Basics of EPR spectroscopy

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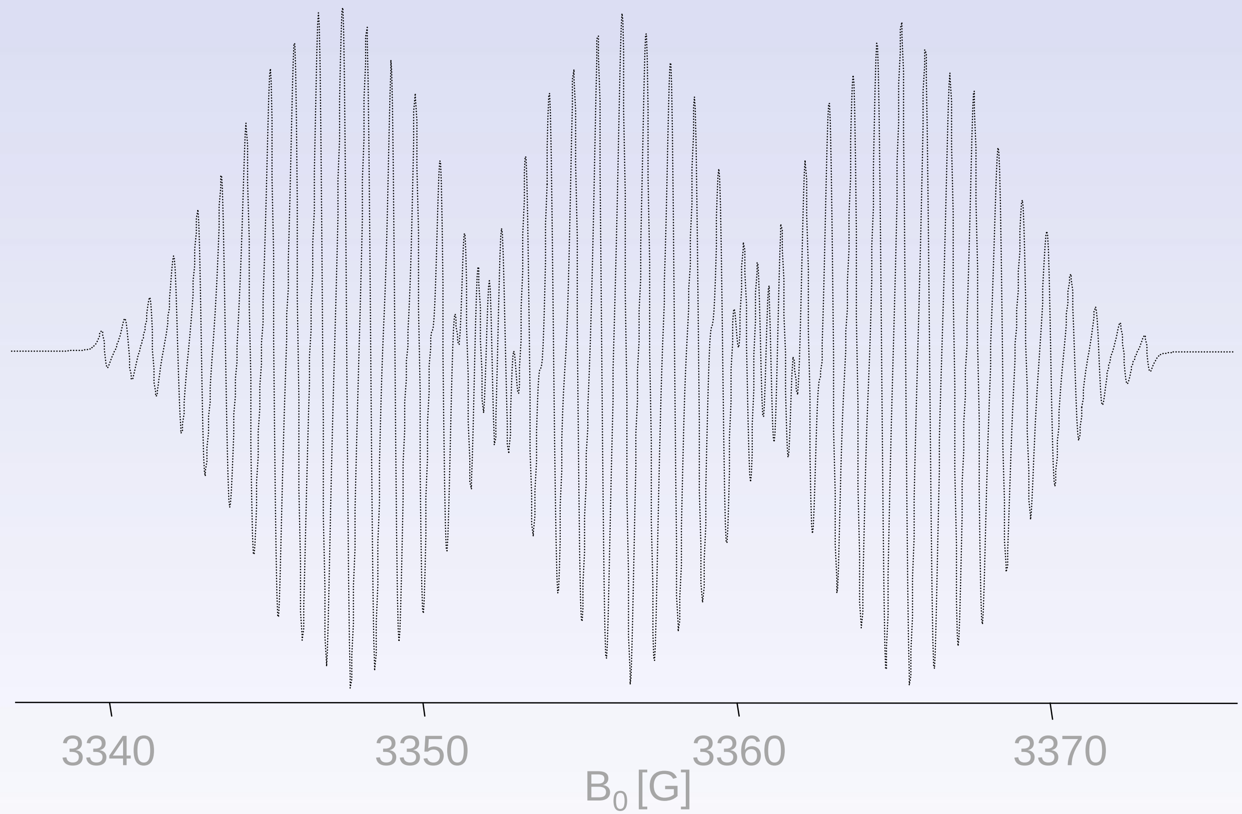


NMR meets Biology 2018

Biologisches Magnet-Resonanz Zentrum

6 Professor
4 Ass. Prof.
20 Postdocs
90 PhDs

Translation from NMR to EPR

NMR Language	EPR Language
Chemical Shift	g-value
Chemical Shift Anisotropy	anisotropic g-Tensor
J-Coupling	J-Coupling Isotropic Hyperfine Coupling
Dipolar Coupling Homonuclear	Dipolar Coupling
Dipolar Coupling Heteronuclear	Anisotropic Hyperfine Coupling
Quadrupole Coupling	Zero Field Splitting
SEDOR	PELDOR/DEER
Solid Echo	SIFTER
MAS	-
Decoupling	-

Typical EPR operation frequencies

Magnetic field	EPR frequency	Band	Wavelength	NMR ^{1}H frequency
1000 G	2.8 GHz	S-band	11 cm	4 MHz
3300 G	9.2 GHz	X-band	3 cm	14 MHz
1.2 T	34 GHz	Q-band	9 mm	50 MHz
3.4 T	95 GHz	W-band	3 mm	140 MHz
6.5 T	180 GHz	G-band	1.6 mm	275 MHz
9.2 T	260 GHz	J-band	1.2 mm	400 MHz

Lower magnetic fields but much higher excitation frequencies

MW Band Nomenclature

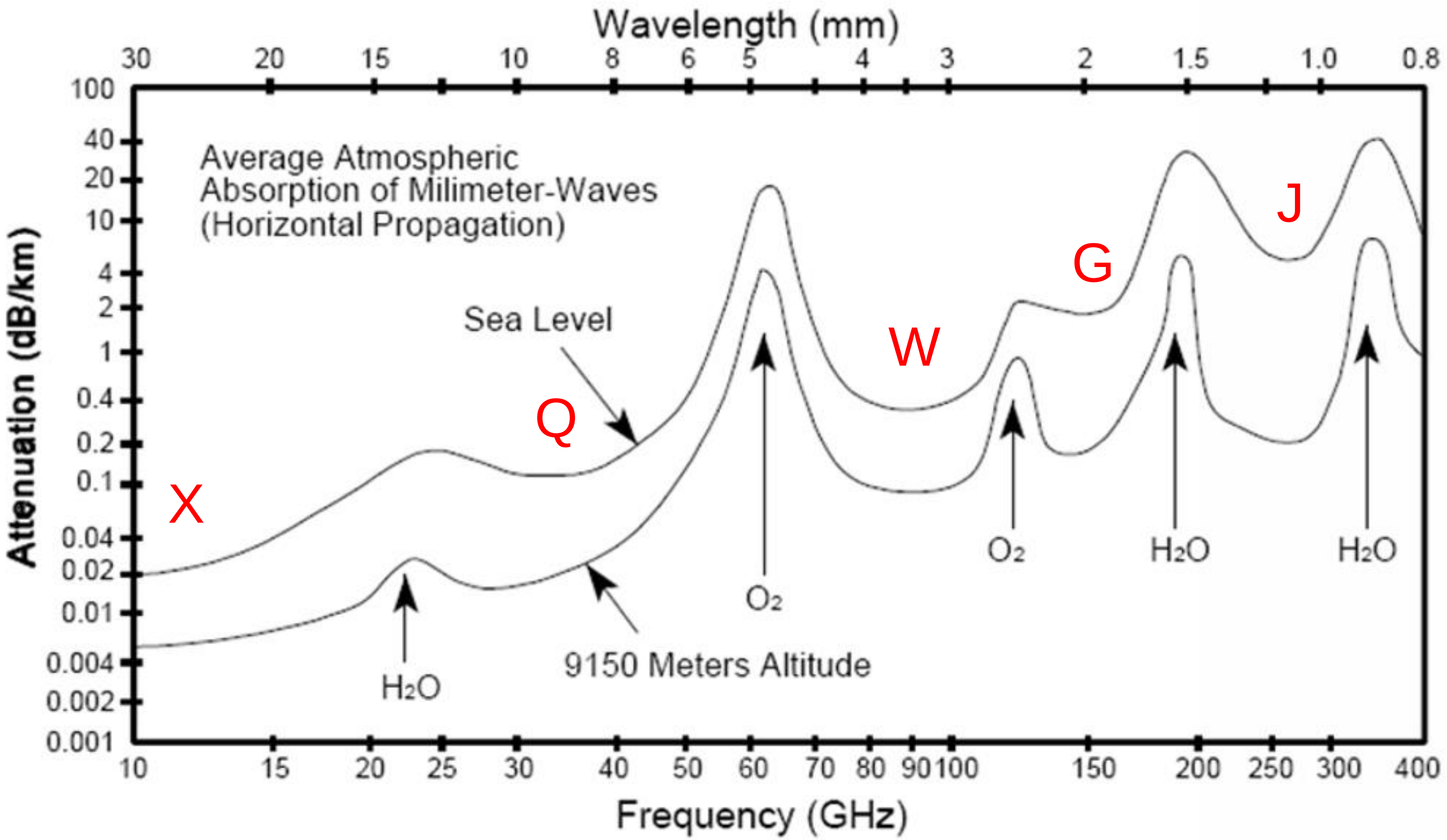


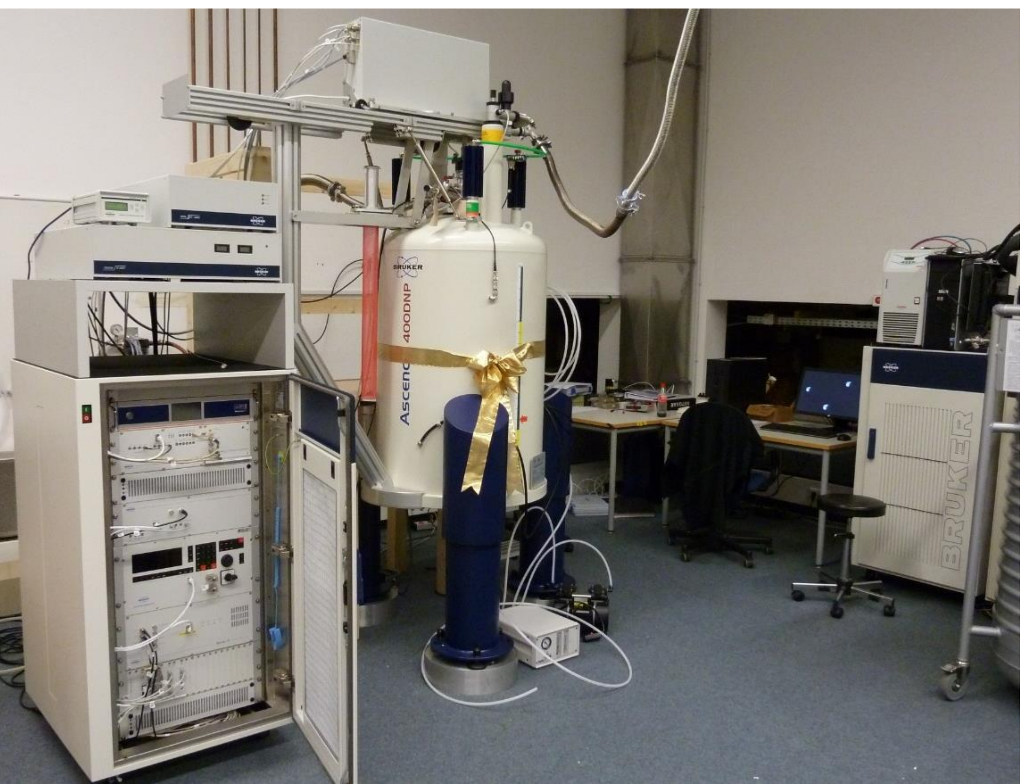
Figure 1. Atmospheric Absorption of Millimeter Waves

