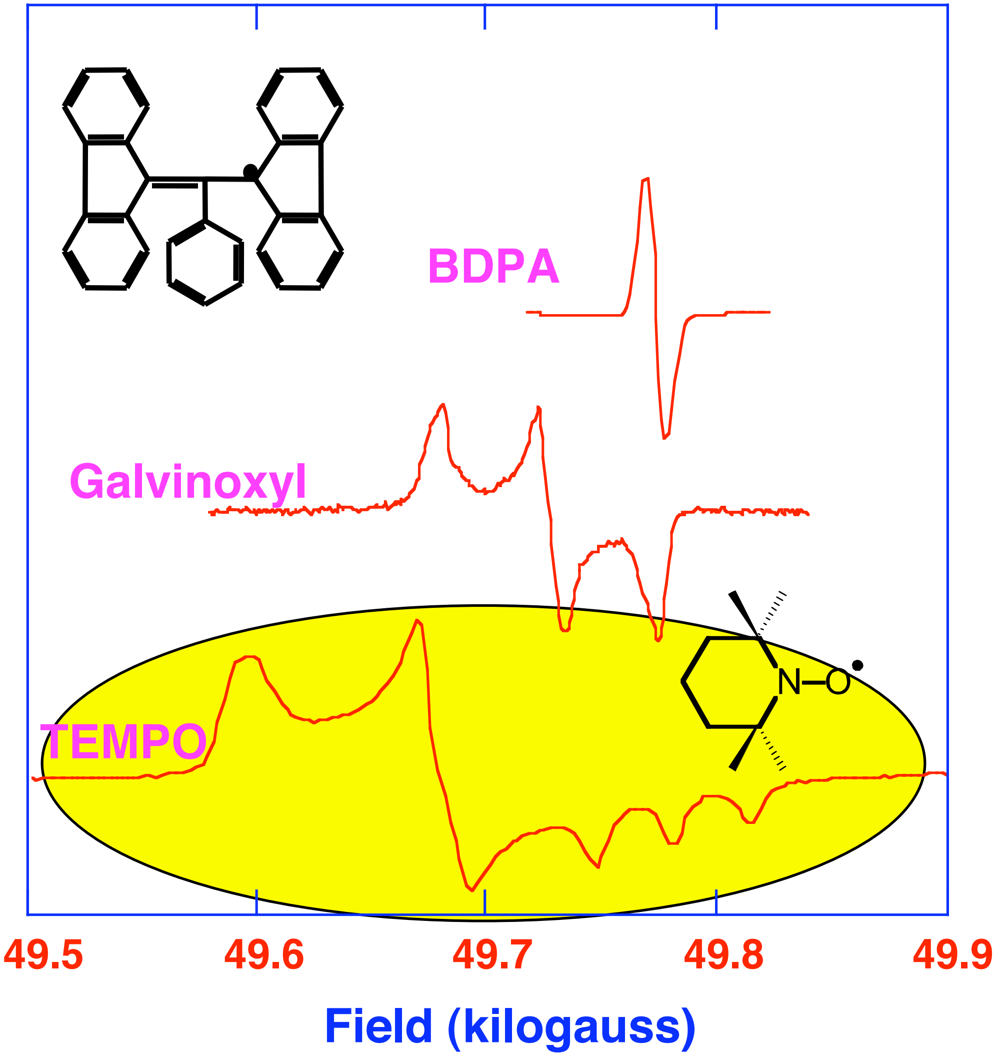
- Enhancements are significant but modest only ±15 ! ☹ **!**

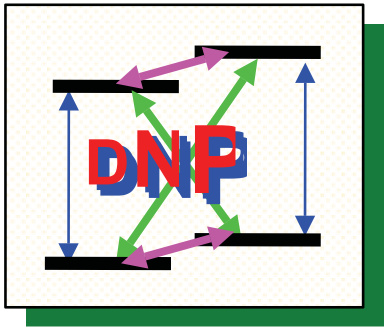
Paramagnetic Centers for DNP

- EPR lineshapes are Dominated by *g- anisotropy*

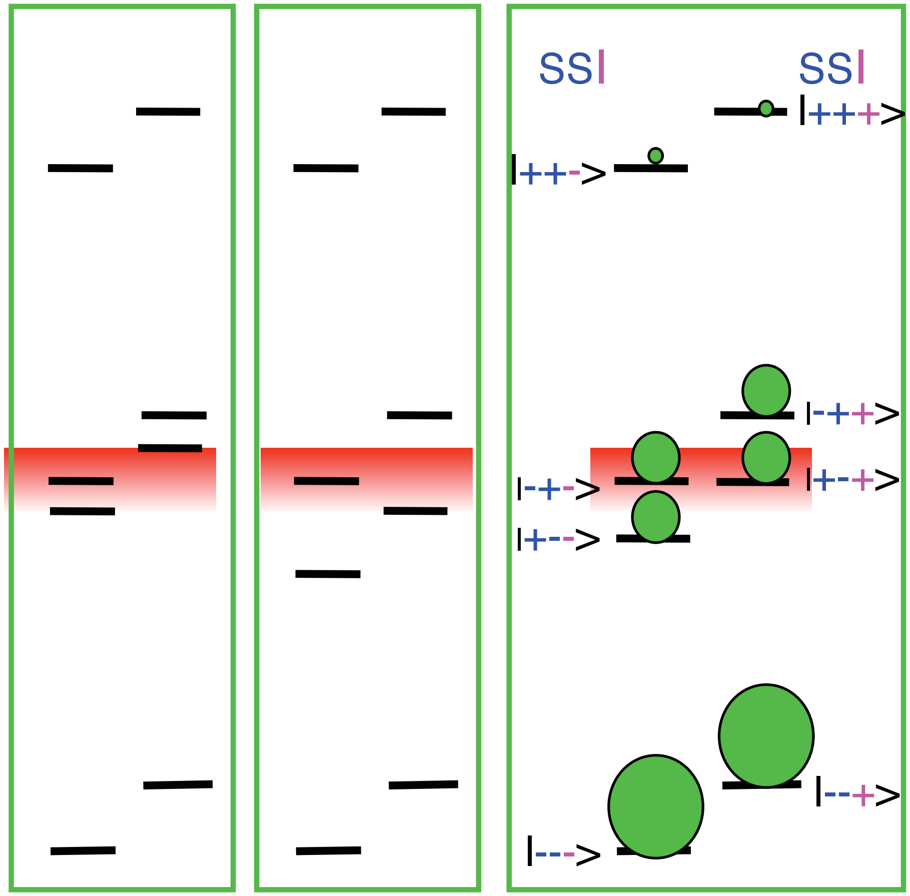
- BDPA linewidth ~21 MHz
  ---- *Solid effect*

- TEMPO powder pattern ~600 MHz
  ---- *Thermal mixing or cross effect*





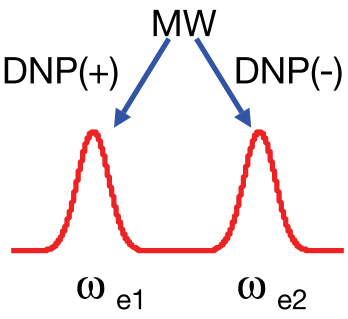
Three-Spin Process in Thermal Mixing/Cross Effect DNP



Equilibrium $\Delta\omega_e < \omega_n$

Equilibrium $\Delta\omega_e > \omega_n$

Equilibrium $\Delta\omega_e = \omega_n$

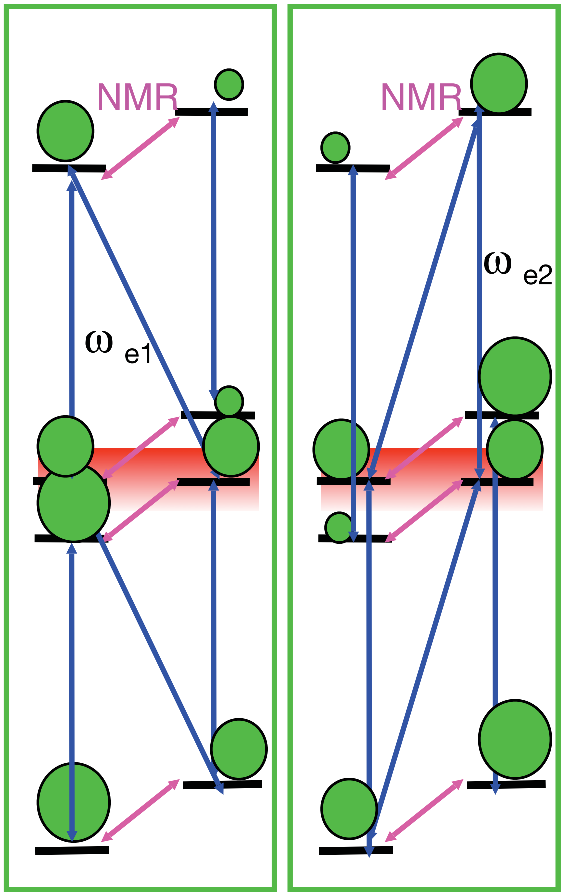


If

$$\omega_{e2}-\omega_{e1}\sim\omega_n$$

|-+->, |+-+> degenerate

Kan Hu
PhD Thesis
2006



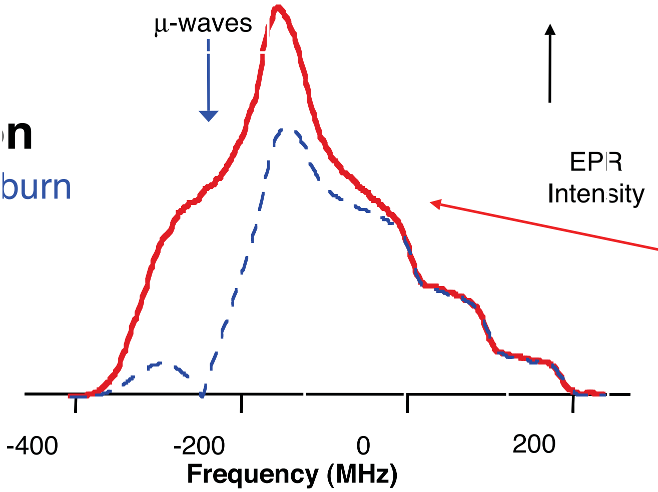
Positive Enhancement

Negative Enhancement

Thermal Mixing/ Cross Effect DNP

1. µwave irradiation

and e$^-$-e$^-$ cross-relaxation burn a hole in the EPR line.