Pulse EPR Spectrometer Setups

X/Q-band EPP

Electromagnet

MW
Semiconductor Technology
Waveguide Transmission

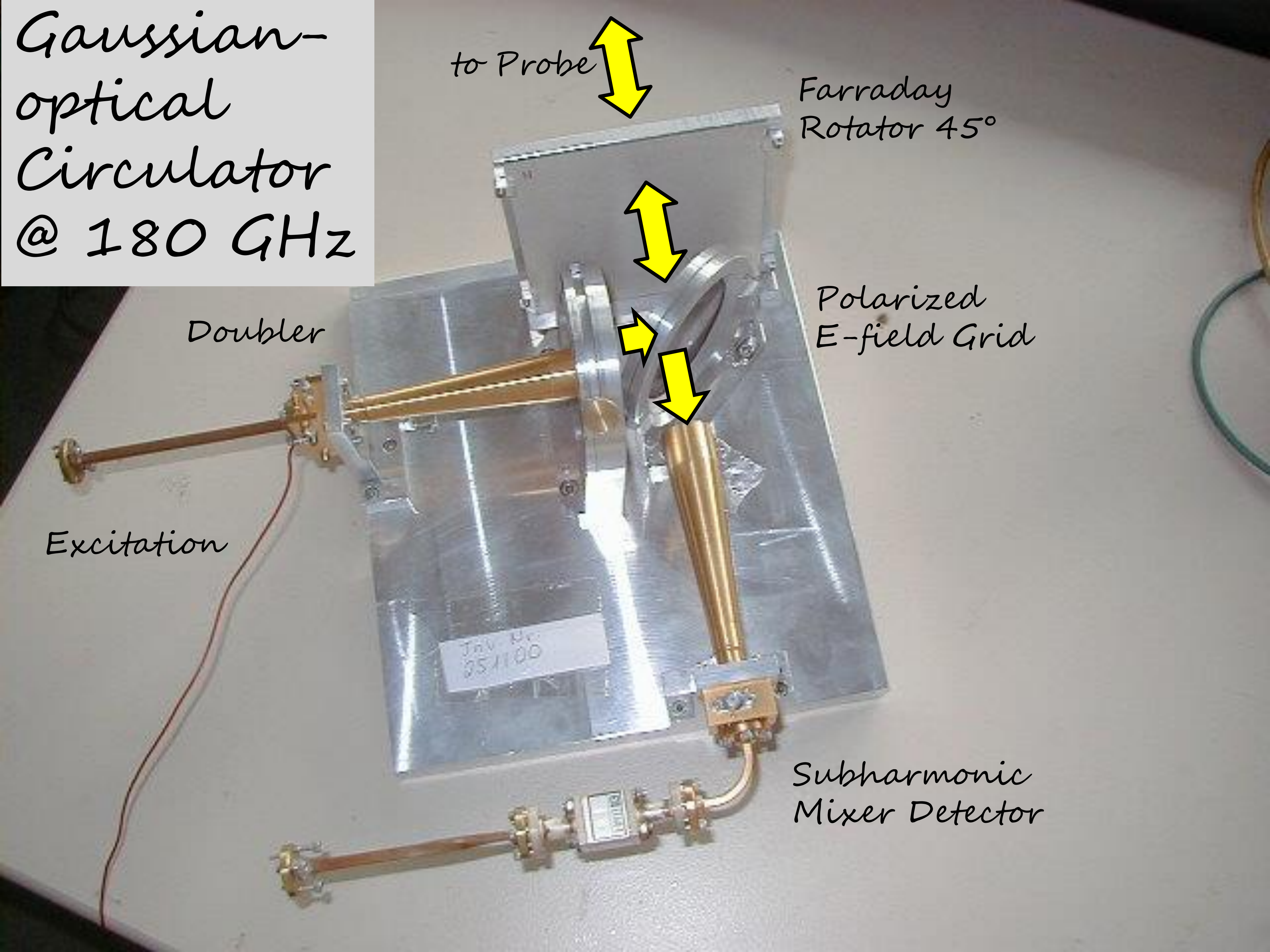


W/G/J-band EPP

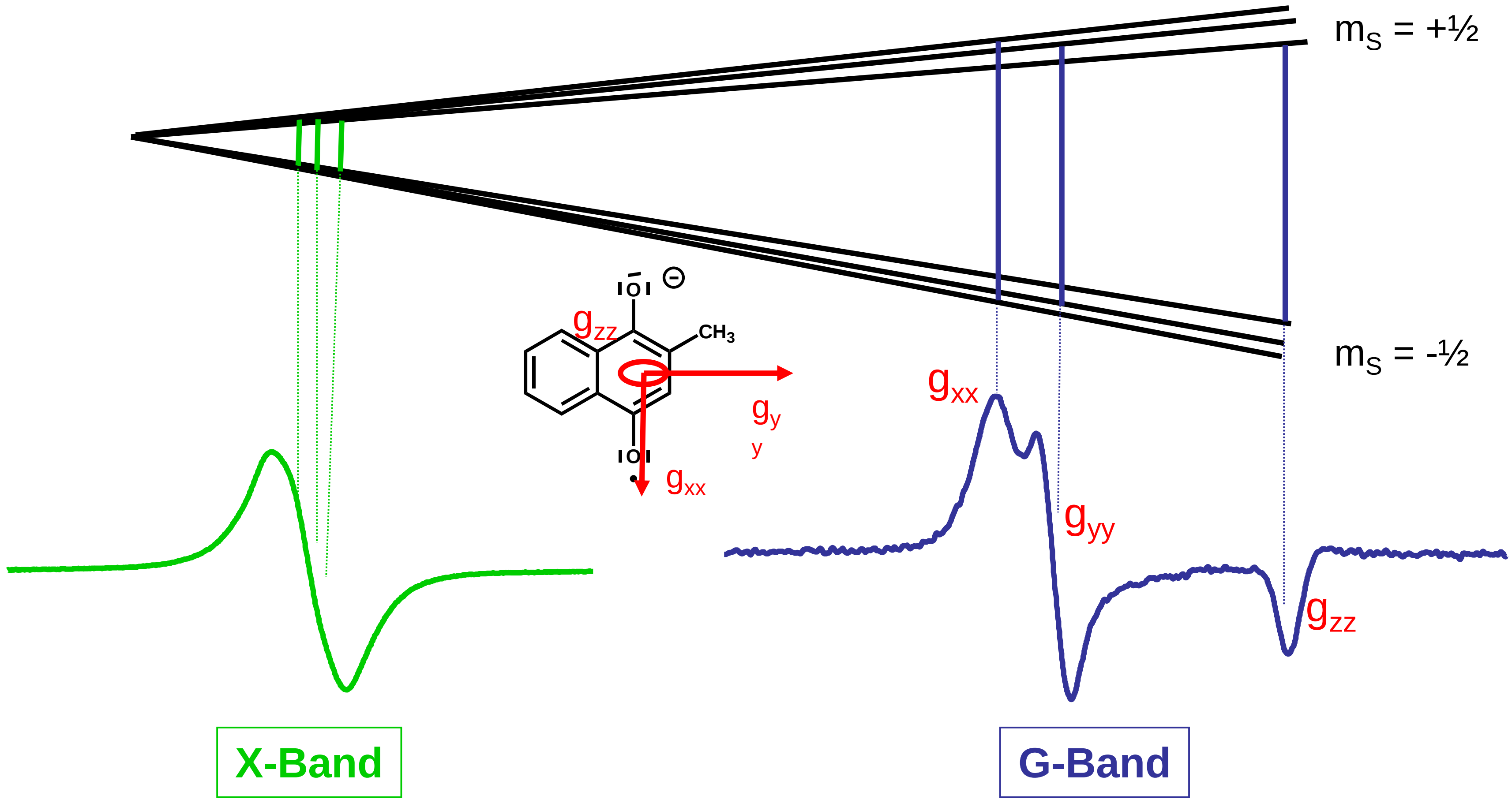
Superconducting Magnet

MW (Far-IR, THz)
Free Electron Tube Devices
or high-harmonic (low power)
Quasioptical Transmission

Gaussian-optical Circulator @ 180 GHz



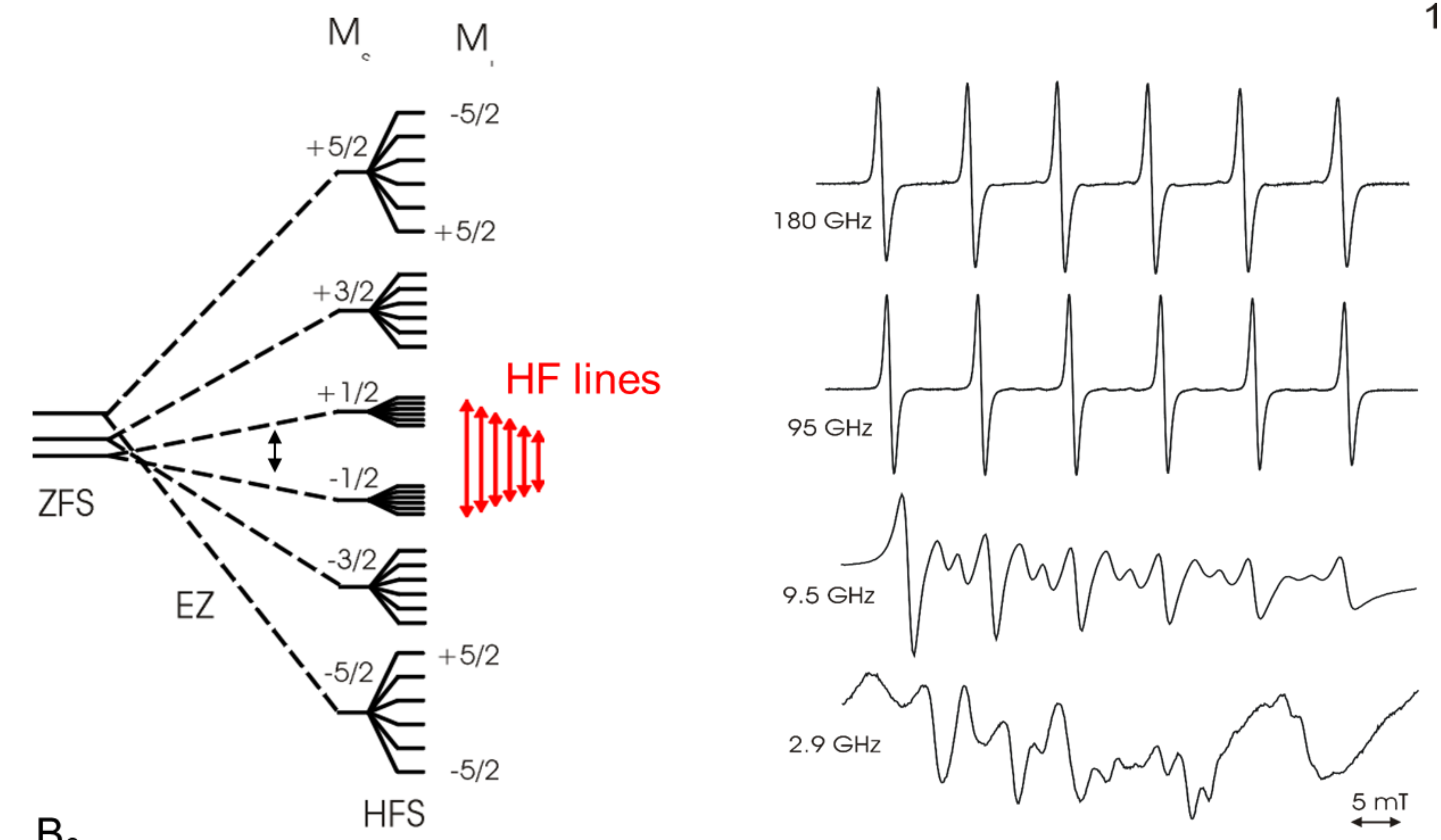
Anisotropic G-Tensor resolution at High Fields



Different orientations can be distinguished at high fields

Higher Sensitivity for half-integer high spins

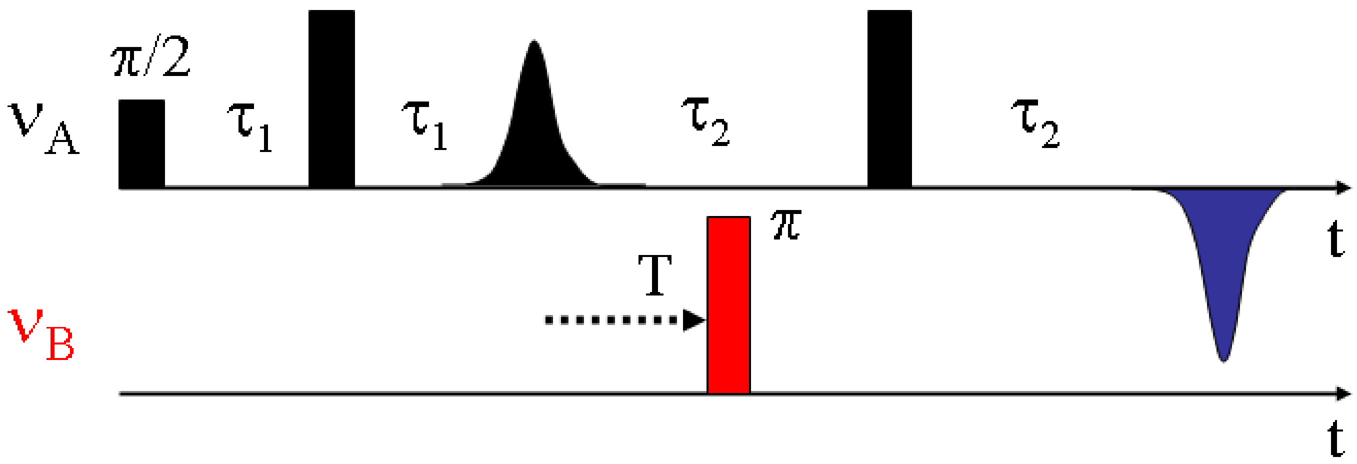
EPR Spectra of Mn^{2+} S = 5/2



Rohrer et al, 2001

The m_s ±1/2 transition is affected by the ZFS only in second order, forbidden transitions are suppressed