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

TEMPO EPR Absorption Lineshape

4. Enhancement

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

657 for ^{1}H

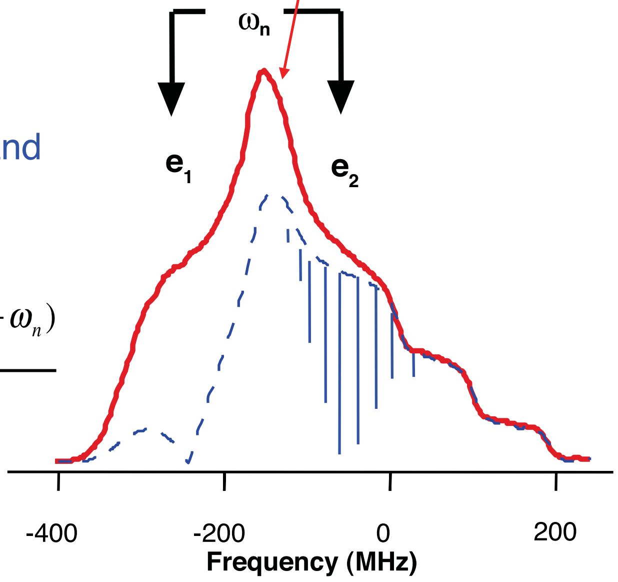
2615 for ^{13}C

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

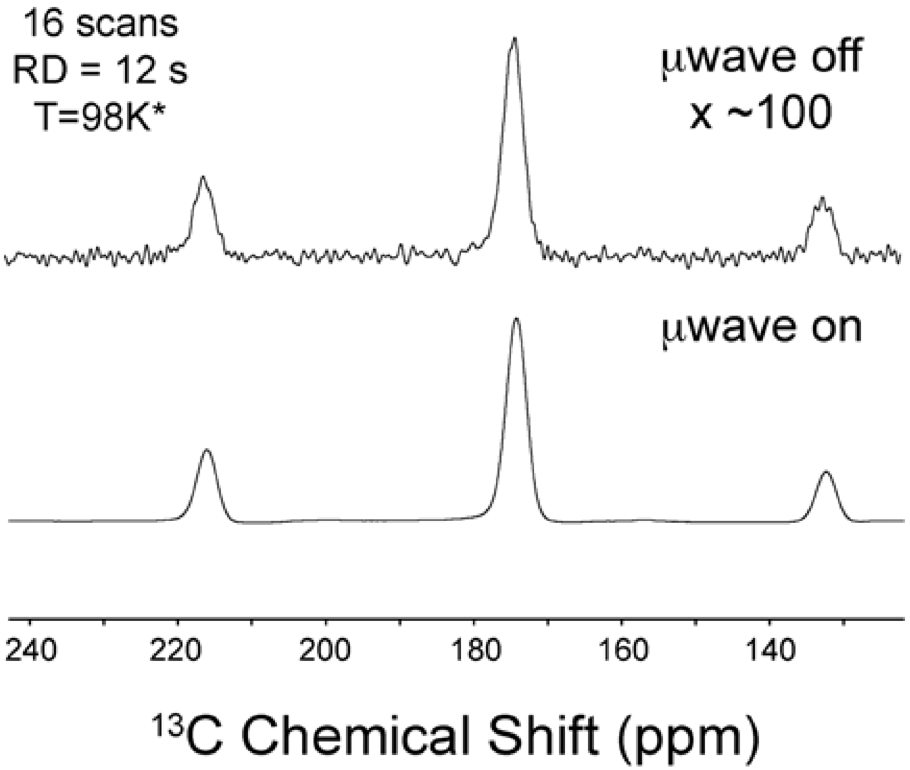
2. Two electrons

separated by ω_n flip-flop and the difference in energy is used to flip a nucleus.

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



DNP Enhancement at 250 GHz
^{13}C-Urea/Glycerol/$D_2$0



Expected enhancement

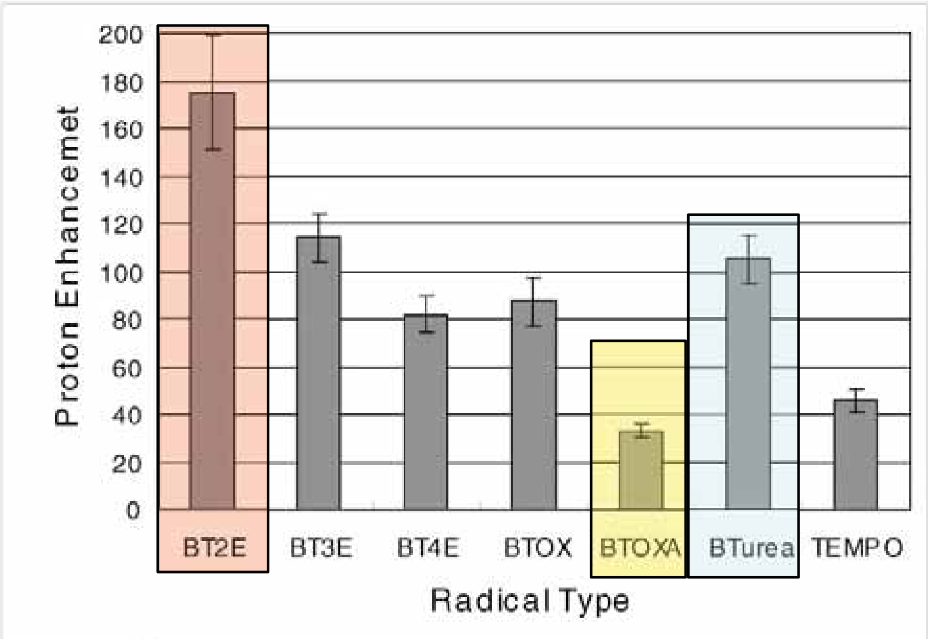
$$\varepsilon_{250} = \varepsilon_{140}\left(\frac{140}{250}\right)$$

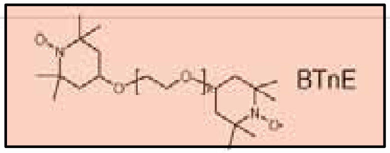
$$170\left(\frac{140}{250}\right) \approx 95$$

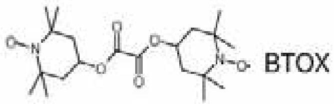
ε~100

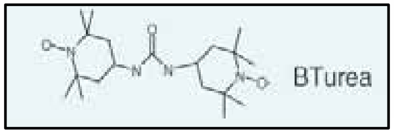
- Cross Effect DNP enhancement scales as ω_0^{-1}

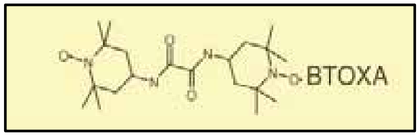
DNP Enhancements from Biradicals











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

BTOXA yields the smallest ε-30!

Coaxial g-tensors !

- An optimal ^1H ε requires a strong e^- - e^- dipolar coupling.

BTurea has shorter $R_{e\text{-}e}$, but no greater ε ?

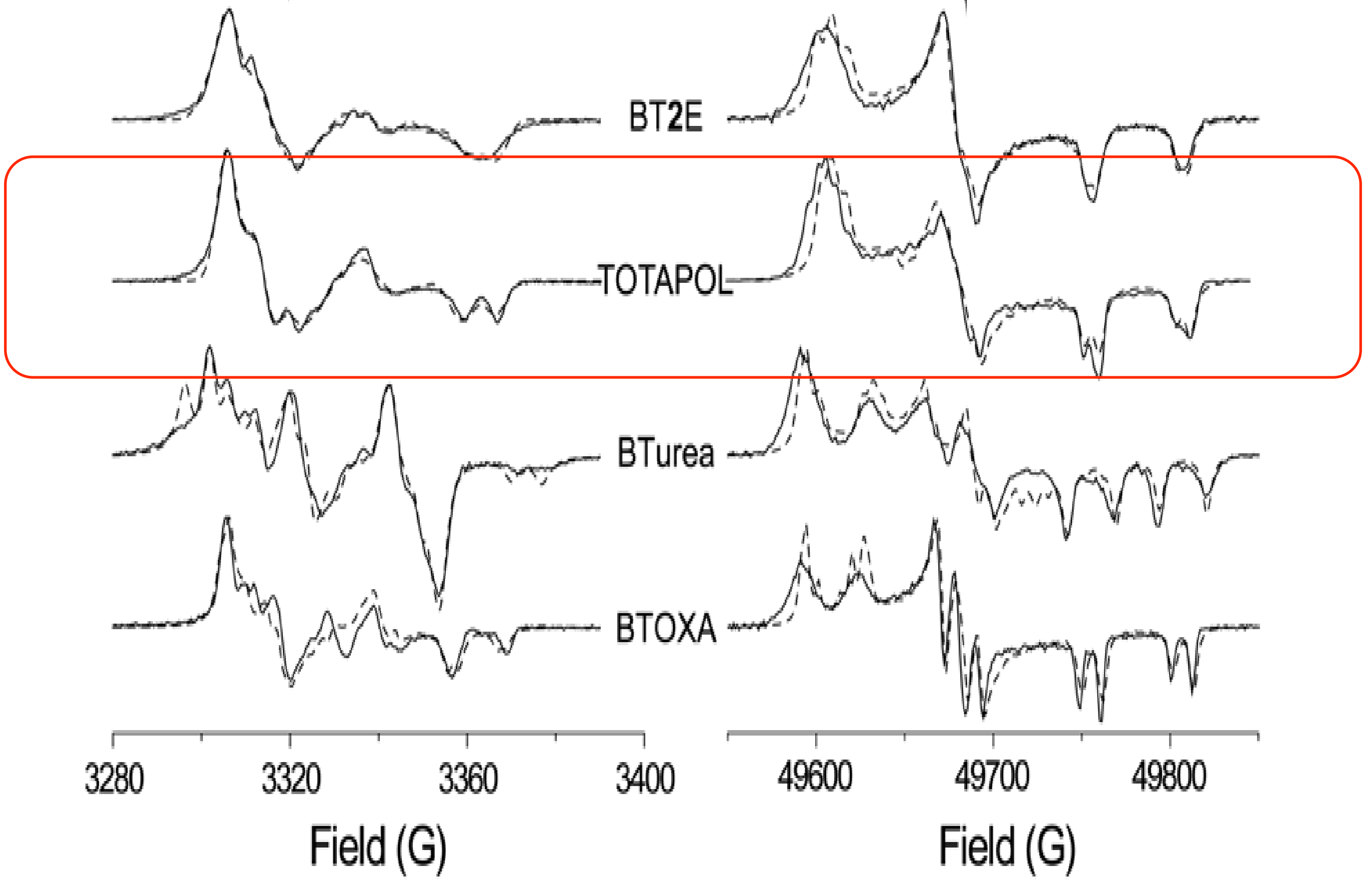
Incorrect tensor orientation ?

- A flexible tether is important.

BT2E yields the largest ε–175!!

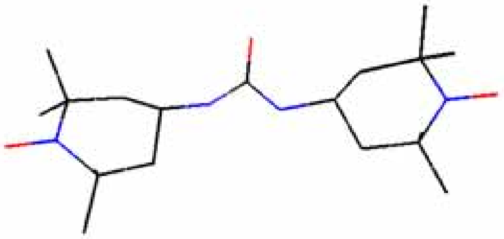
Short distance and correct g-tensor orientation !