Time Scale of pulsed EPR



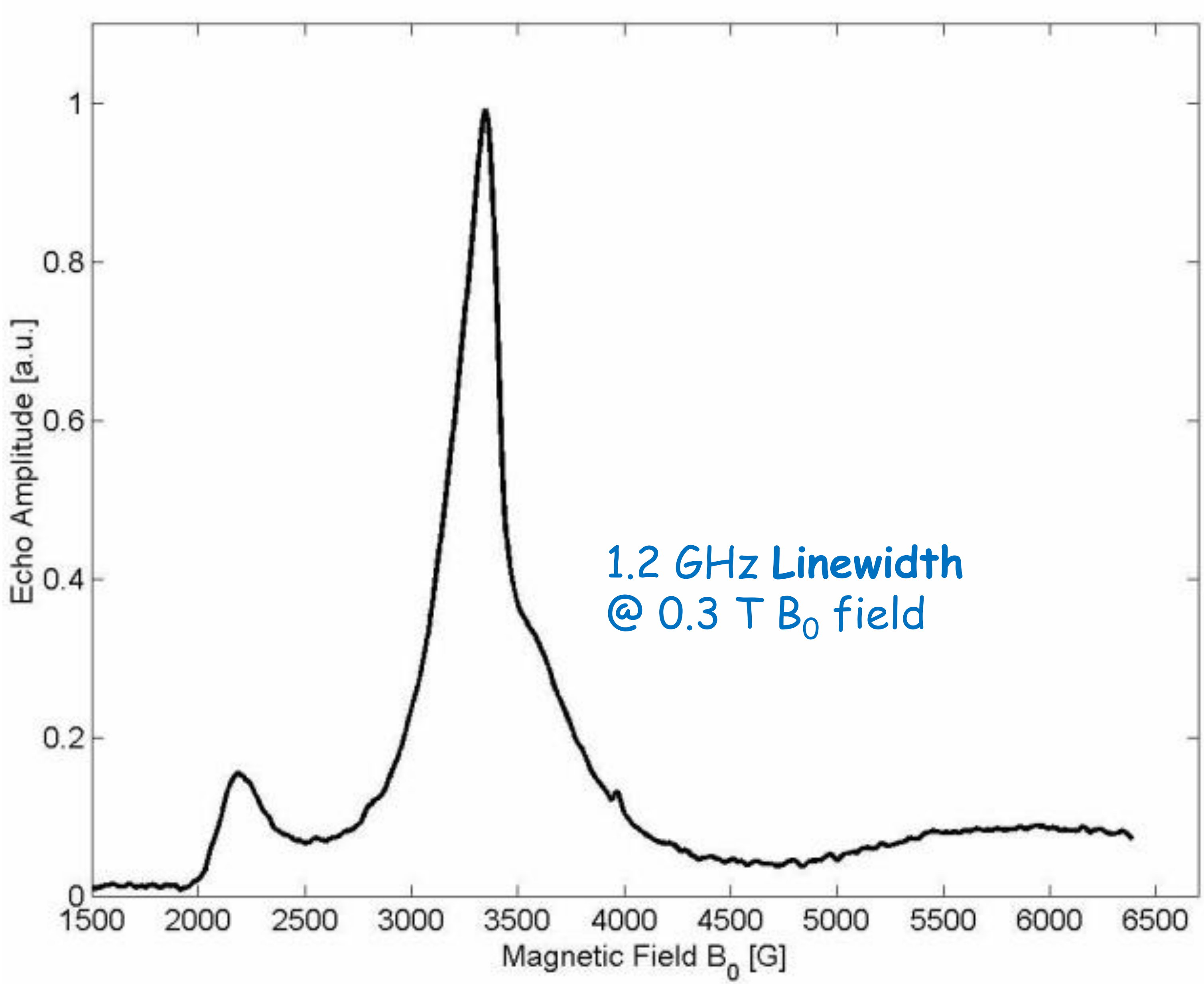
Pulse lengths : 2-20 ns

Deadtime after pulse: 20-50 ns

Pulse delays: 50 ns-10 μs

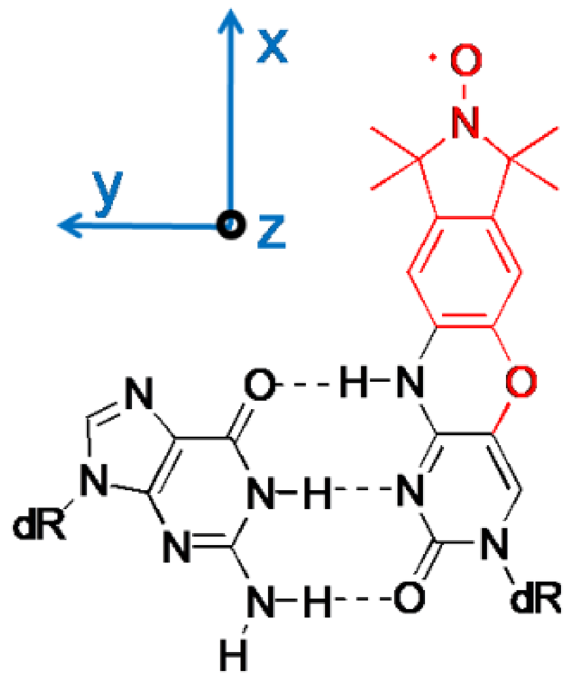
Repetition rates: 1-100 KHz

Linewidth in EPR Spectroscopy



1.2 GHz **Linewidth**
@ 0.3 T B_0 field

Coupling strengths in EPR Spectroscopy



Hyperfine Coupling Nitrogen: 90 MHz

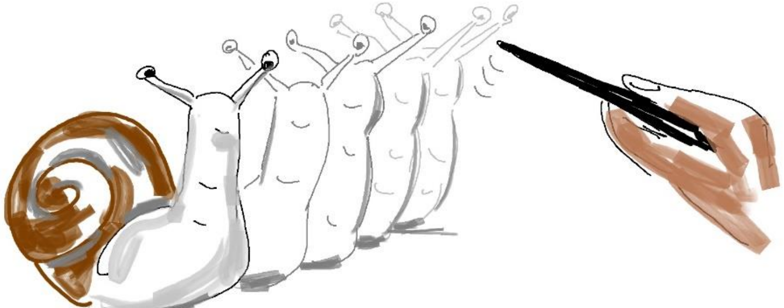
Hyperfine Coupling Protons: < 1 MHz

Dipolar Coupling S – S: 0.1-50 MHz

Comparison nuclear spin I versus electron spin S

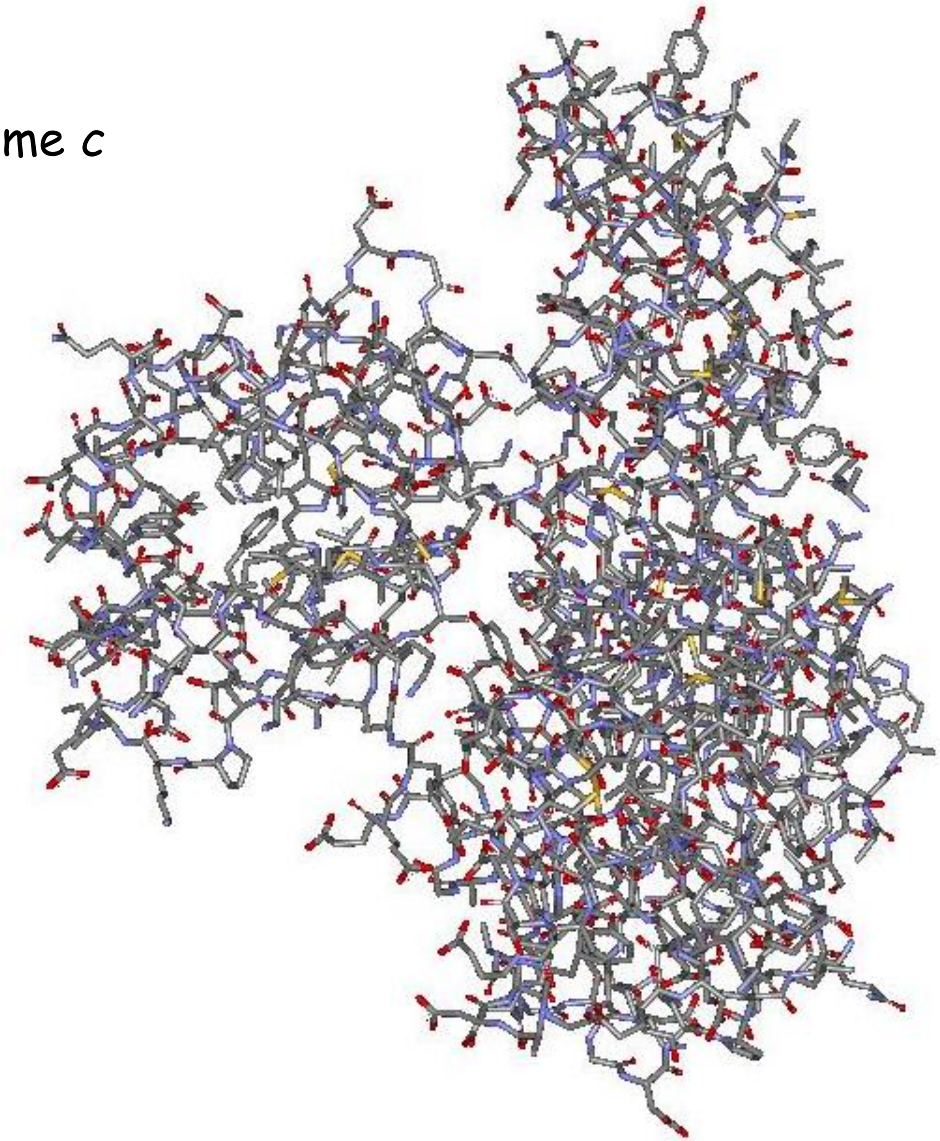
Weight: 30 g 50 kg

Speed: 3 m/h



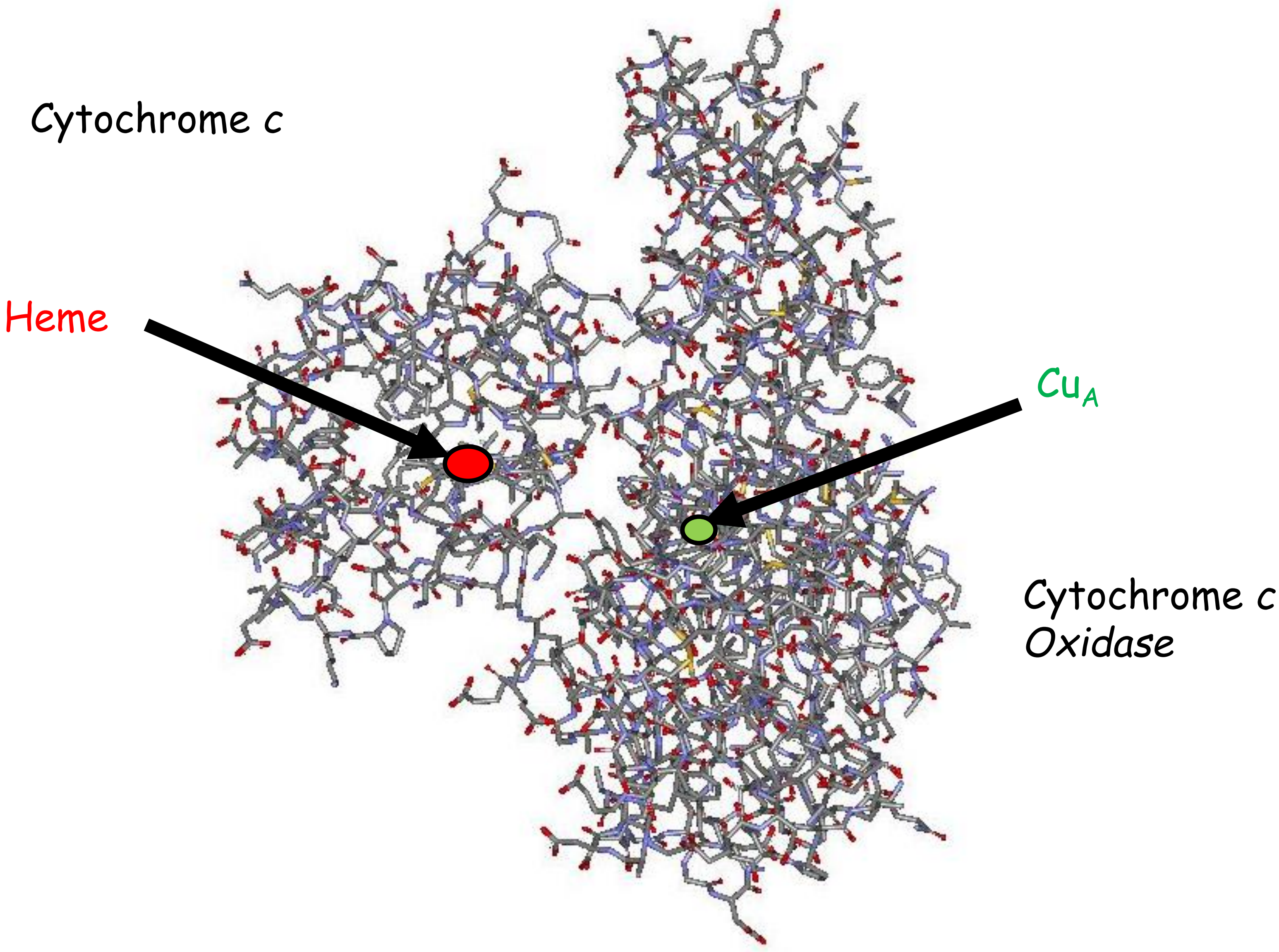
Biological Applications of EPR

Cytochrome *c*

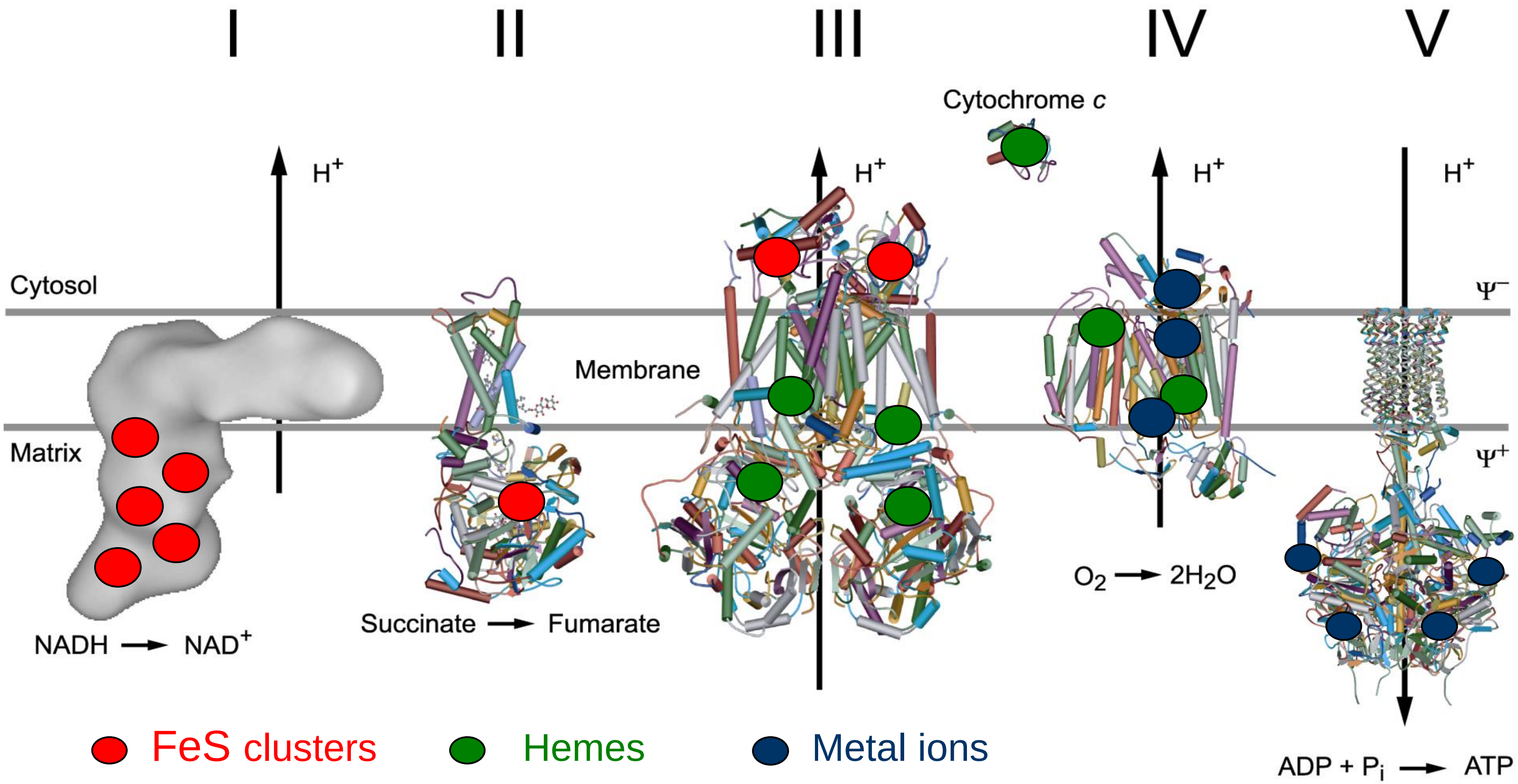


Cytochrome *c*
Oxidase

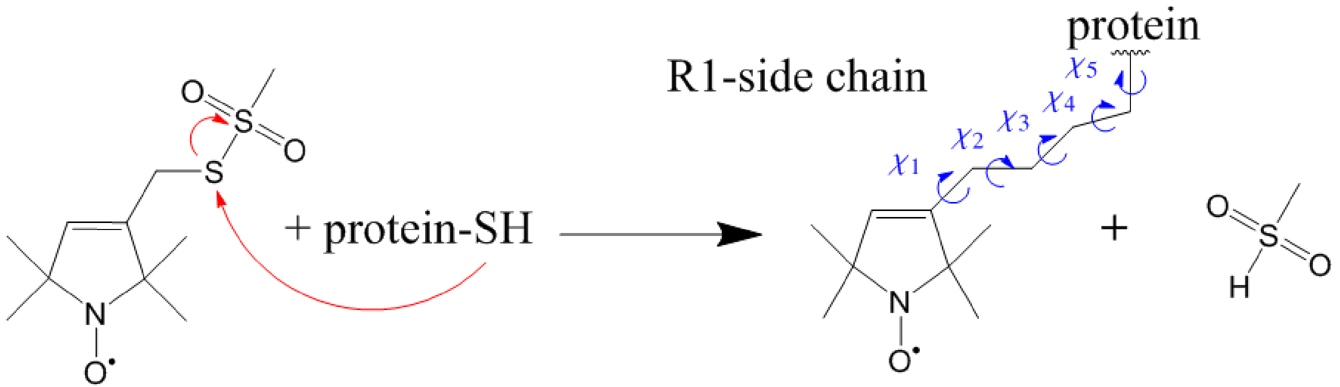
Biological Applications of EPR



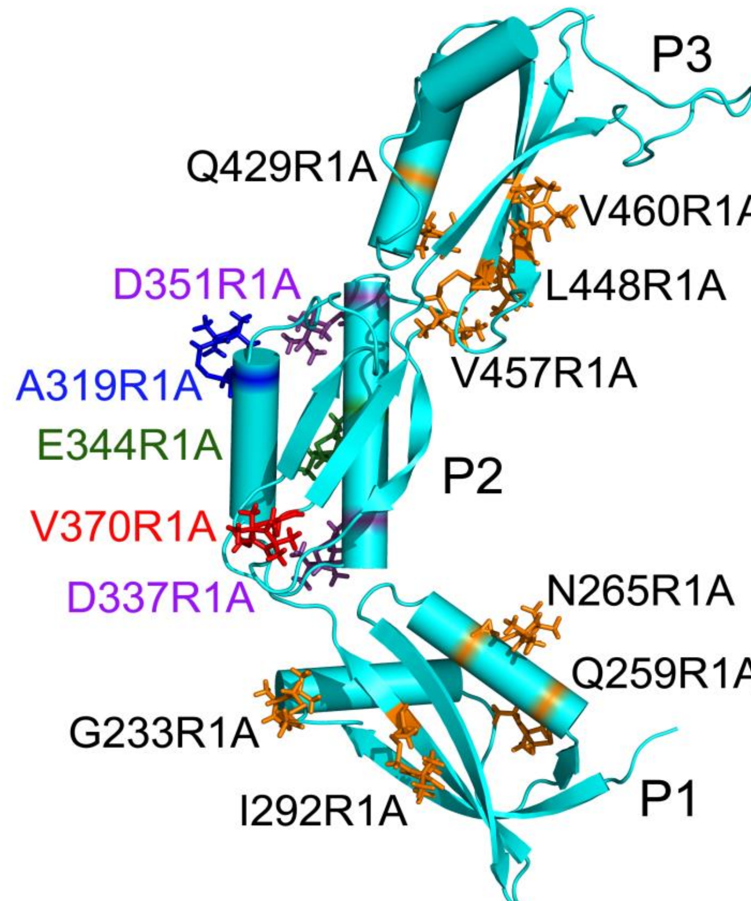