Powder EPR Spectrum of TOTAPOL

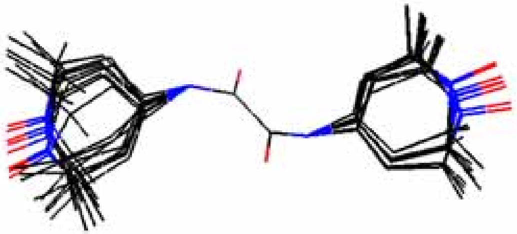


- Rigidity of the tether of TOTAPOL improves DNP performance.
- Narrower distribution of e^- - e^- distance, but no constraint of relative g-tensor orientation

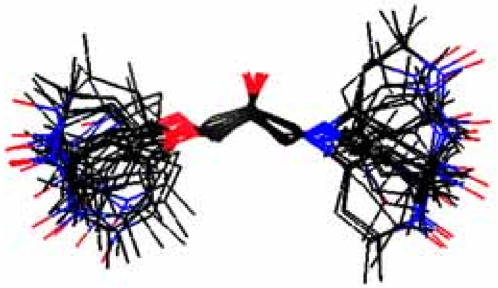
Biradical Models from EPR Analyses



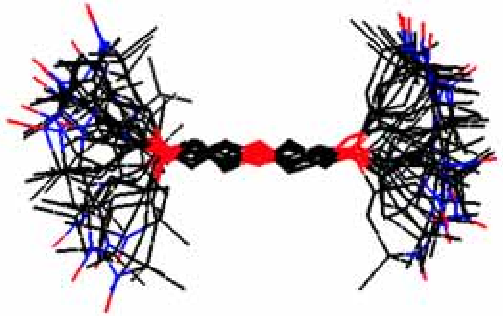
BTurea
RMSD(rings) = 0.03 Å



BTOXA
RMSD(rings) = 0.50 Å



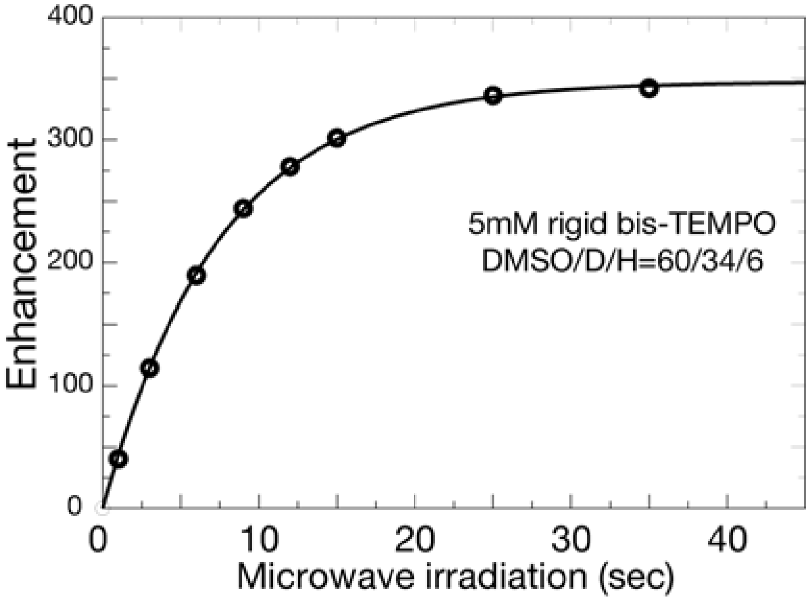
TOTAPOL
RMSD(rings) = 2.15 Å

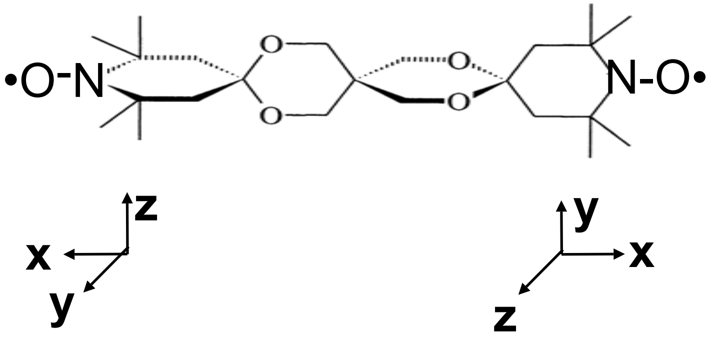


BT2E
RMSD(rings) = 2.82 Å

A longer tether reduces constraints on relative g-tensor orientation.

Rigid *bis*-TEMPO (**Tordo/ Prisner**) Biradical
^{13}C-Urea/DMSO/D_20/H_2O





ε~275-340

Yoh Matsuki, Thorsten Maly
Galia Deblouchina
MIT

Thomas Prisner, Sevdalina Lyubenova -*Frankfurt*
Paul Tordo - *Marseille*

- Rigid biradical satisfying ω_{1e}-$\omega_{2e} \sim \omega_n$*! e^--e^-~30 MHz !*
- *Preliminary result -- errors large !*

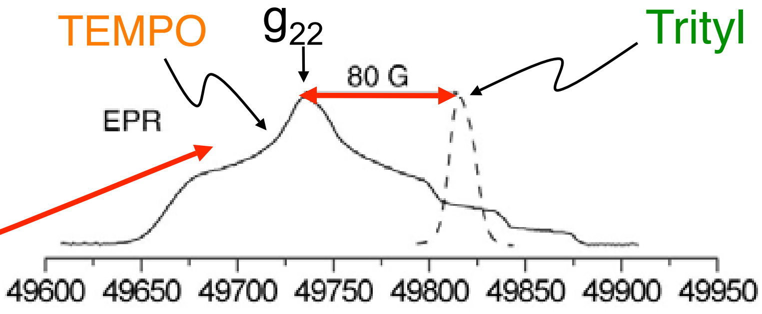
115,600 in time !

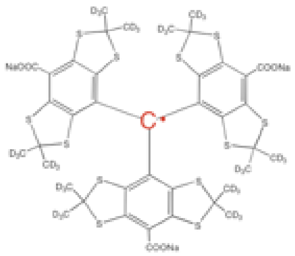
- *ε ~ 340/660 ➞ 51% efficient !*

Cross Effect in **TEMPO** / **Trityl** Mixtures

An Approximation to an Ideal Polarizing Agent

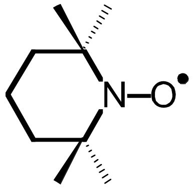
- Ideal polarizing agent --
  - tethered radicals
  - *small g-anisotropies*
  - $\omega_{1e}-\omega_{2e} \sim \omega_n$





trityl

+



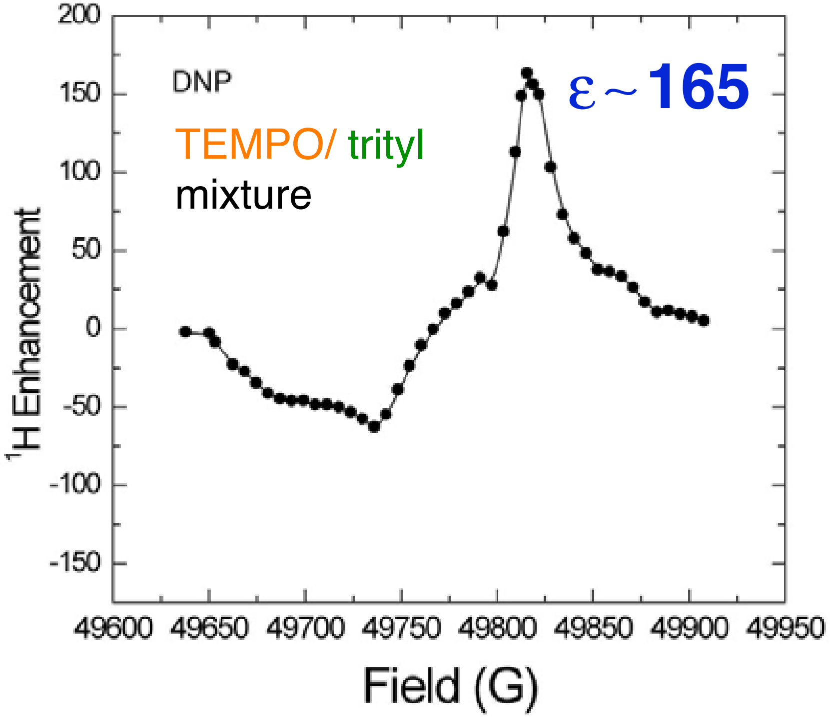
TEMPO

- Notice that ….

  $g_{22(\mathrm{TEMPO})}$-$g(\mathrm{trityl})$ ≈80 G = 224 MHz !

--- which is comparable to

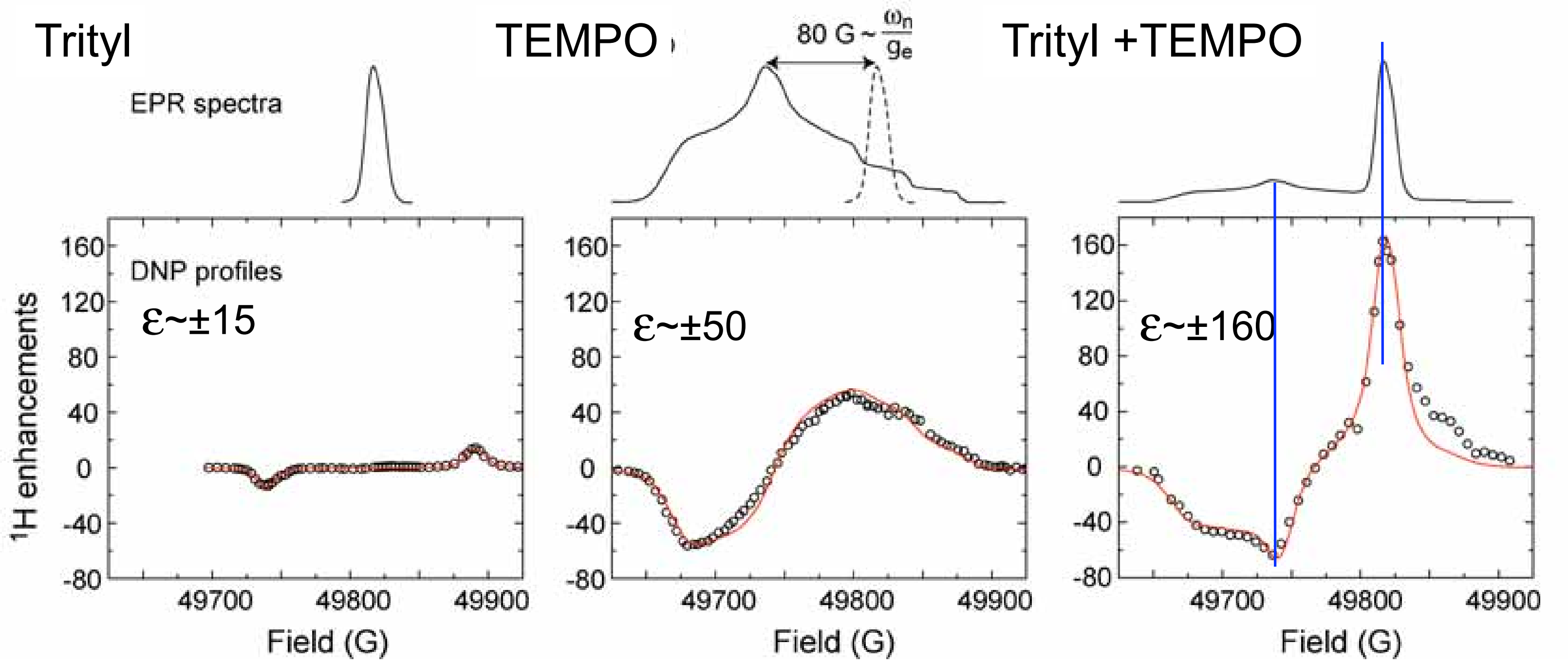
$\omega_{1H}/2\pi$ = 211 MHz !



- *Demonstrates the importance of satisfying $\omega_{1e}-\omega_{2e} \sim \omega_n$!*

DNP in Trityl-TEMPO Mixtures

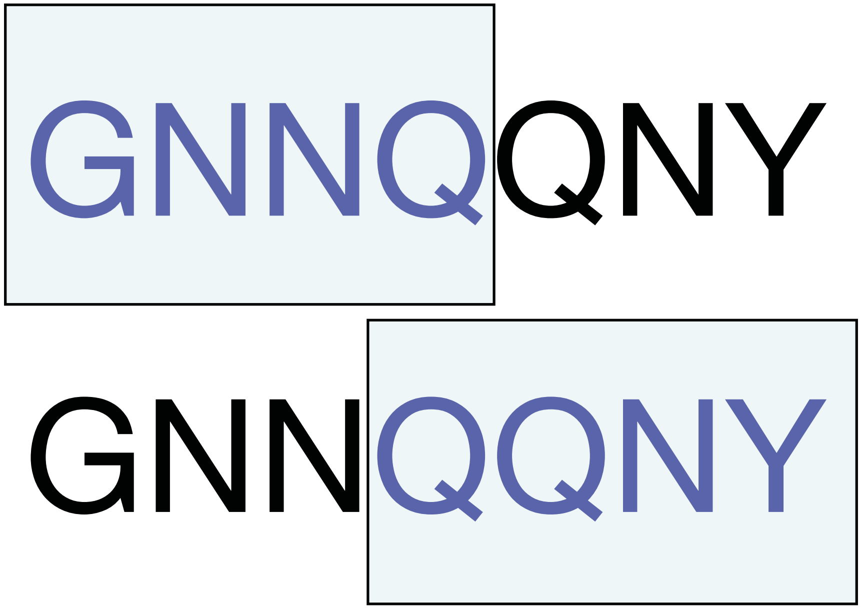
Approximation to an “Ideal Polarizing Agent”



- *Notice that* $g_{22(\mathrm{TEMPO})}-g(\mathrm{trityl})$ *≈80 G = 224 MHz !*
- *Demonstrates the importance of satisfying* $\omega_{1e}-\omega_{2e} \sim \omega_n$ *!*

Hu, Bajaj, Rosay, Griffin, J. Chem. Phys. (2007)

Labelling Scheme GNNQQNY$_{7-13}$

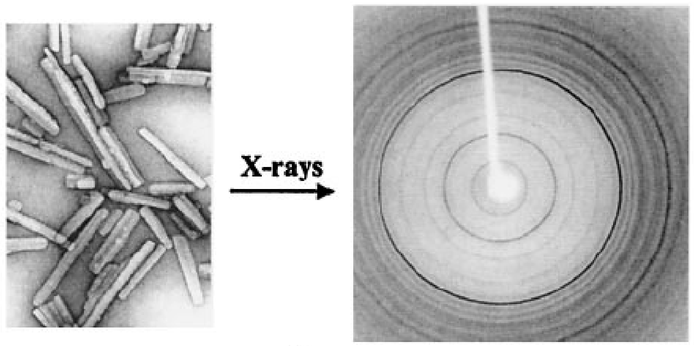


GNNQQNY

GNN**QQNY**

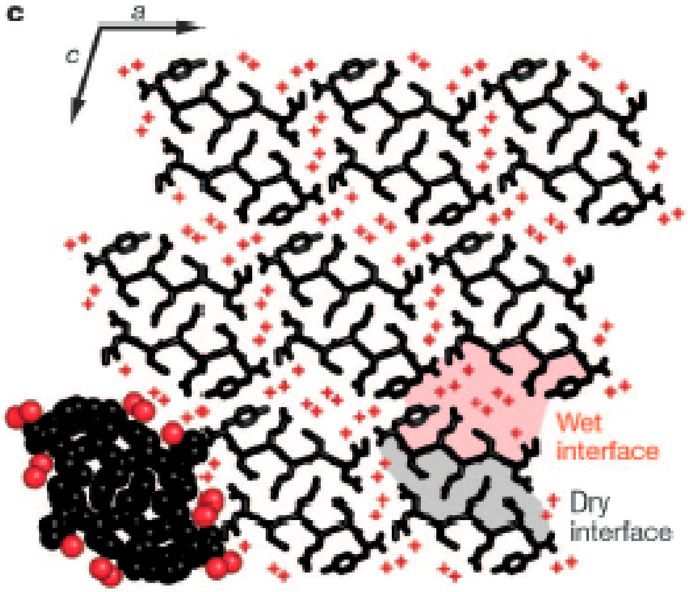
- ➢ Solid phase peptide synthesis
- ➢ Two segmentally, overlapping U-^{13}C,^{15}N labeled peptides

GNNQQNY$_{(7-13)}$ from Sup35 (Yeast Prion)



Crystal width ~ 280 Å

Nanocrystals

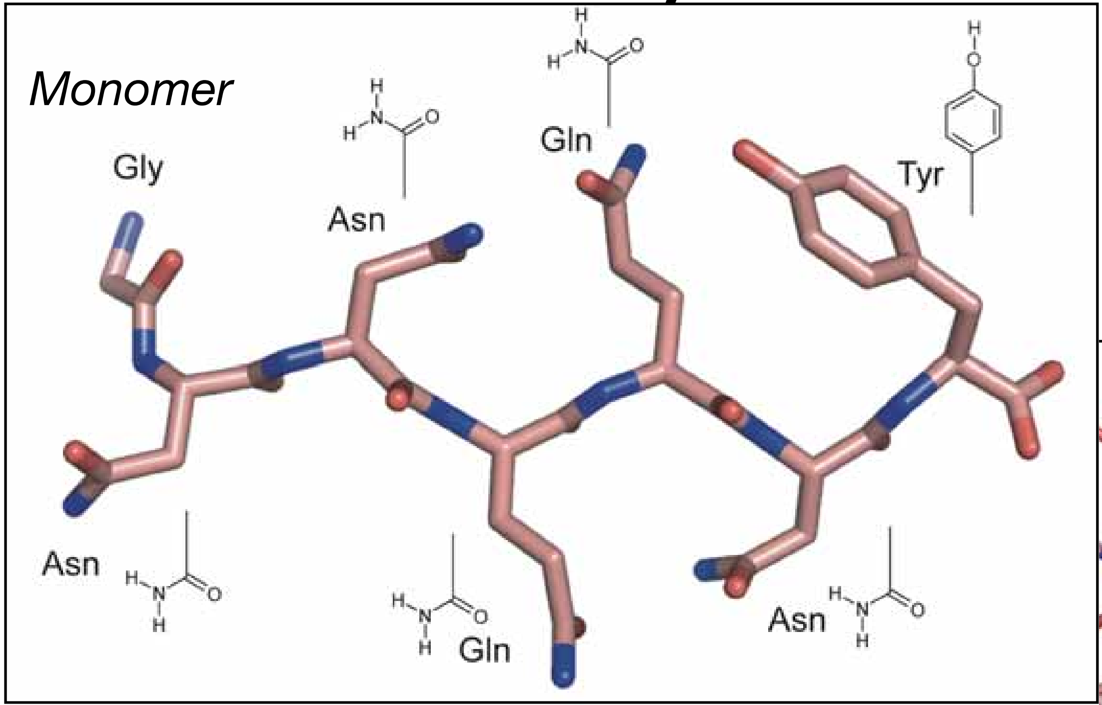


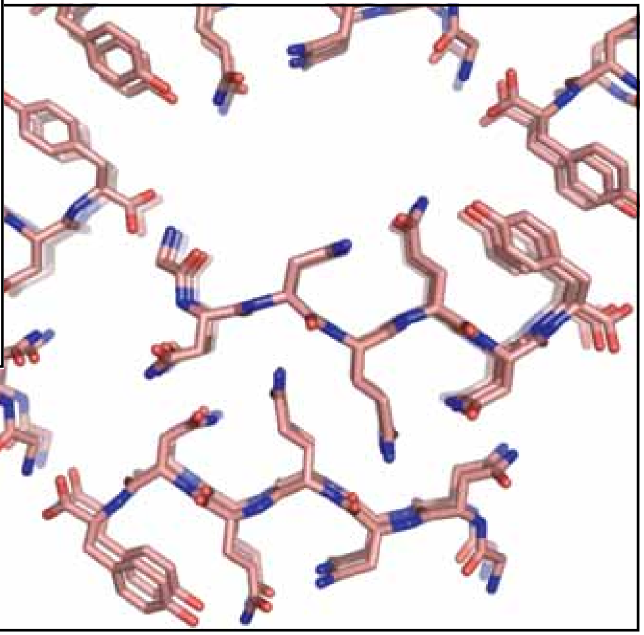
X-ray structure

Eisenberg et al (2005)

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

Monoclinic Crystals - X-ray Studies

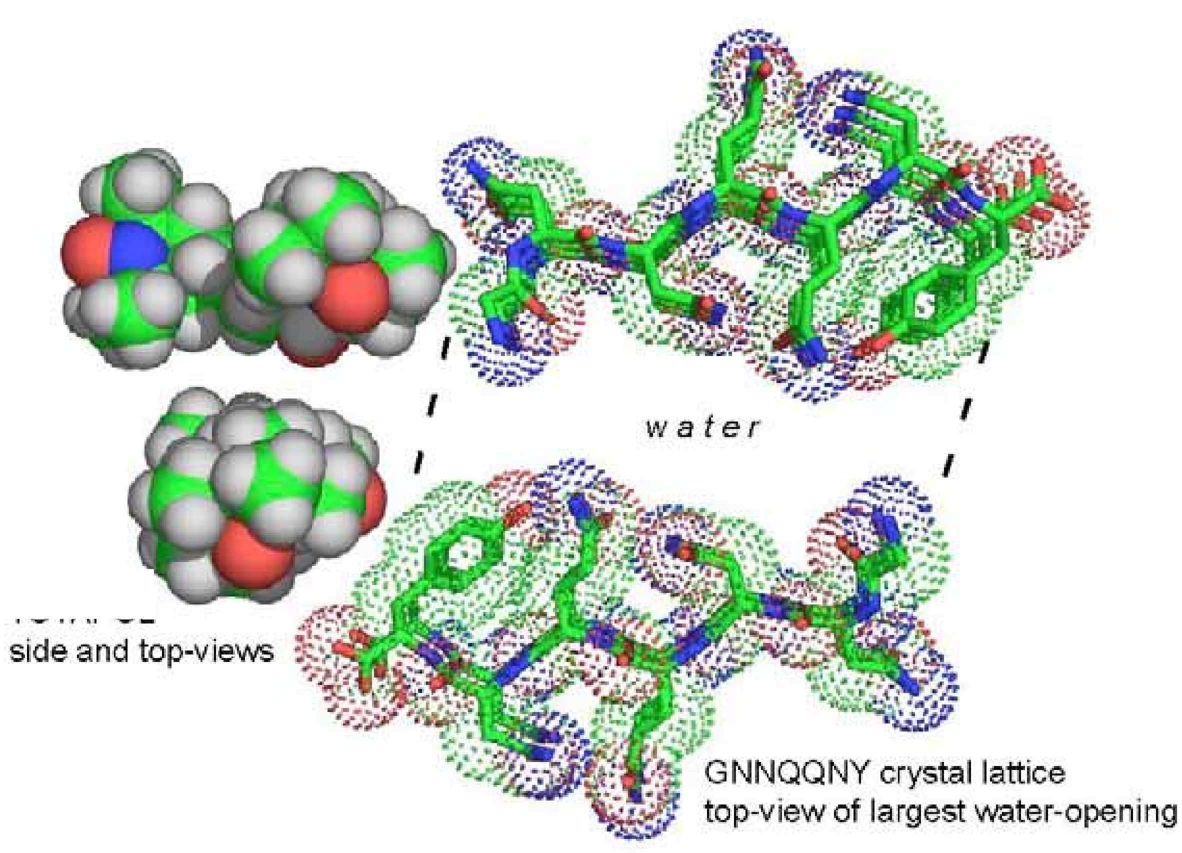




How does this structure relate to the actual prion aggregates?