Intrinsic Paramagnetic Centers



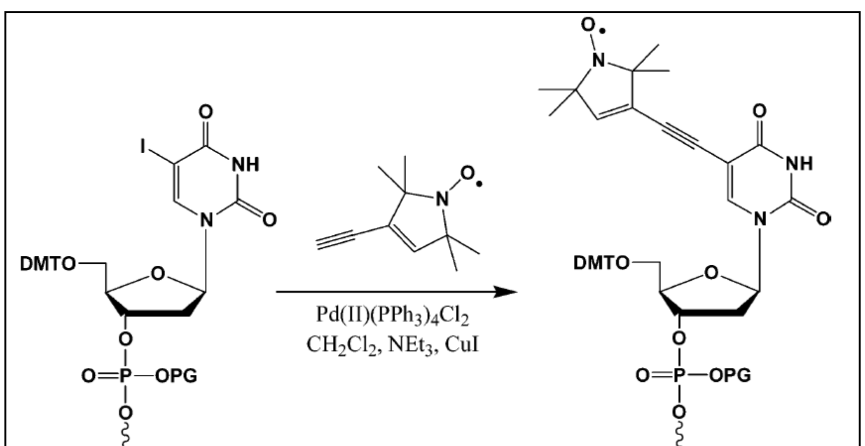
Spin-labeling of biomolecules



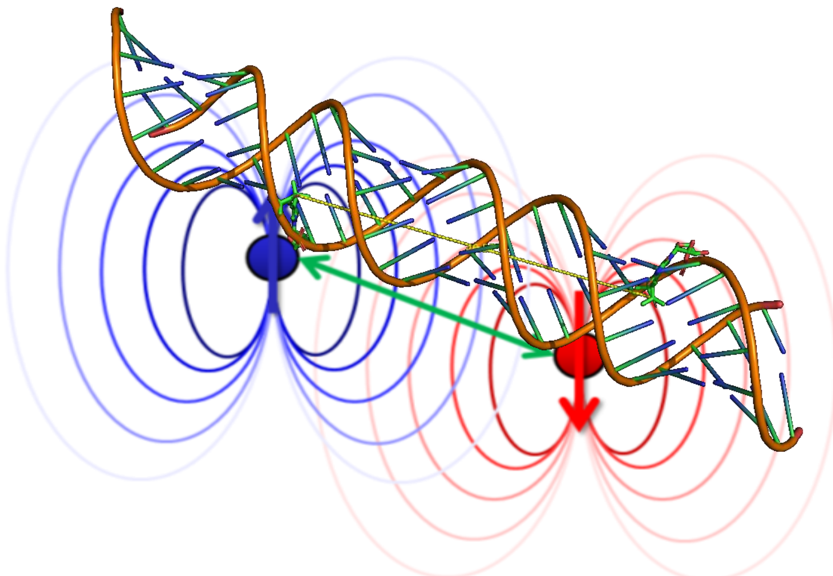
MTSL



Nucleic Acids

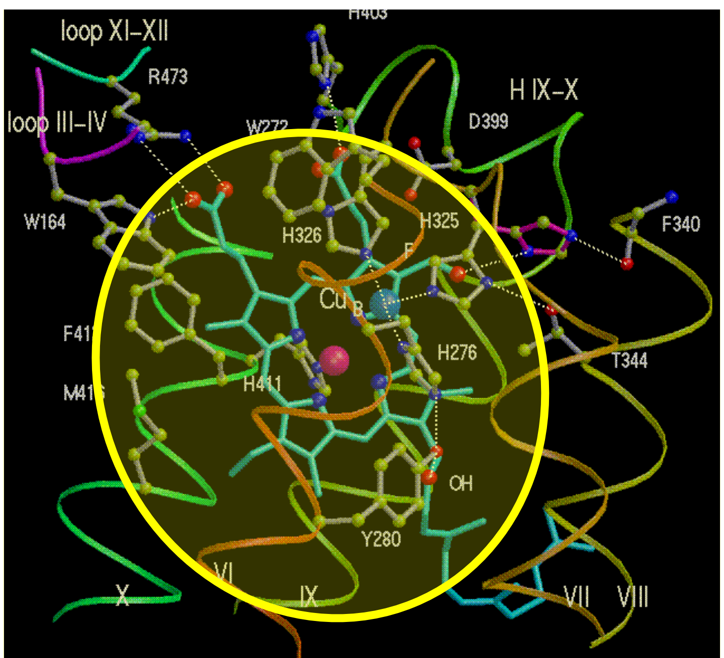


Applications of pulse EPR in Biology



Dipolar Spectroscopy

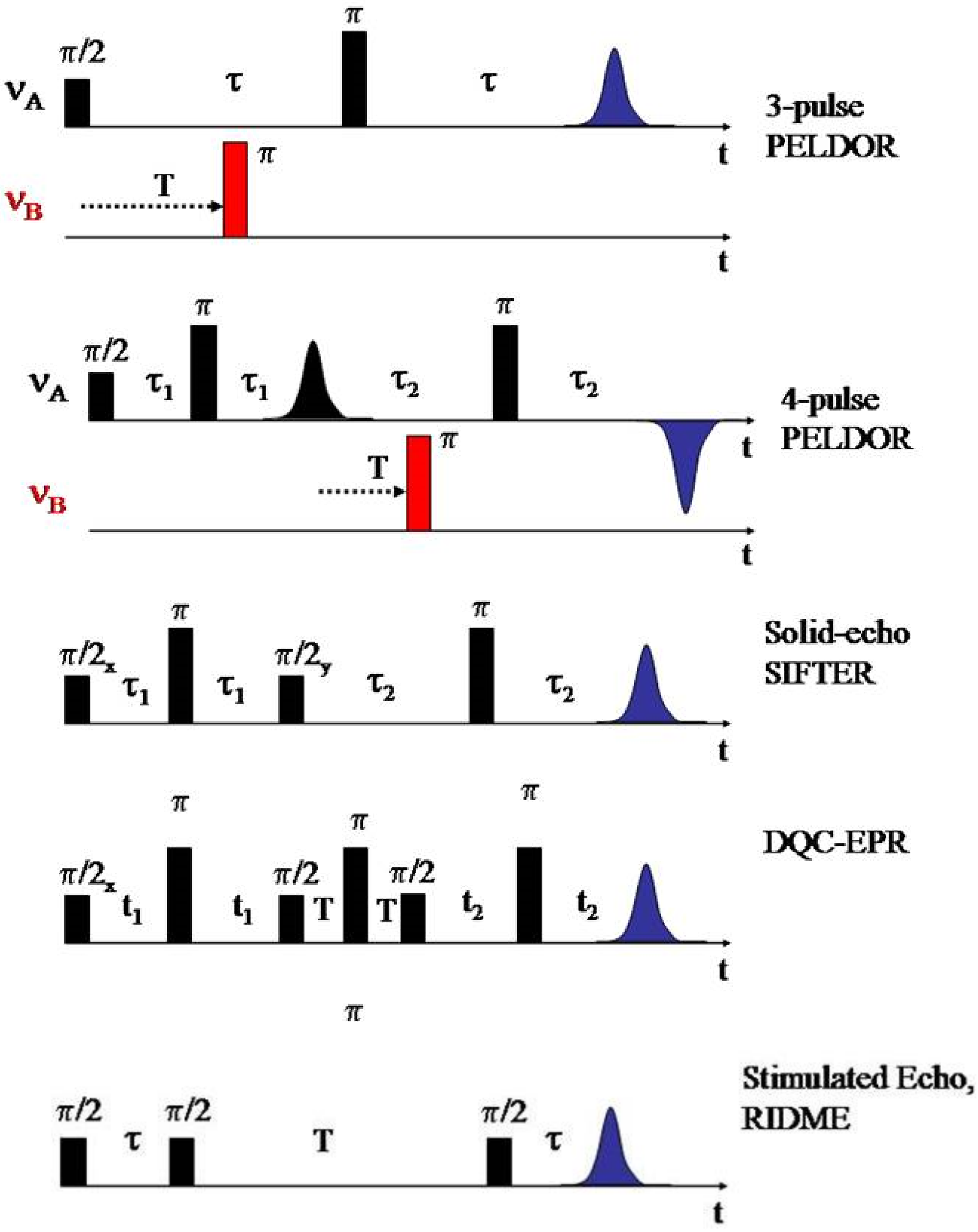
nm Distance determination in Biomolecules



Hyperfine Spectroscopy

Local nuclear spin surrounding of natural paramagnetic cofactors in Biomolecules

Dipolar Experiments



Milov CPL 1984

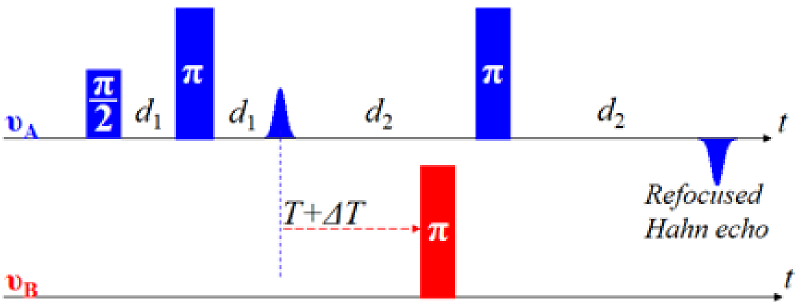
Martin Angewandte 1998

Jeschke CPL 2000

Borbat CPL 1999

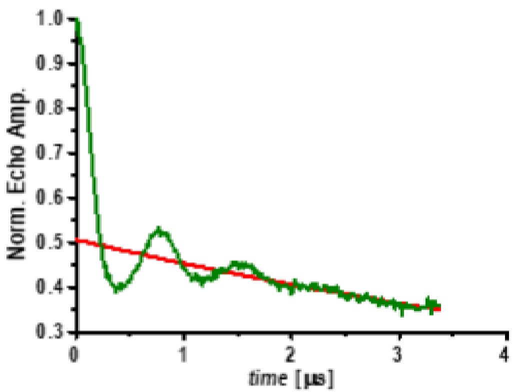
Milikisyants JMR 2008

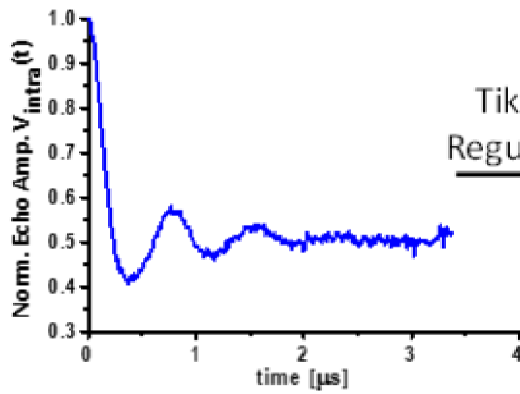
PELDOR / DEER

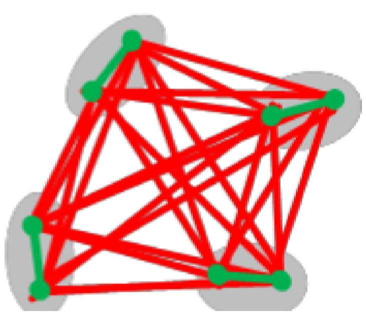


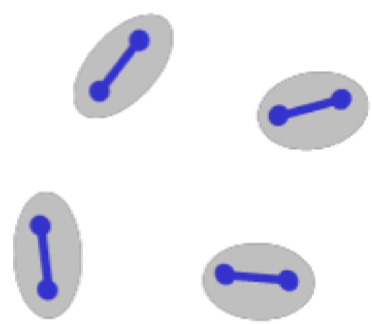
Distance determination between two electron spins