- Structure of monoclinic nanocrystals of GNNQQNY -- *R. Nelson et al. (2005) Nature,* ***435****: 773*

Biradical and the GNNQQNY Lattice

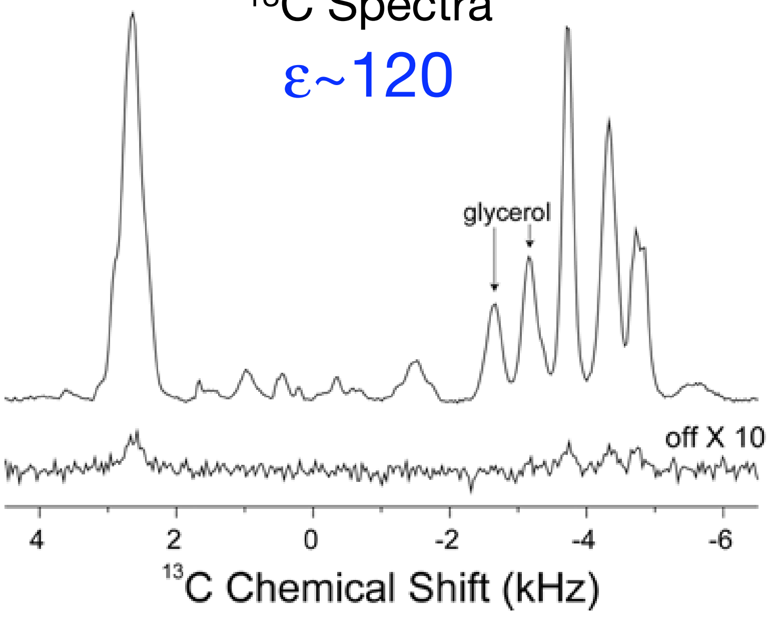


- Biradical is larger that the water pore (~7Å)
  in the GNNQQNY lattice

140 GHz ^{13}C and ^{15}N DNP/MAS Spectra
GNNQqny Nanocrystals

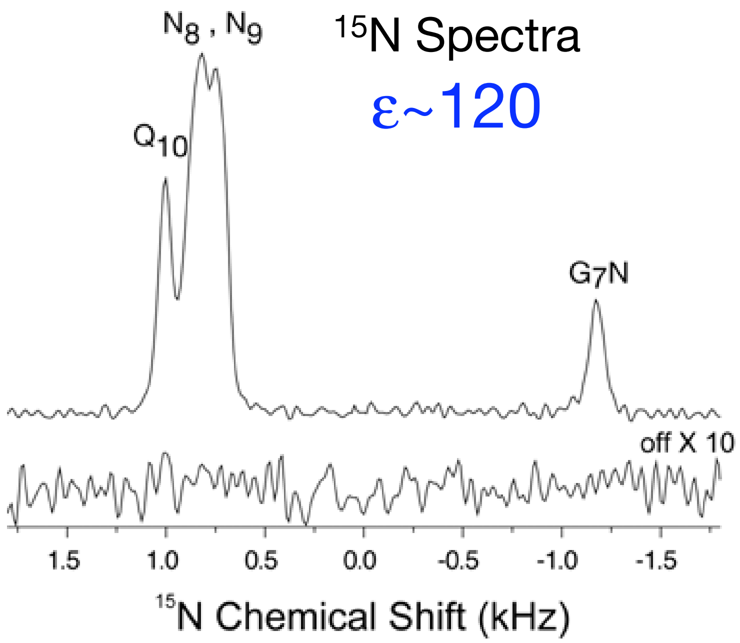
^{13}C Spectra

ε~120



^{15}N Spectra

ε~120



- Both the ^{13}C and ^{15}N spectra are enhanced by factors of ***~120*** !
- (U-^{13}C,^{15}N)-GNNQqny in 60% glycerol/H_2O, $\omega_r/2\pi$= 4.10 kHz,T=90 K

140 GHz ^{13}C and ^{15}N DNP/MAS Spectra
GNNQqny Nanocrystals

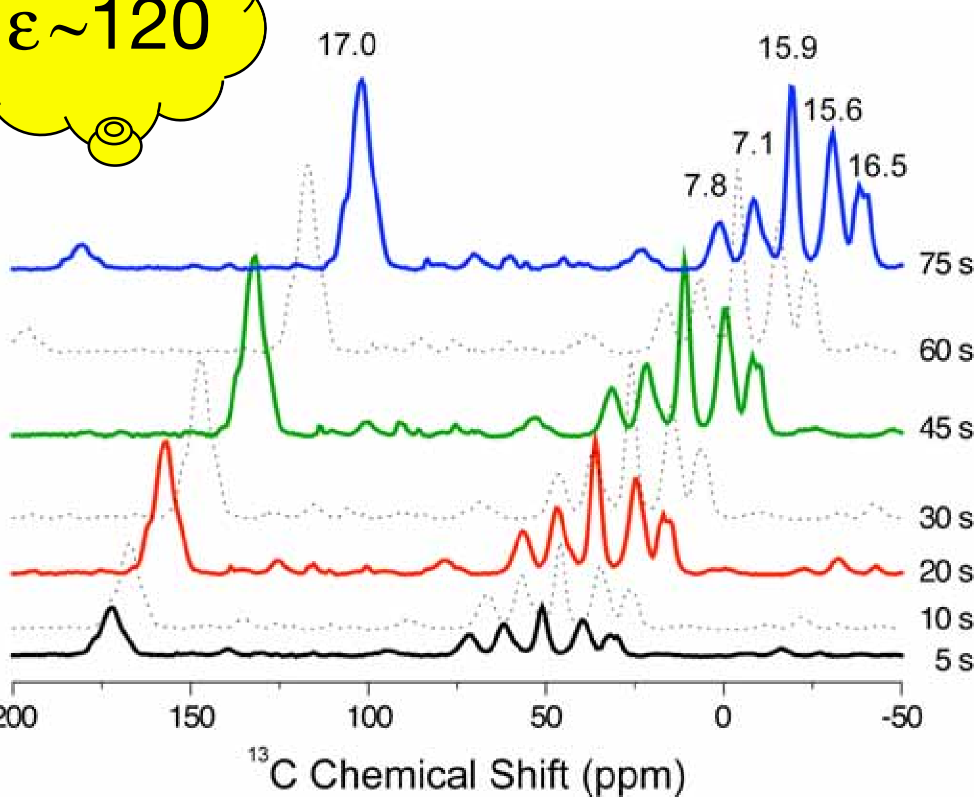
- Biradical polarizing agent -- TOTAPOL -- employed in the experiment

- TOTAPOL is probably too bulky to penetrate the nanocrystals

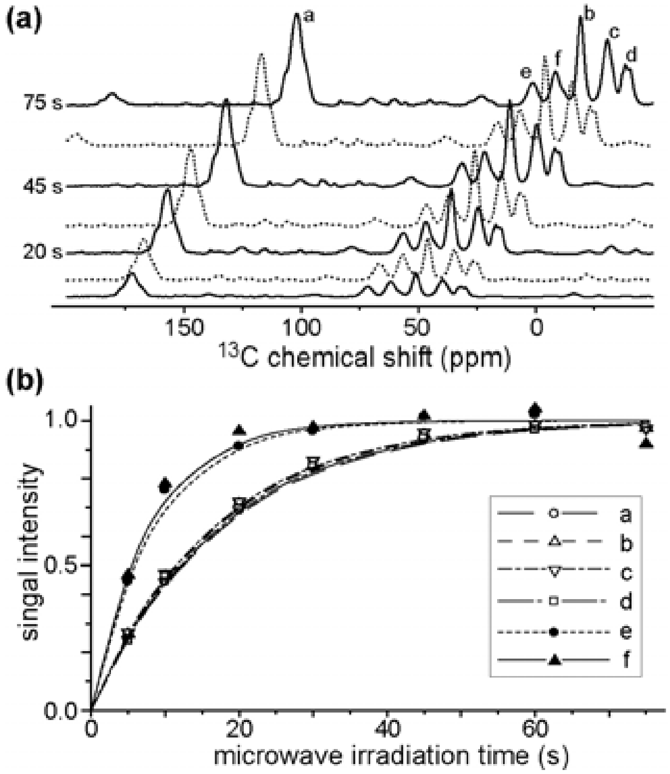
- Solvent polarization likely diffuses from the surface to the interior of the nanocrystals

- Polarization builds up with ~T_1 of the crystals

ε~120



T_1 Values for GNNQQNY



- TOTAPOL is probably too bulky to penetrate the nanocrystals

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

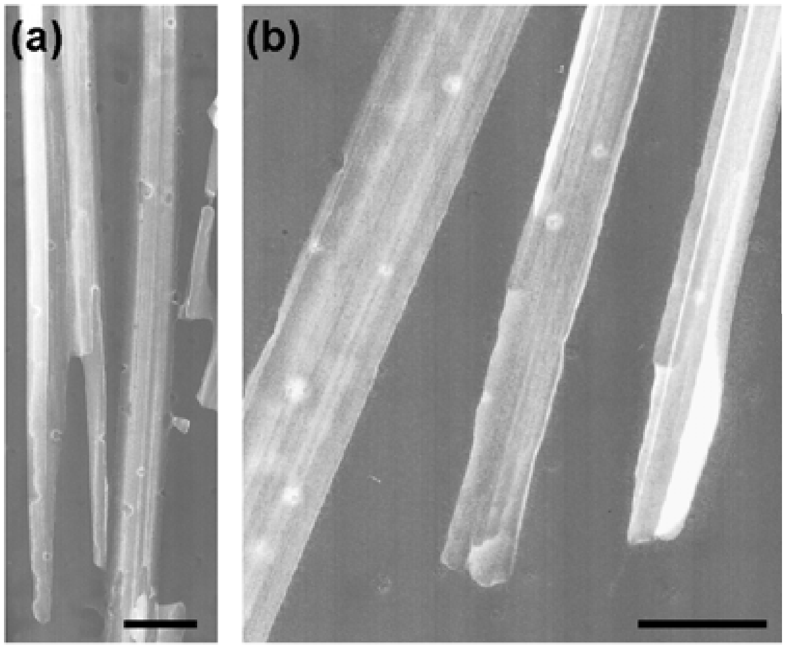
- Polarization builds up with ~T_1 of the crystals

DNP in GNNQQNY Nanocrystals

TEM's of GNNQQNY nanocrystallites

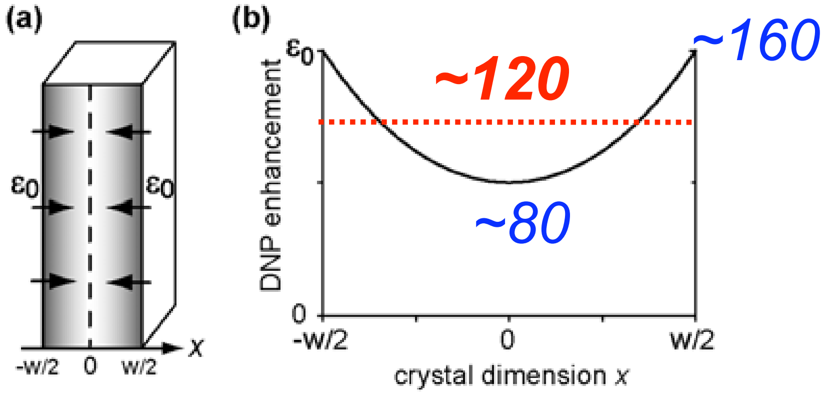
x 55,000

x 110,000



2000 Å.

~160



Fick's Law:

$$\frac{\partial P}{\partial t} = D\frac{\partial^2 P}{\partial x^2} - \frac{P}{T_{1n}}$$

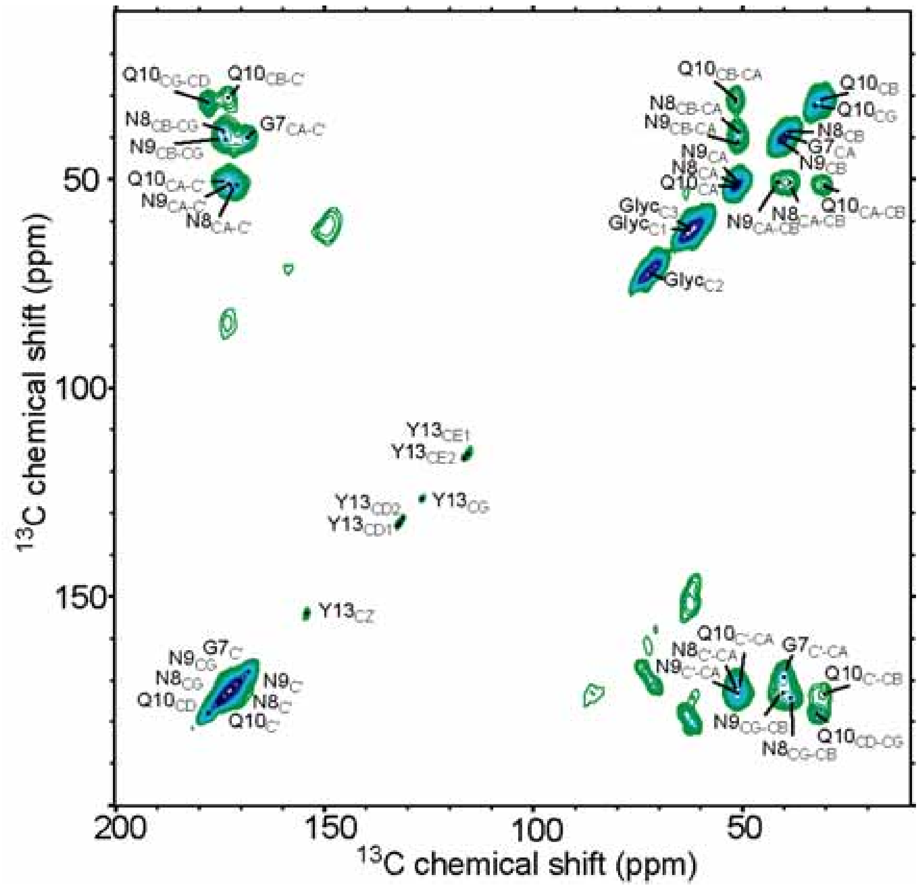
Steady state and boundary conditions:

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

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

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

Outline

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

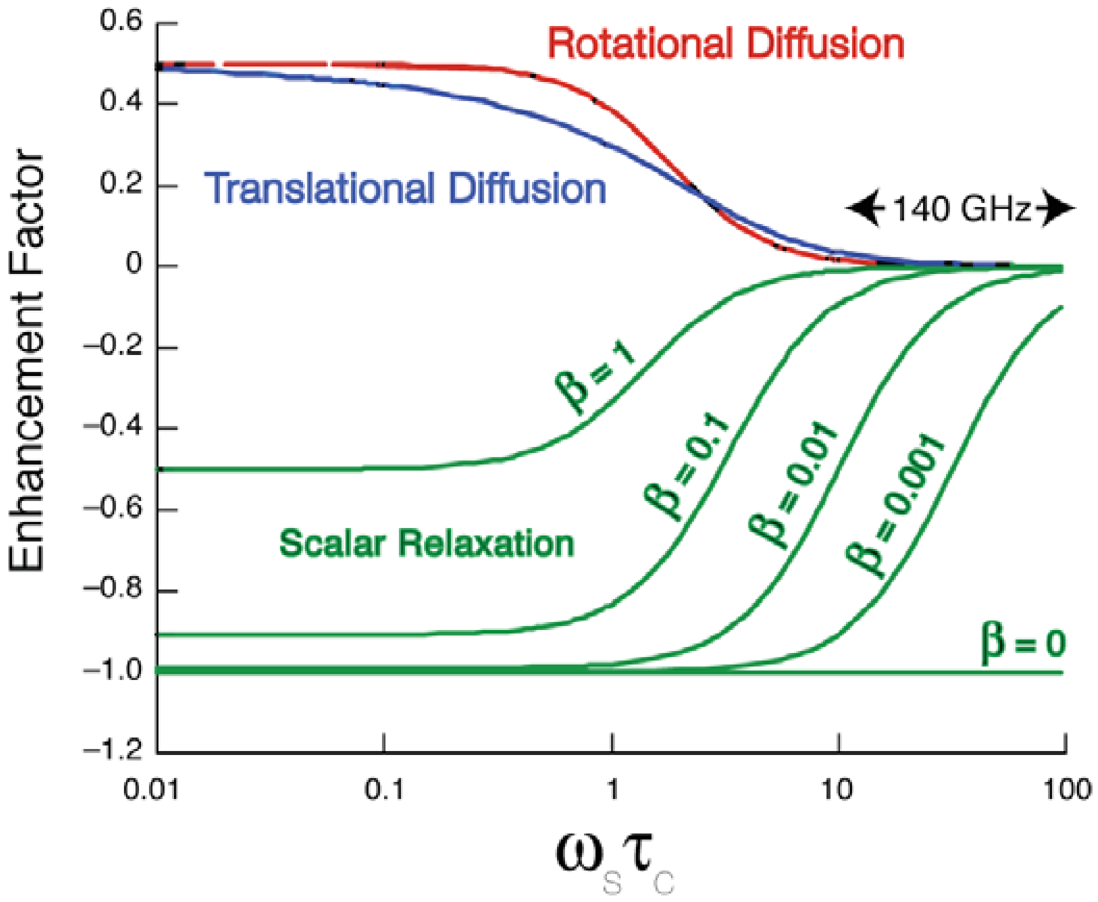
- Instrumentation for DNP
  Quadruple Resonance, LT MAS Probes
  Gyrotron Microwave Sources

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

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

- DNP in Solution (and for Metabolomics)
  Solid state polarization and laser T-jump
  $\varepsilon^{\dagger} = \varepsilon(T_{obs}/T_{polar}) = 130\text{-}330$ for ^{13}C

Cross-Relaxation in Liquids



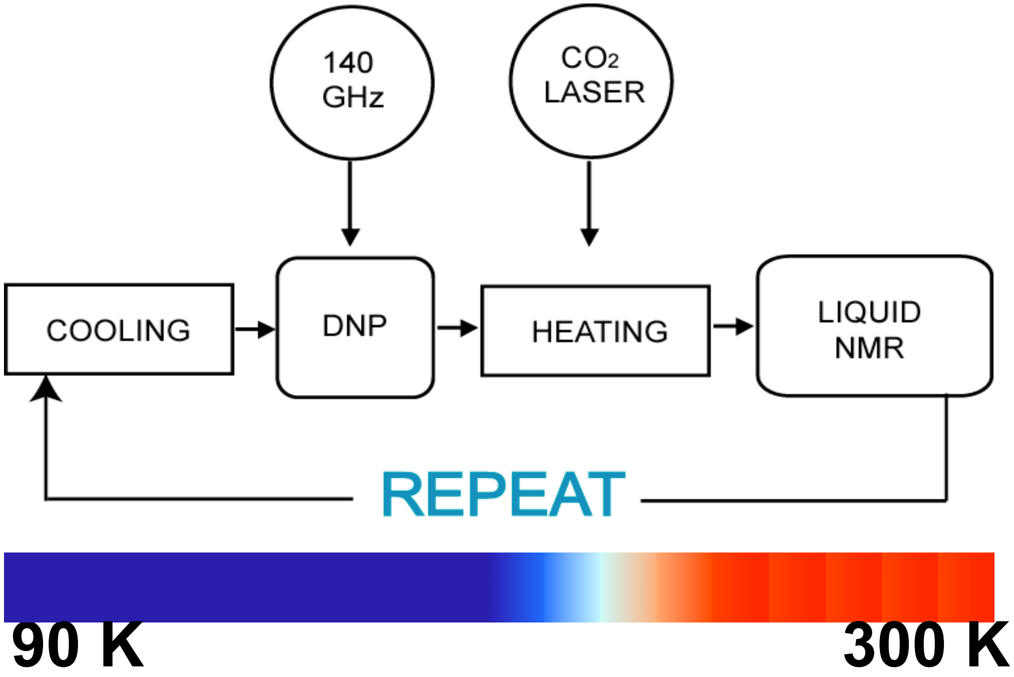
- •Dipolar relaxation is not useful for electron-nuclear polarization transfer -- no spectral density.
- •Scalar relaxation (with transient complex) is effective for producing DNP enhancements

Loening, Rosay, Weis and Griffin (2002)

TJ-DNP (in brief)

Principle:

Efficient transfer of DNP polarization from solid state to liquid state

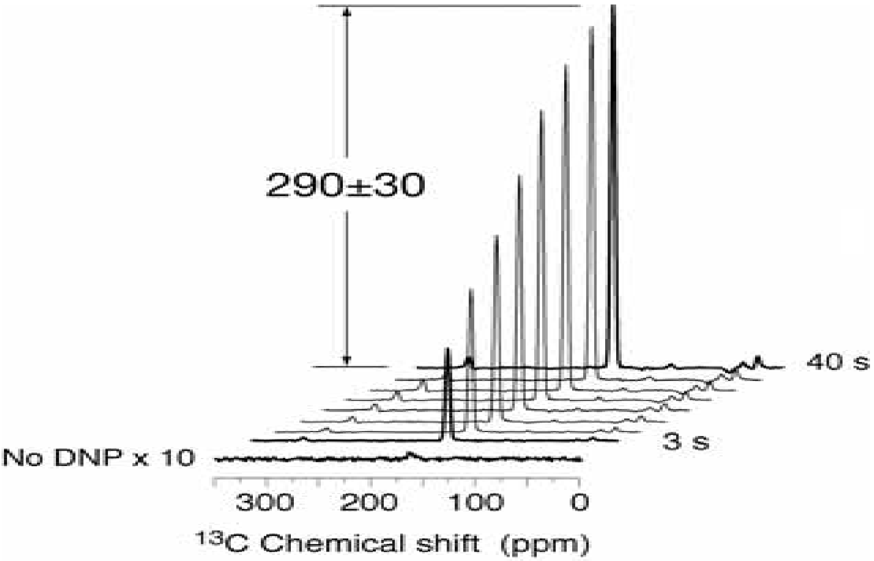


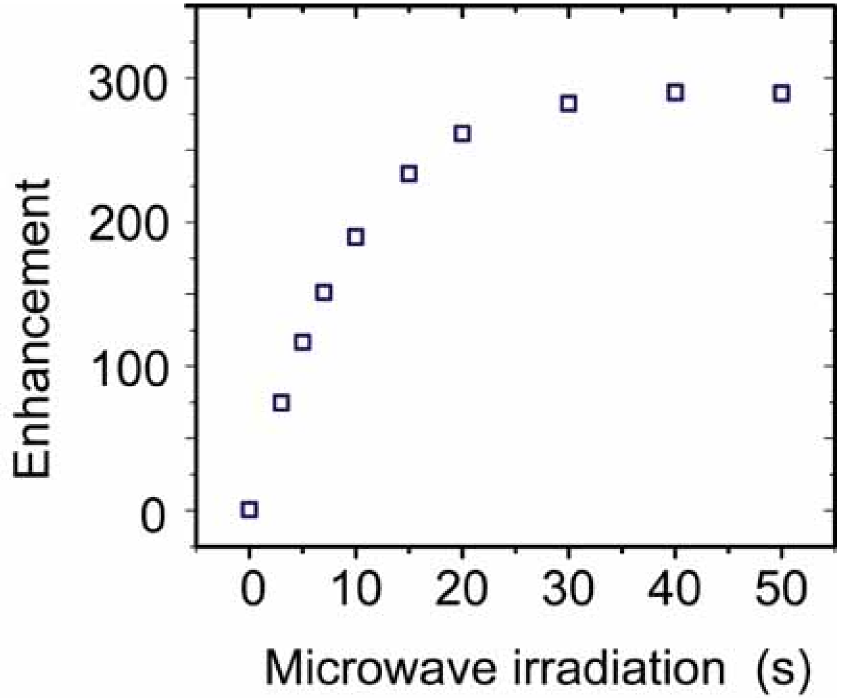
Laser heating: Ferguson, Krawietz, Haw, *J. Magn. Reson. Ser. A* (**1994)**
Ferguson, Haw, *Anal. Chem.* (**1995)**