R= 1.5-12 nm



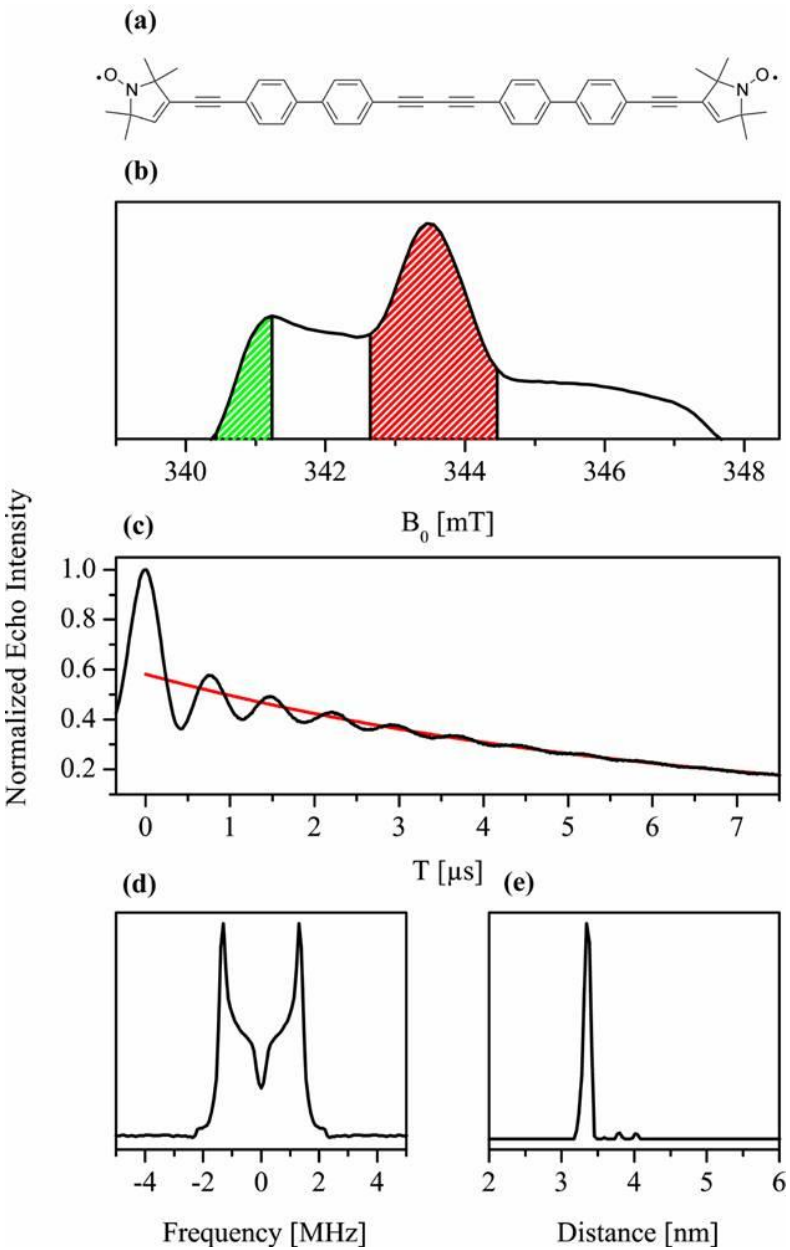






Typically T= 50 K

Distance accuracy of PELDOR / DEER

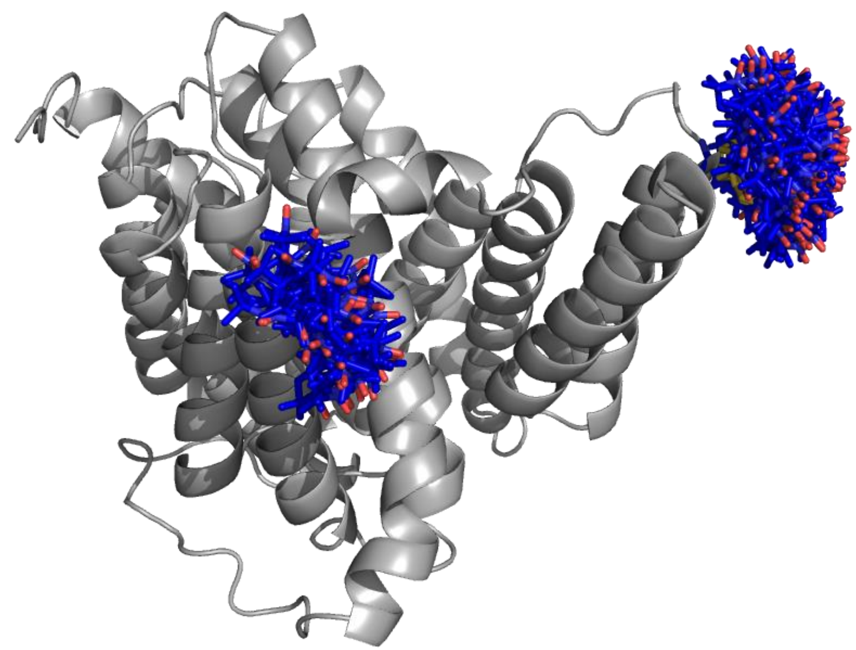


Accuracy intrinsically very high

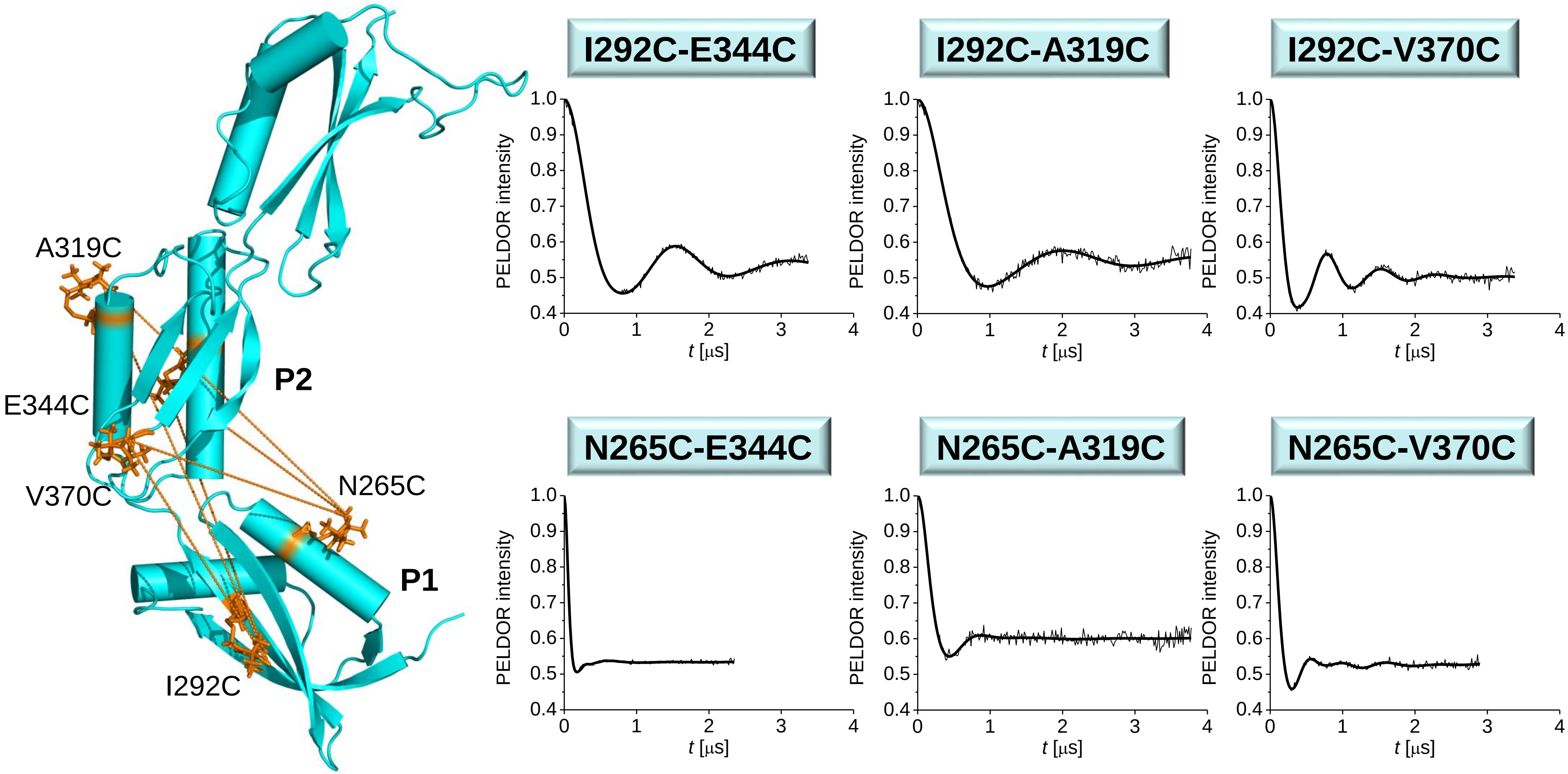
<< 1 Å

In Proteins with MTSSL

~ 3 Å



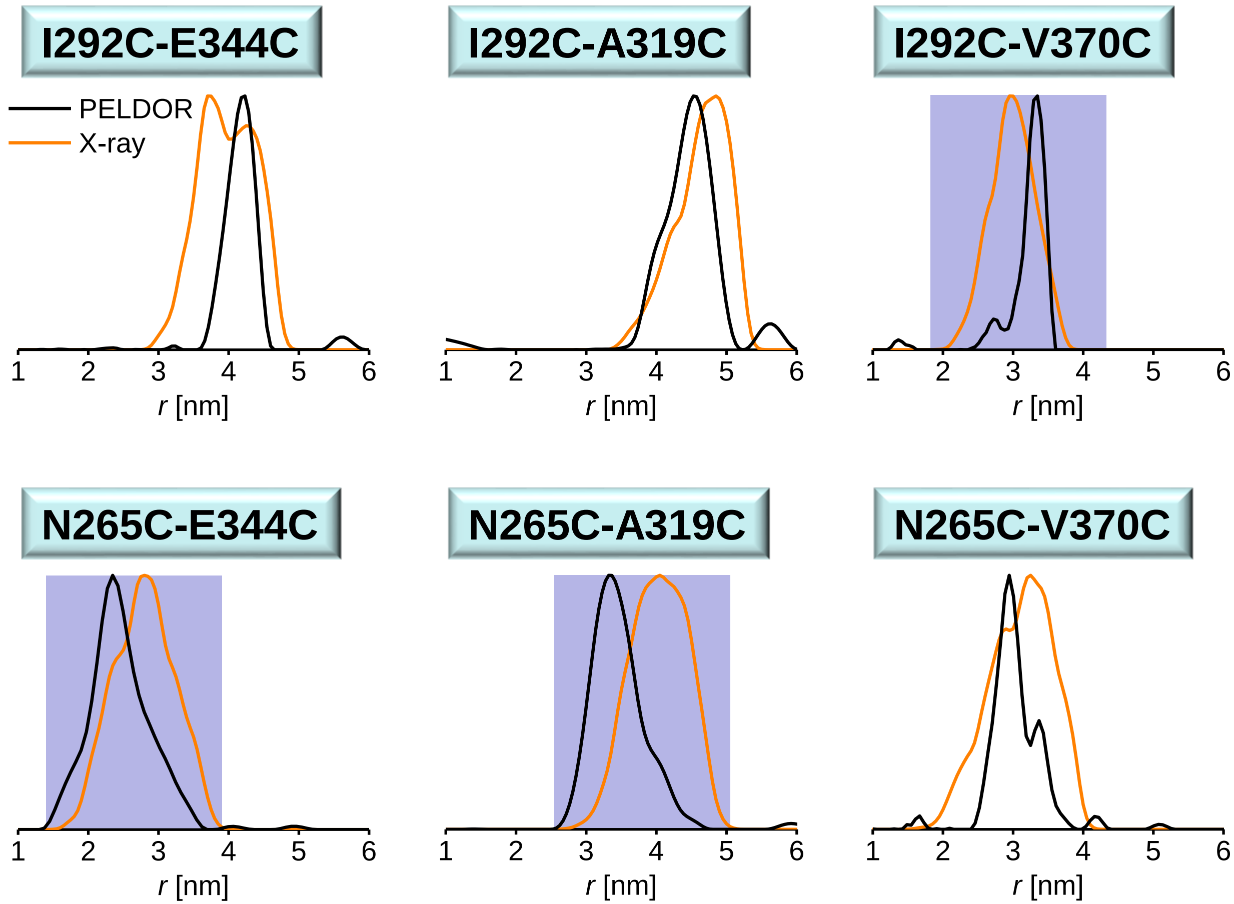
Translation from NMR to EPR



Dastvan et al. Biophys. J. 2016

Translation from NMR to EPR

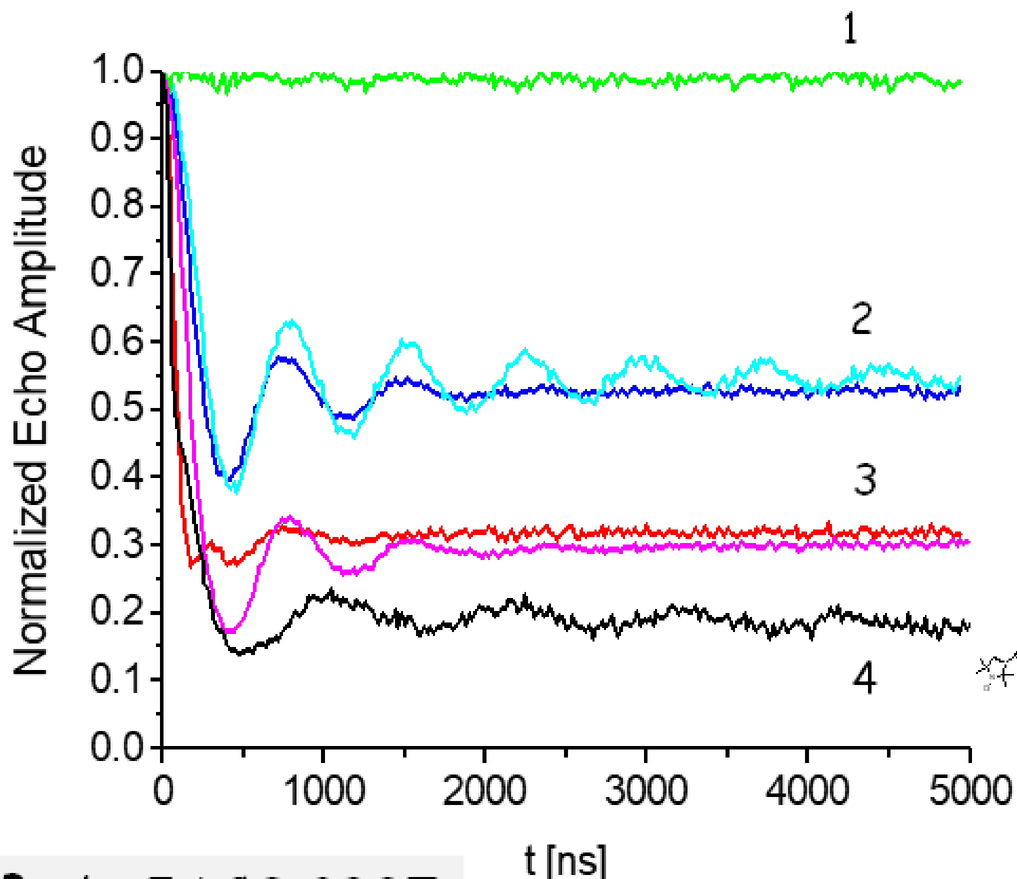
X-ray
PELDOR

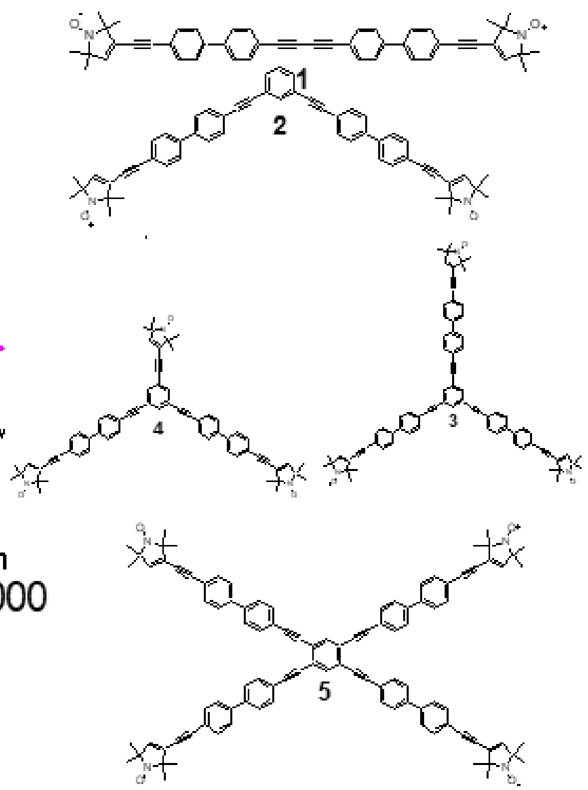


Deviations from X-ray structure observed !