DNP enhancement in the solid state

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

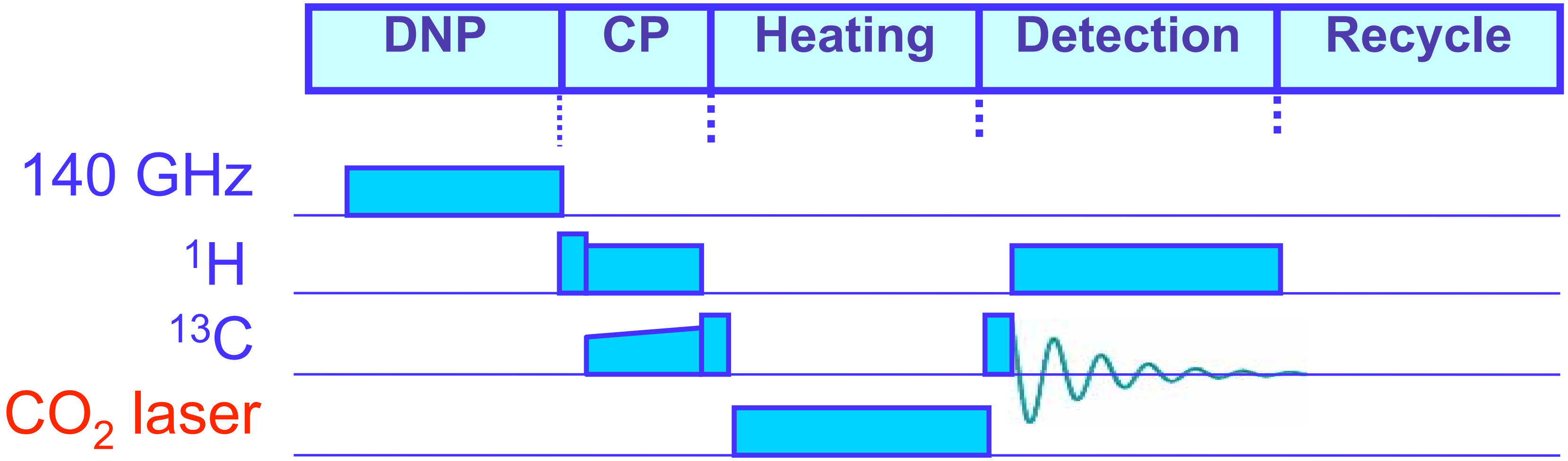
$\varepsilon_H = 290 \pm 30$





$\varepsilon^{\dagger}_{C}$ (expt) = 290 x 4 x 3.3 ~ 3800

TJ-DNP Experimental scheme

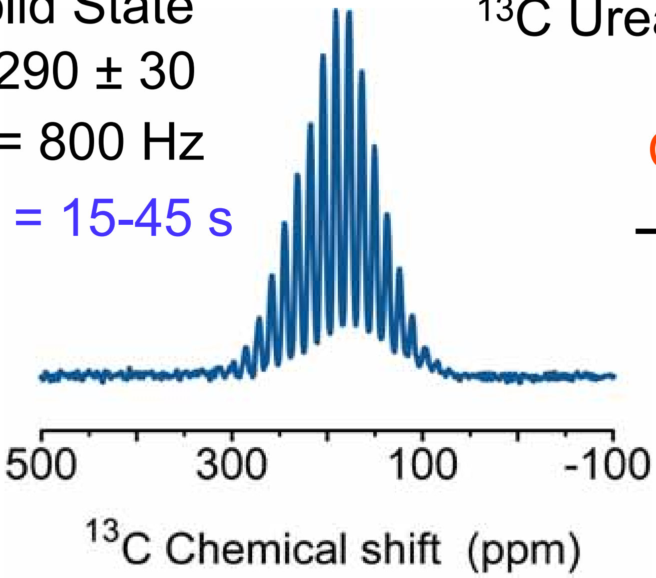


Solid State

ε~290 ± 30

ω_r = 800 Hz

τ_{DNP} = 15-45 s



^{13}C Urea/DMSO/water

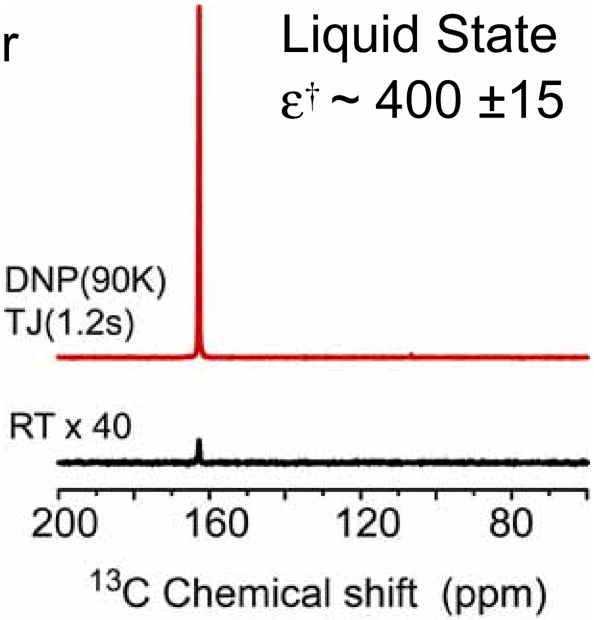
CO_2 Laser

→

Melting

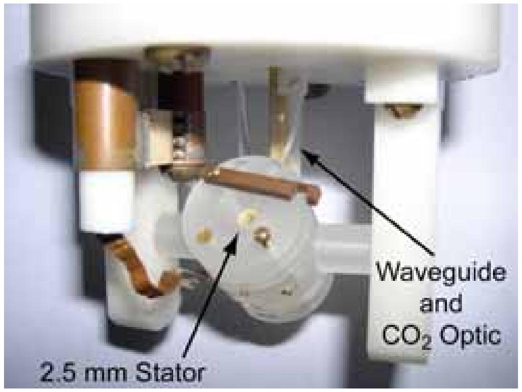
Liquid State

$\varepsilon^{\dagger}$ ~ 400 ±15

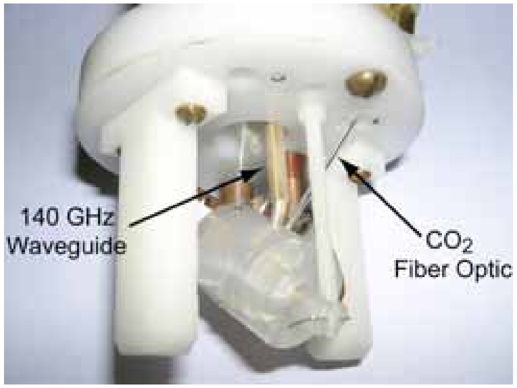


TJ-DNP Melting Probe

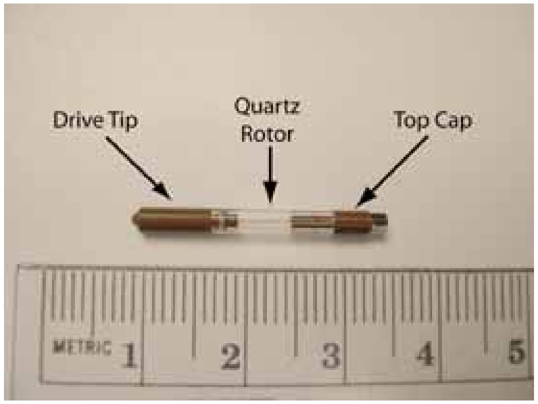
Front



Rear

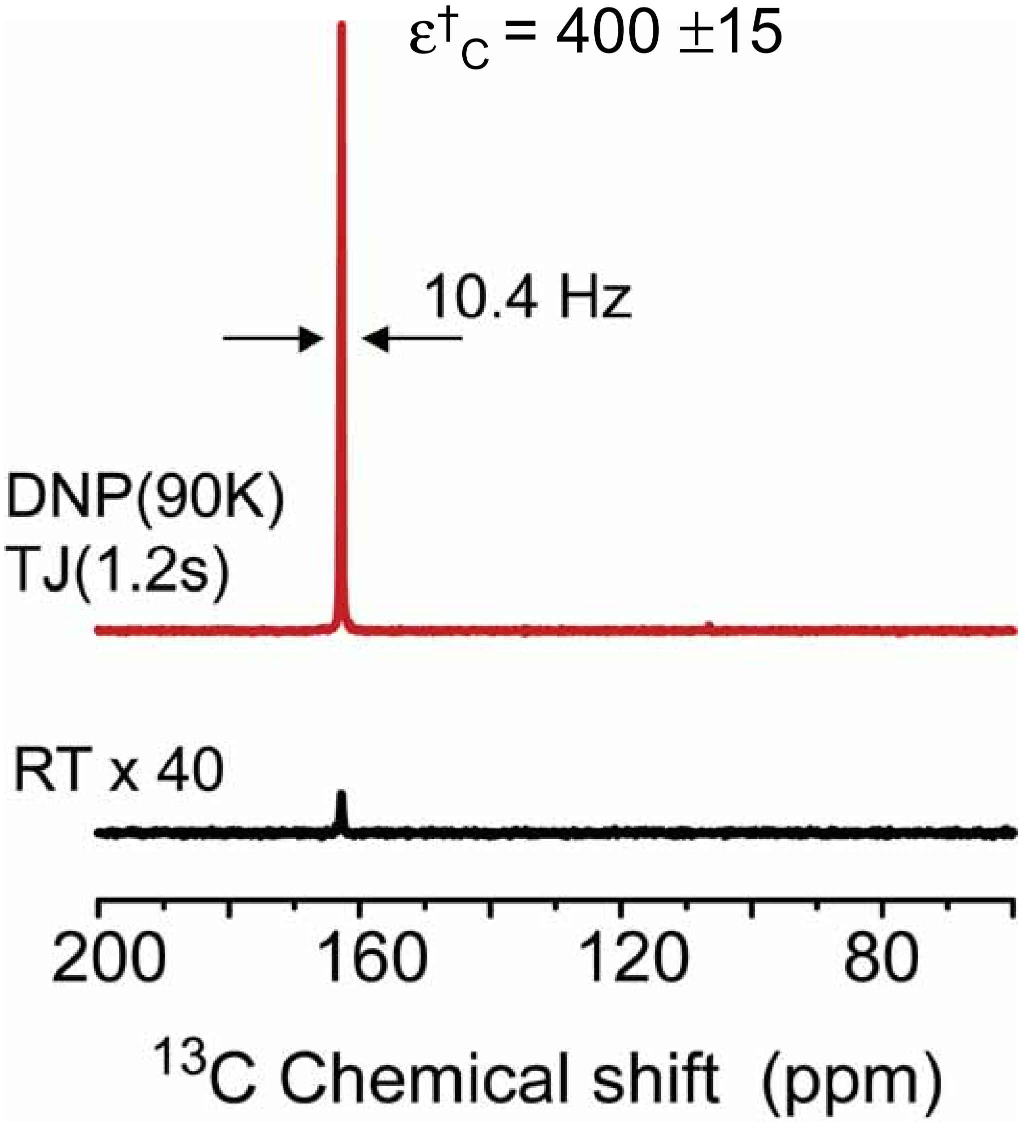


Rotor

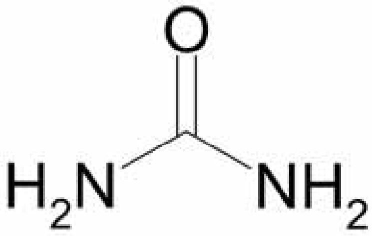


- Polarization is performed at 140 GHz and 90 K with slow rotation -- 300-800 Hz
- Melting is achieved with a 10 watt CO_2 laser