Dastvan et al. Biophys. J. 2016

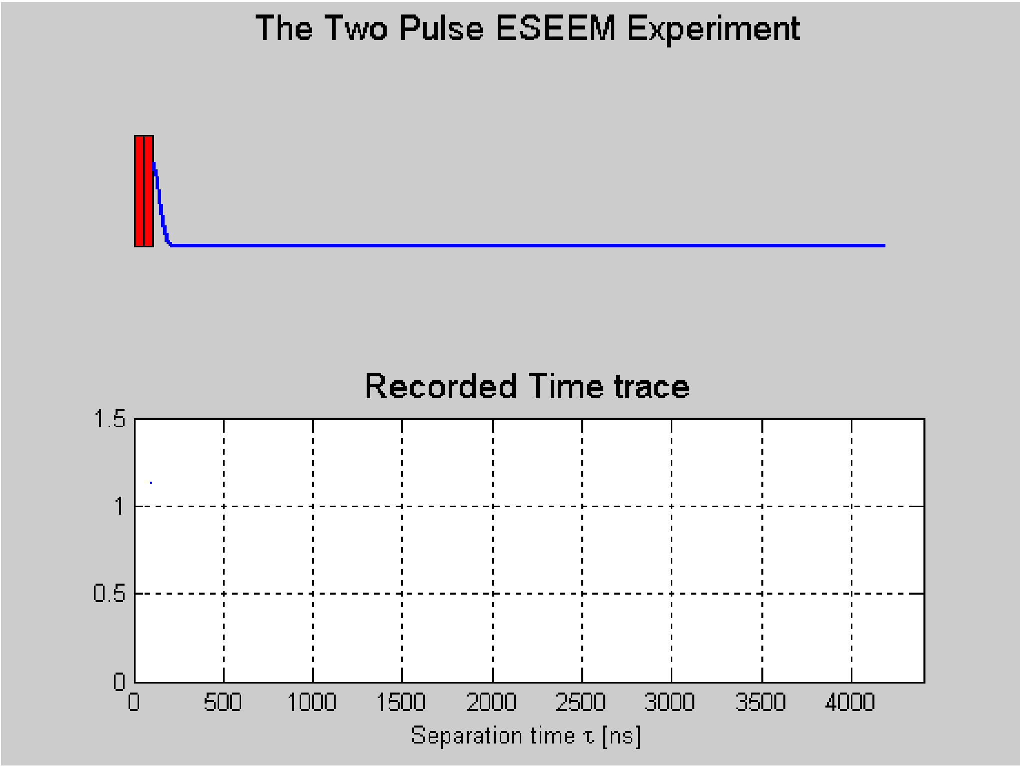
Determination of the number of coupled spins





Bode JACS 2007

Hyperfine Spectroscopy 1: ESEEM



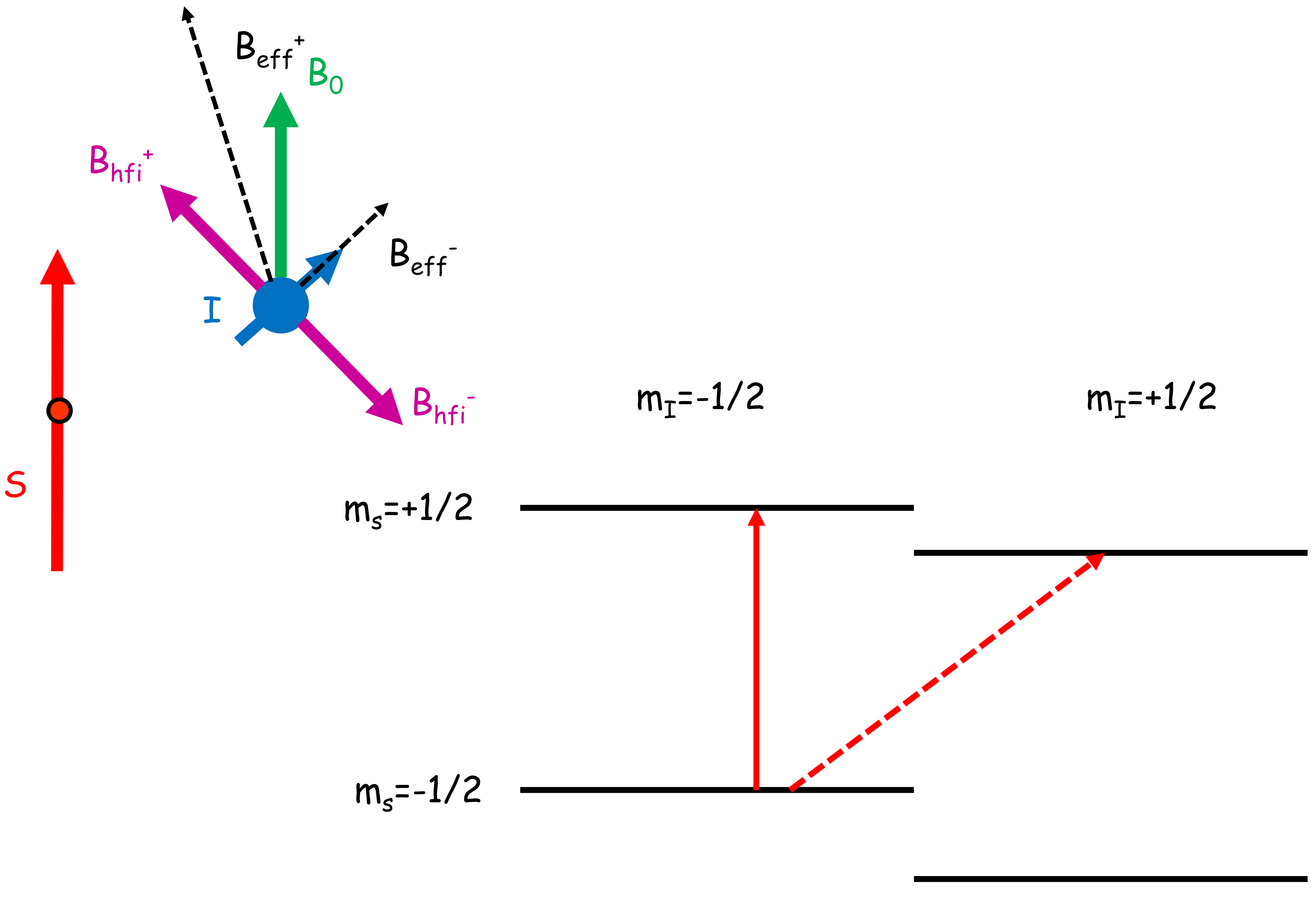
Modulation of
Electron Spin Echo Intensity
by Anisotropic hf Coupling to
Nuclear Spins (R < 1 nm)

Mims Phys Rev. 1961

Stimulated Echo

Better Resolution

Mixing of Nuclear Eigenstates by Hyperfine Field

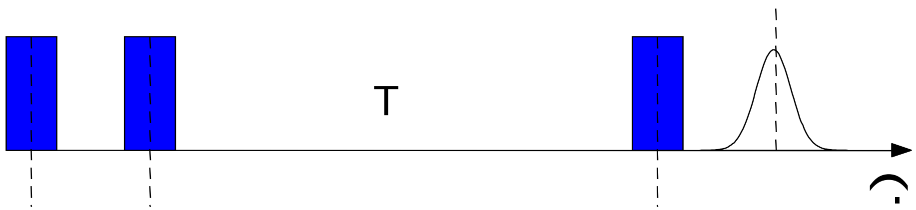


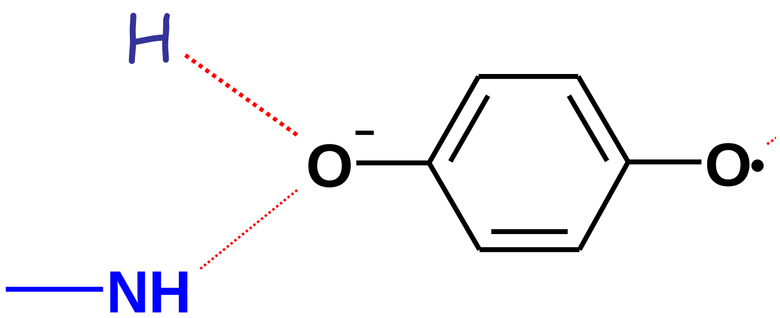
Interaction between electron and nuclear spins

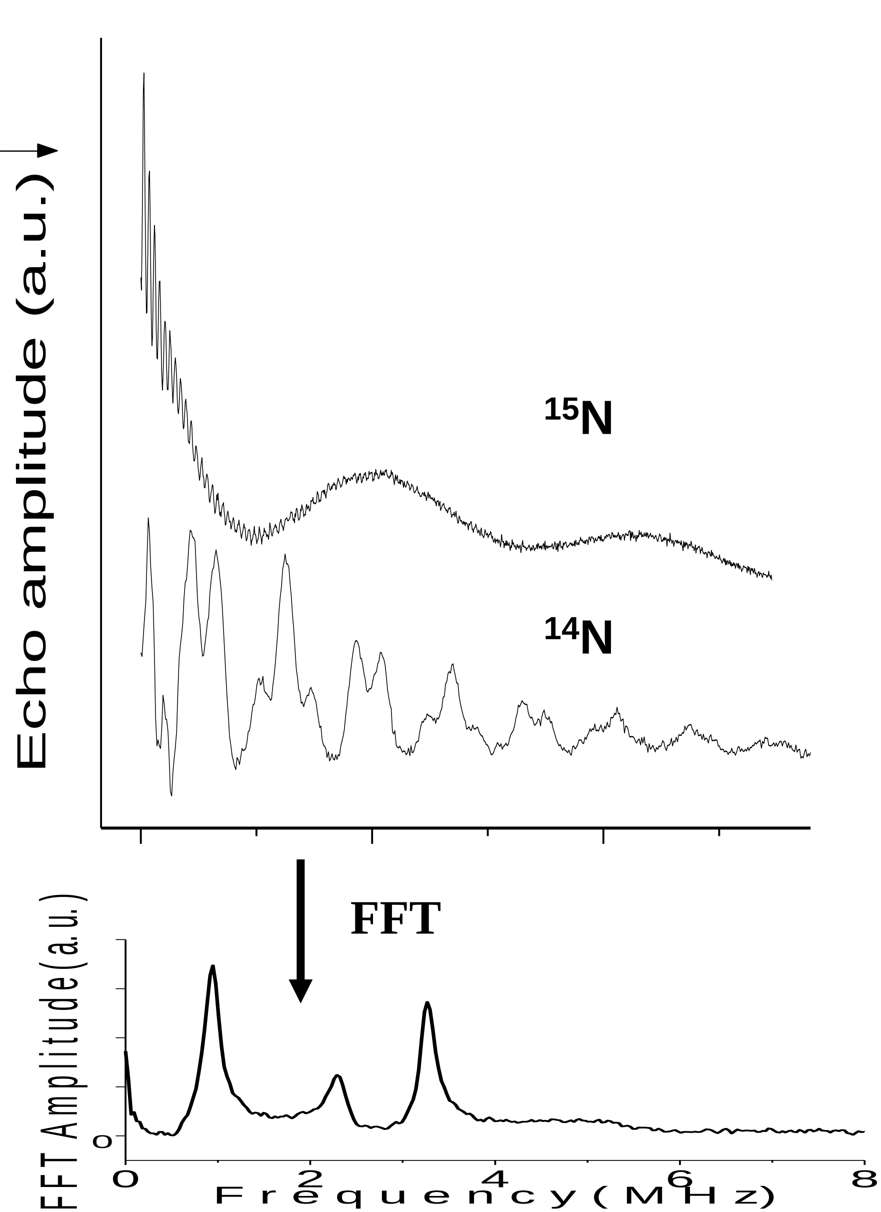
EPR (ENDOR)

Paramagnetic NMR (PRE)

Hyperfine Spectroscopy 1: ESEEM





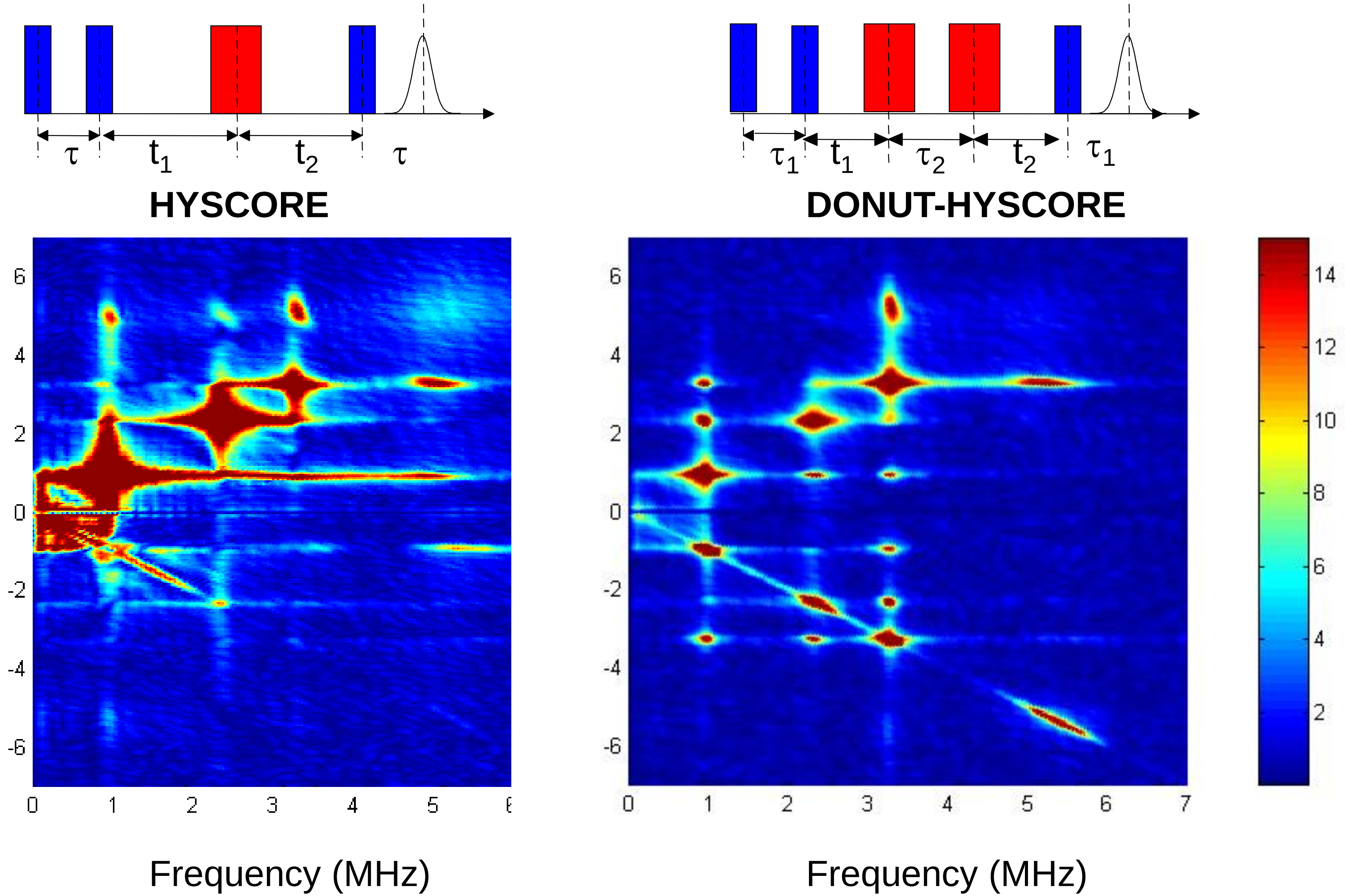


⇒ O—N Distance = 2,5 Å

⇒ O—H Distance = 1,6 Å

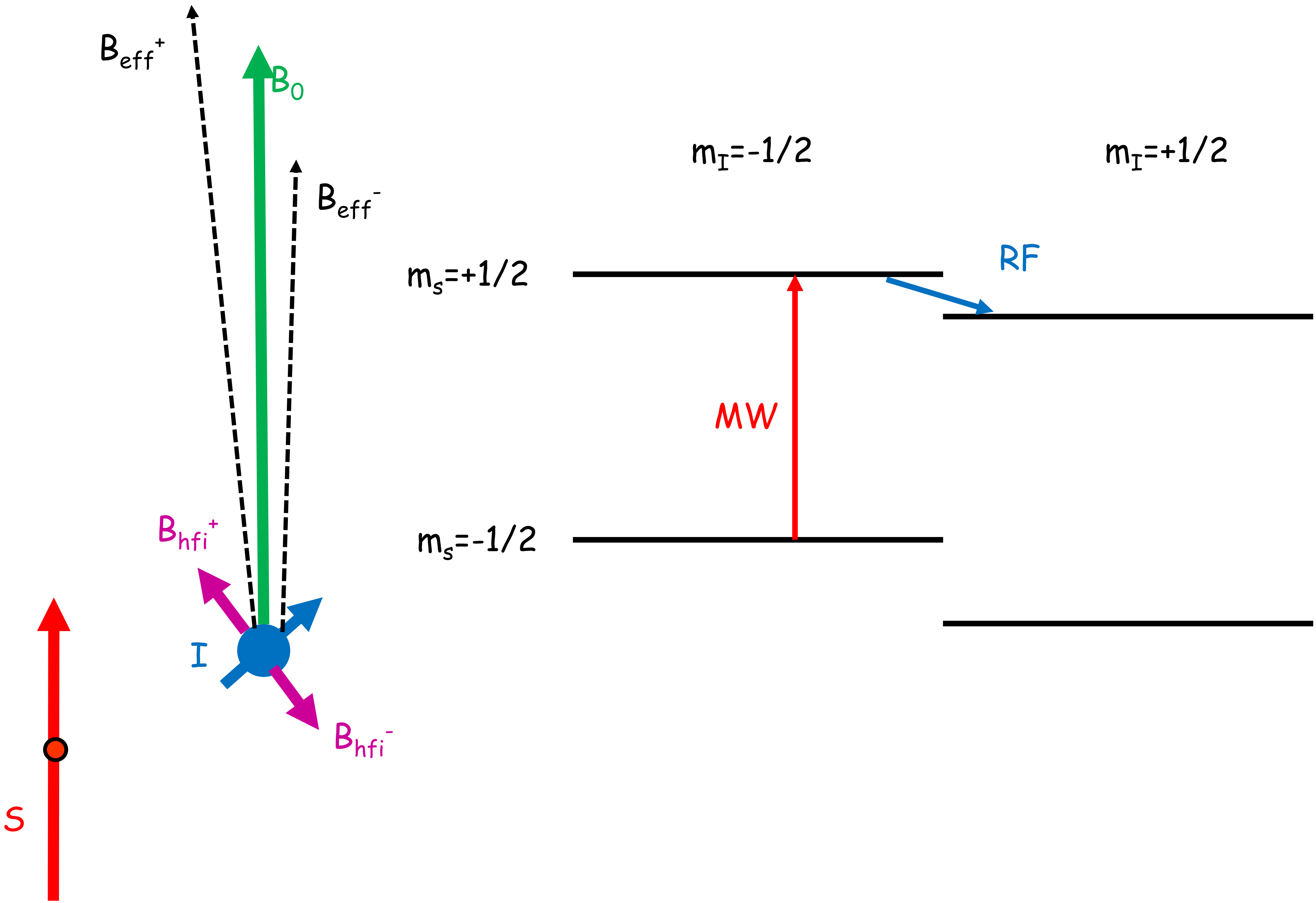
Grimaldi et al. Biochem. 2001

Hyperfine Spectroscopy 2: HYSCORE



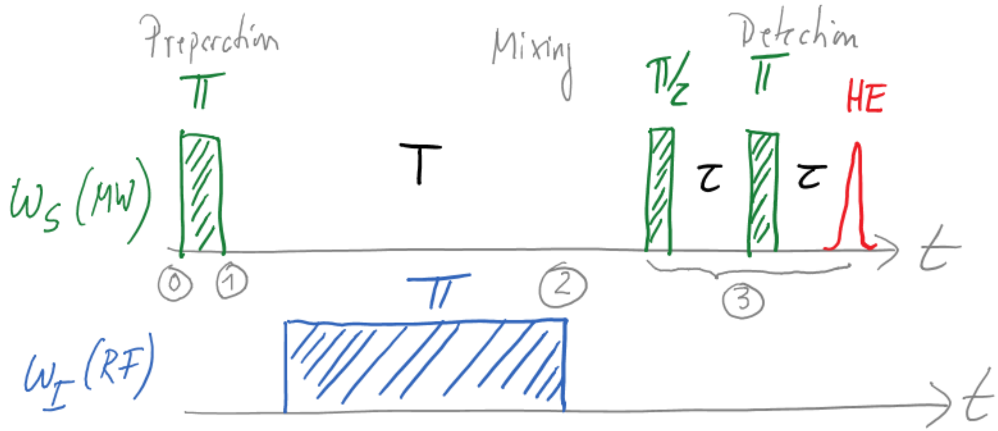
Grimaldi et al. Biochem. 2001

High field Condition with hf coupling



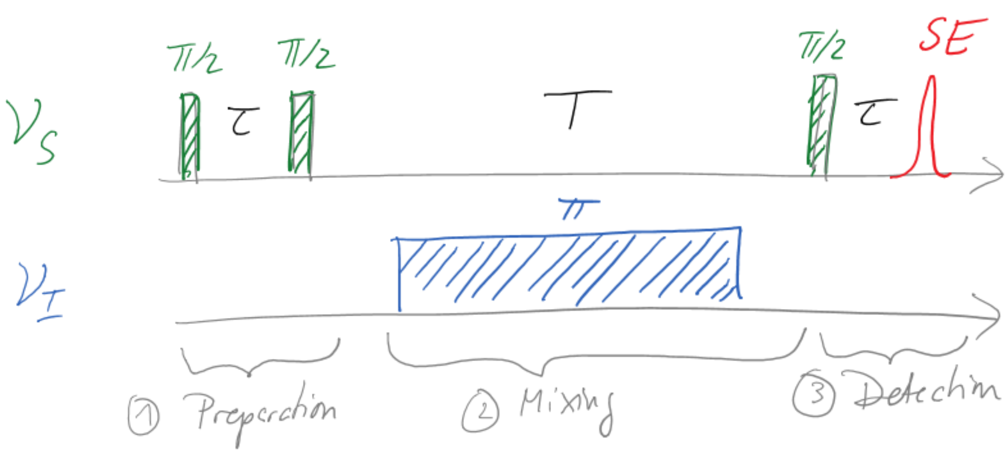
Hyperfine Spectroscopy 3: ENDOR

Davies ENDOR



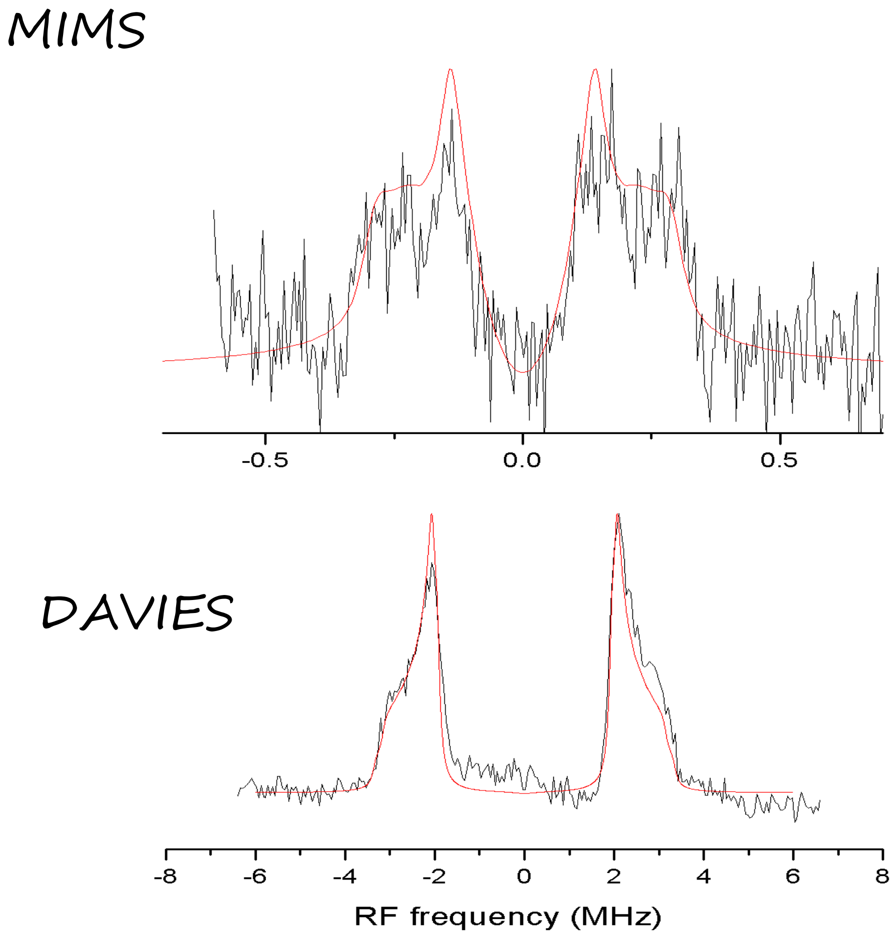
Large Hyperfine Couplings

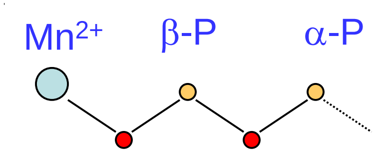
Mims ENDOR



Small Hyperfine Couplings

Hyperfine Spectroscopy 3: ENDOR

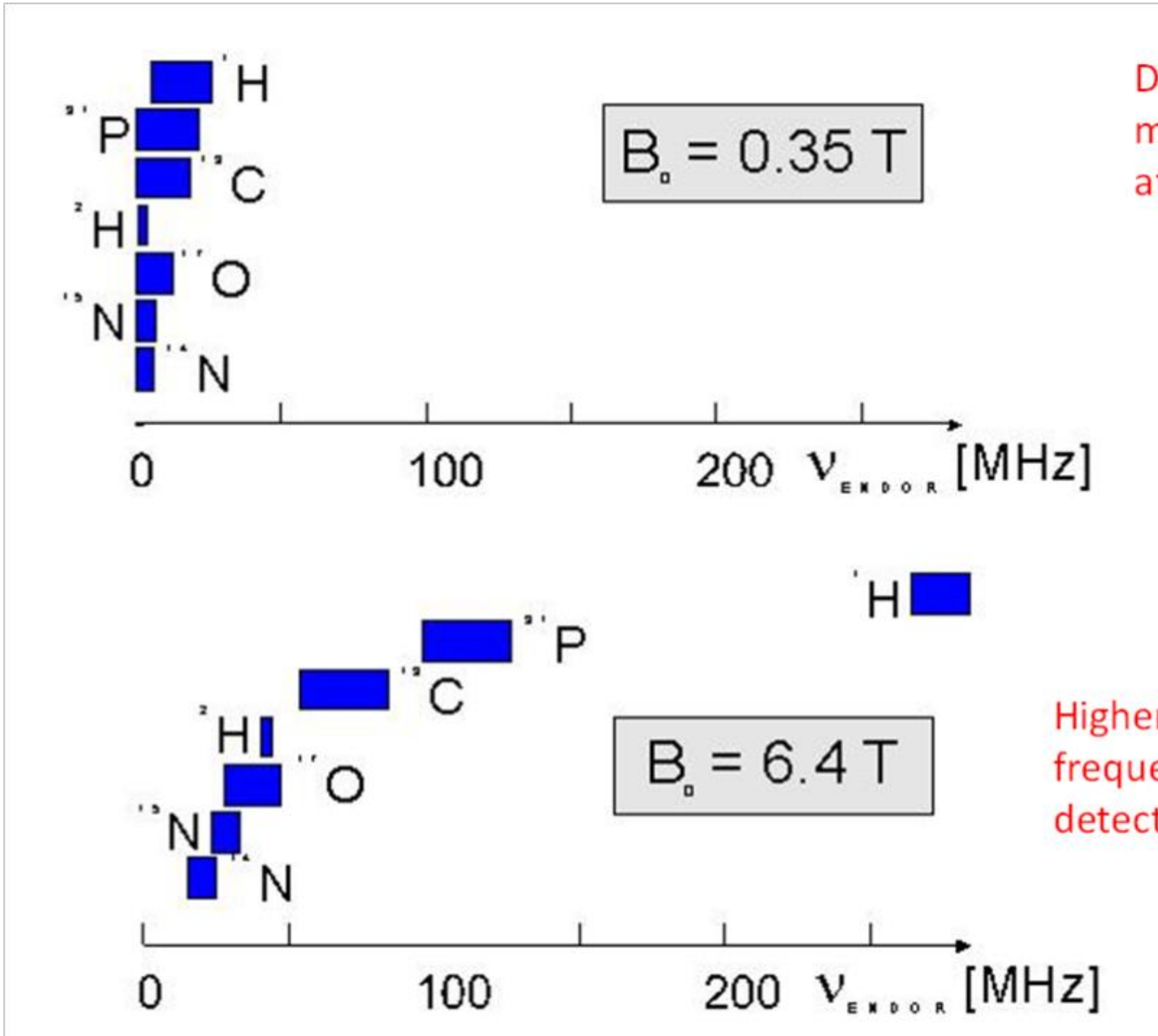




^{31}P ENDOR at W-band

Bennati et al. Biochem. 2006

Hyperfine Frequencies at different B0



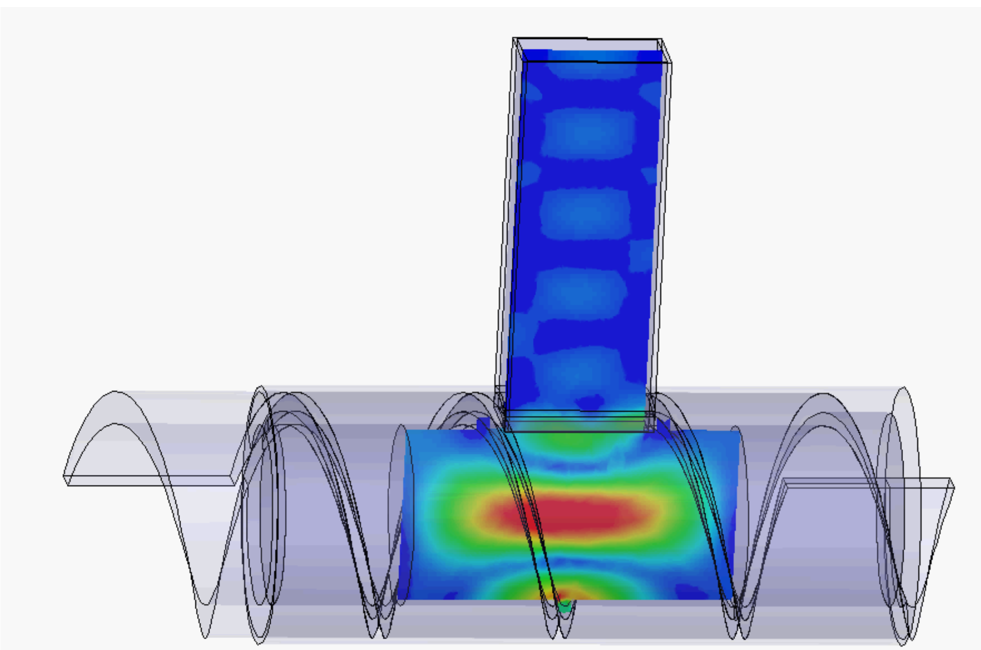