DNP in liquids -- urea

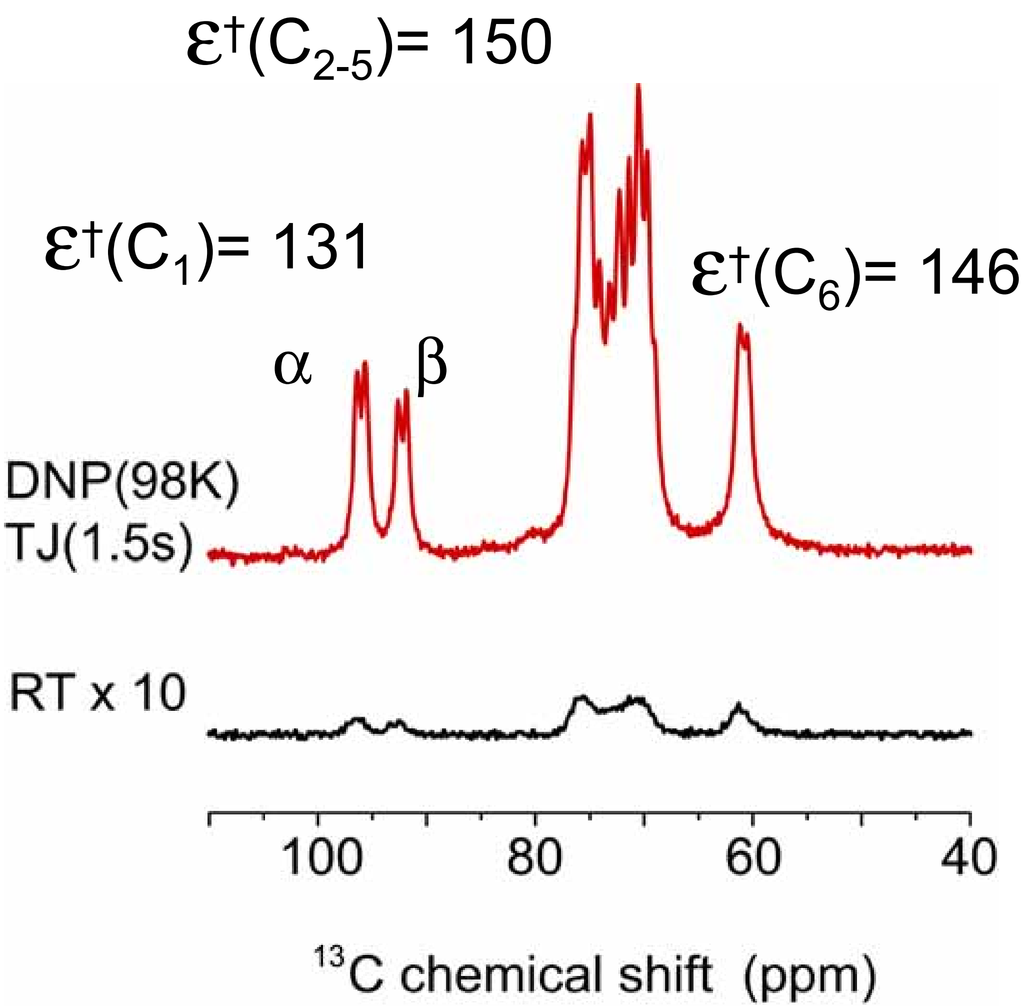


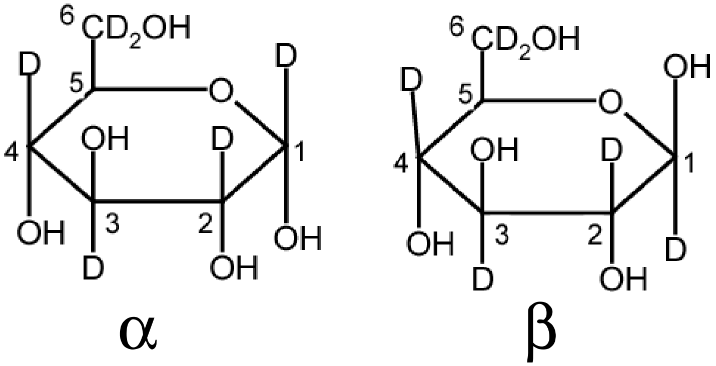
2M ^{13}C urea, DMSO/water
5.35mM biradical
90 K, $\omega_r/2\pi$ = 800 Hz



Non-protonated carbons

Effect of deuteration: Glucose-$^2H_7$





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

$^{13}C_6$,$^2H_7$ glucose/water 8/2
10 mM biradical

Enhancement in liquids ($\varepsilon^{\dagger}$) and solids (ε)

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

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

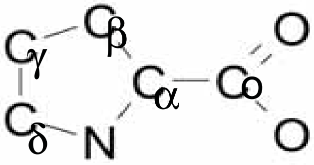
$$\varepsilon^{\dagger} = \varepsilon \left(\frac{T_{final}}{T_{initial}} \right)$$

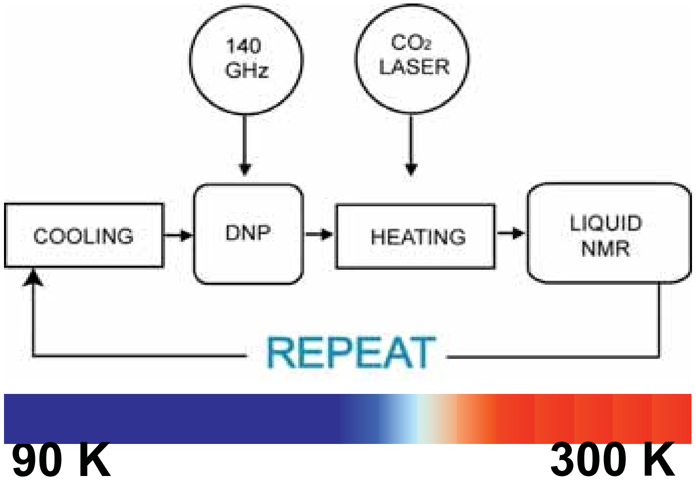
$$\boxed{\varepsilon^{\dagger}\,(max) = 660 \times 4 \times 3.3 \sim 8700}$$

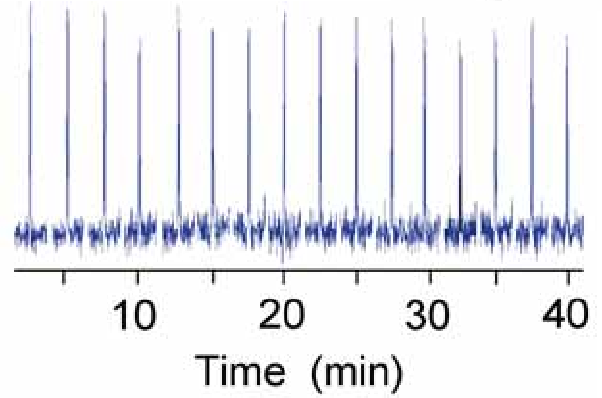
Hype your polarization - multiply by the Boltzmann factor !

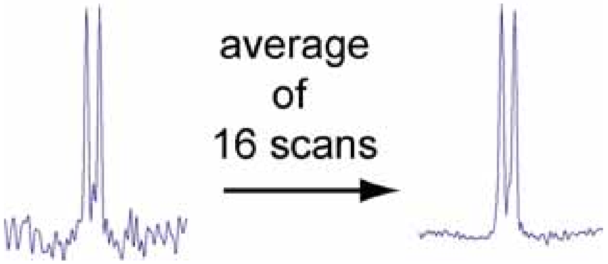
TJ-DNP: Recycling

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





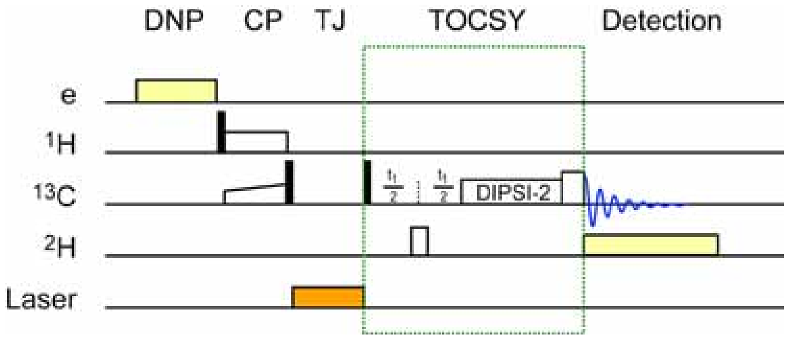




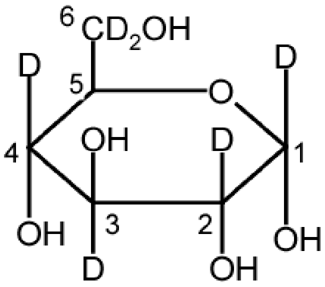
Applications:

Signal averaging and multidimensional NMR

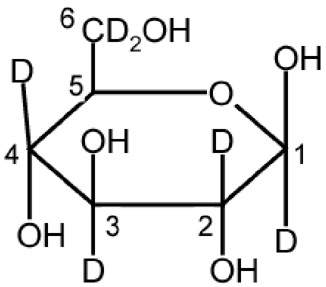
2D spectrum: Glucose-$^2H_7$



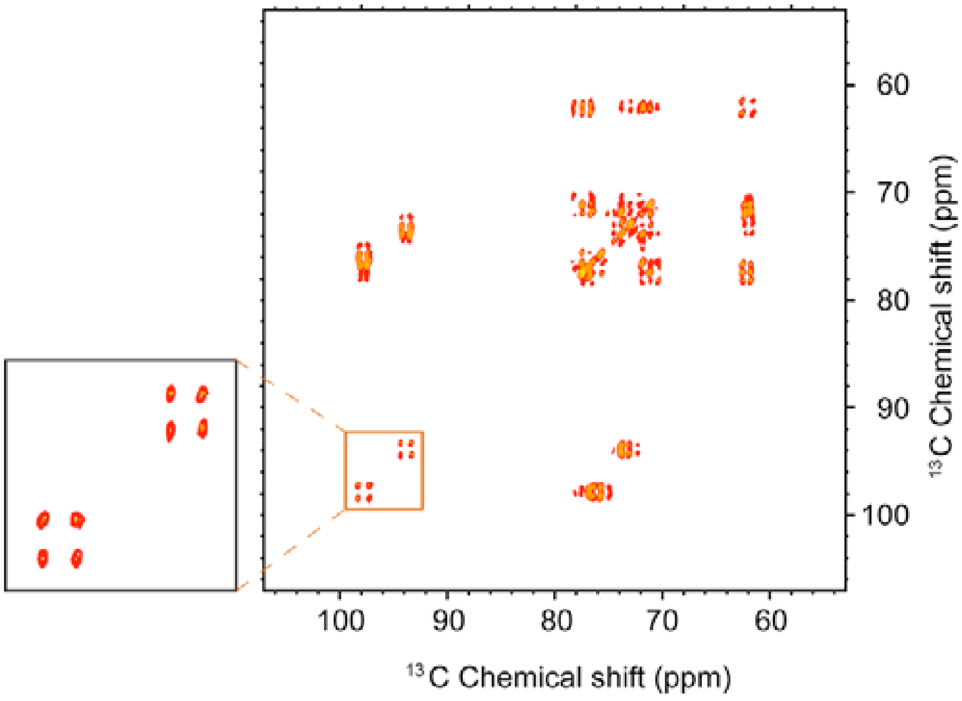
α



β



$^{13}C_6,^2H_7$ glucose/water 8/2
10 mM biradical



$\varepsilon^{\dagger}$ ~120-170

Thank you
for
your attention!