Different nuclei can be much better separated at HF

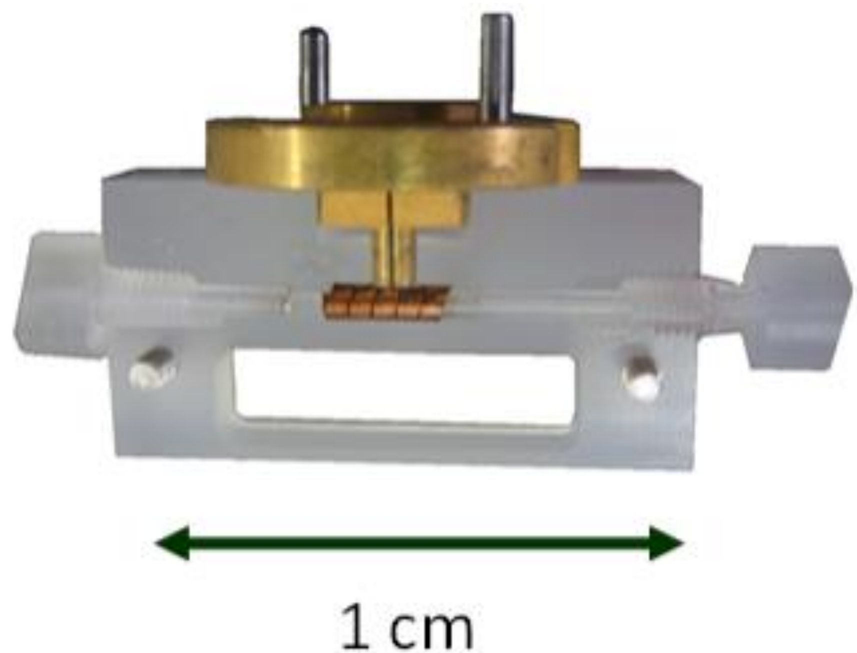
Higher nuclear Zeeman frequencies allow better detection of low γ nuclei

ENDOR Resonator at high frequencies

MW Resonators at 260 GHz (J-band, corresponding to 9.2 T, 400 MHz proton)

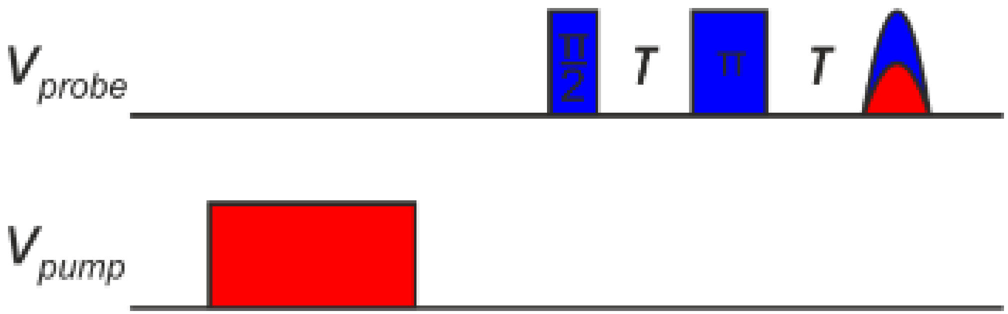
Sample Capillary 0.1 mm diameter
Sample Volume 10 nl
Protein : 1 pMol



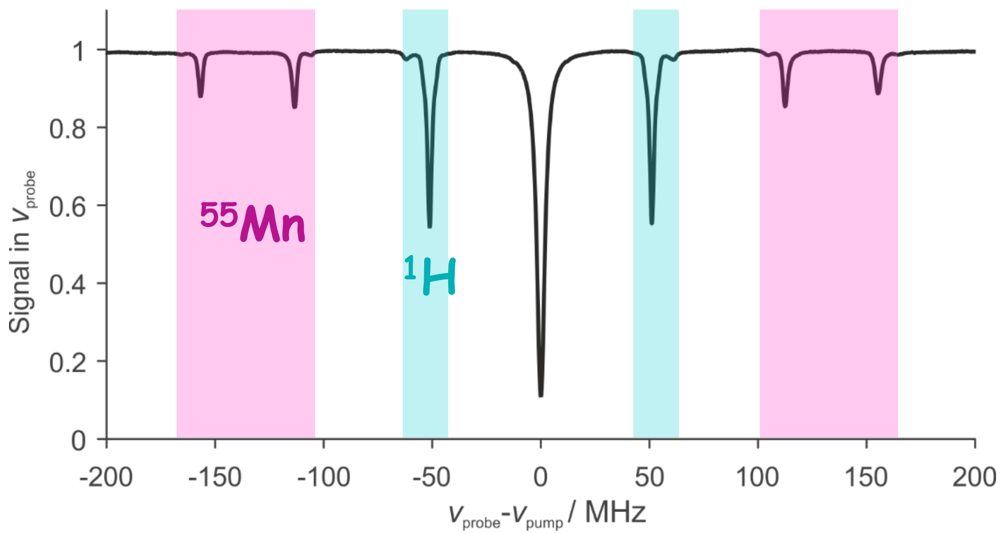


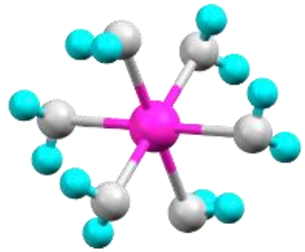
Prandolini et al. JACS 2009

Hyperfine Spectroscopy 4: ELDOR detected NMR



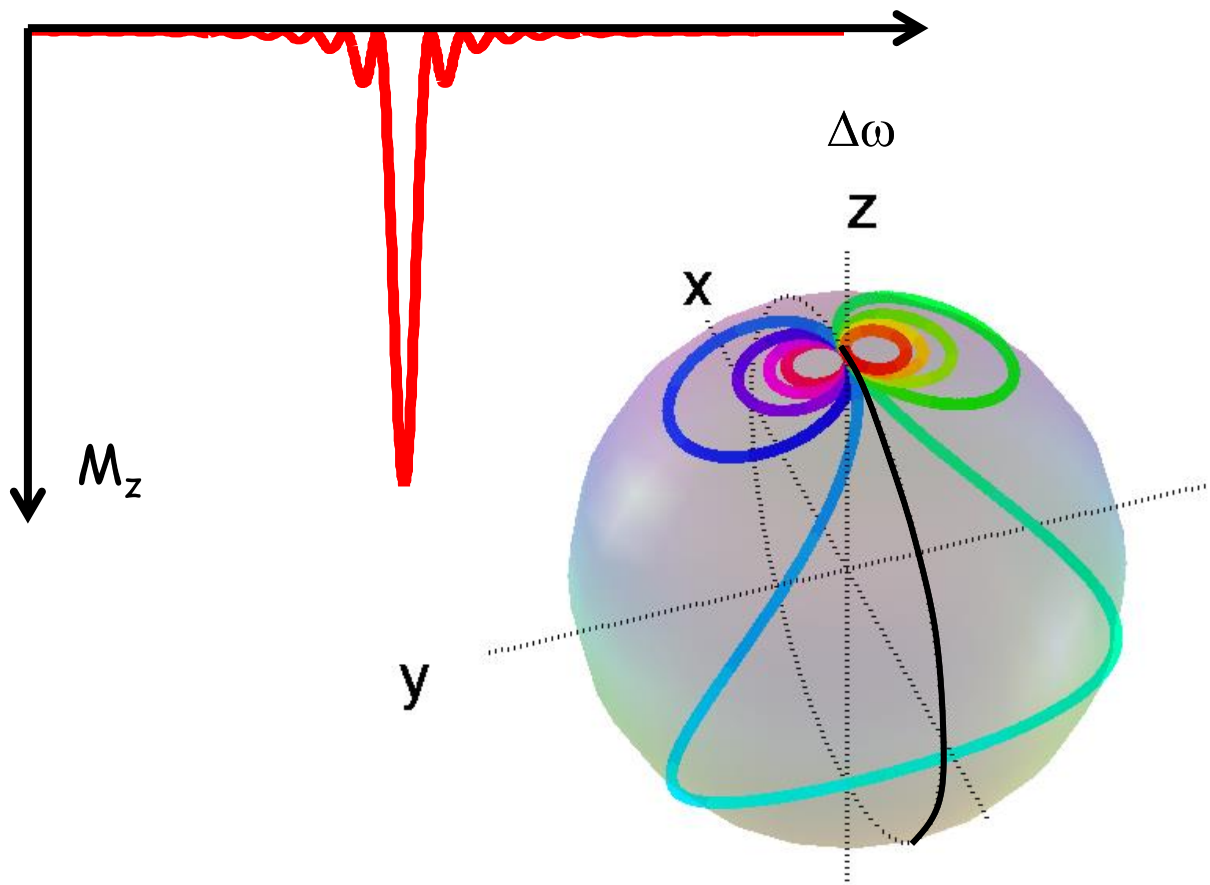
Q-band EDNMR (34 GHz/1.2 T) of $Mn(H_2O)_6^{2+}$ recorded at 5K





Hetzke et al. Appl. Magn. Reson. 2017

Rectangular inversion pulse

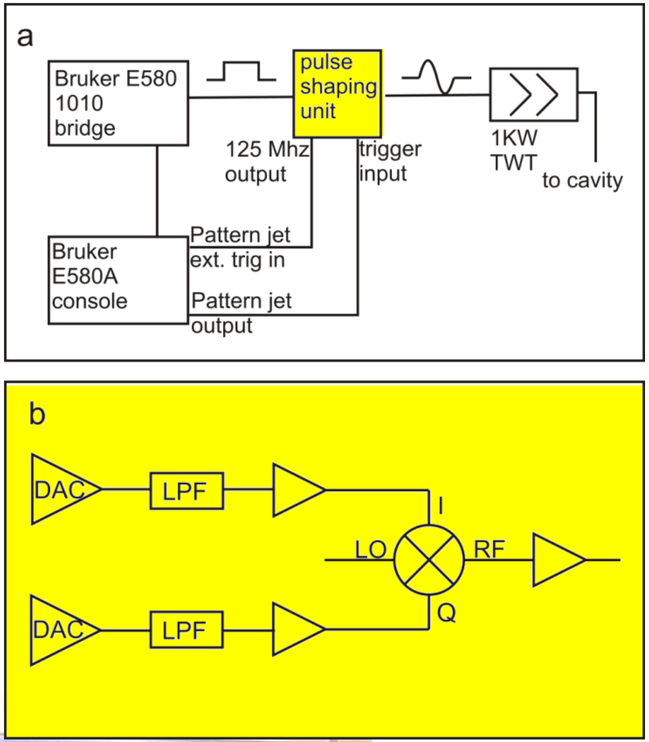


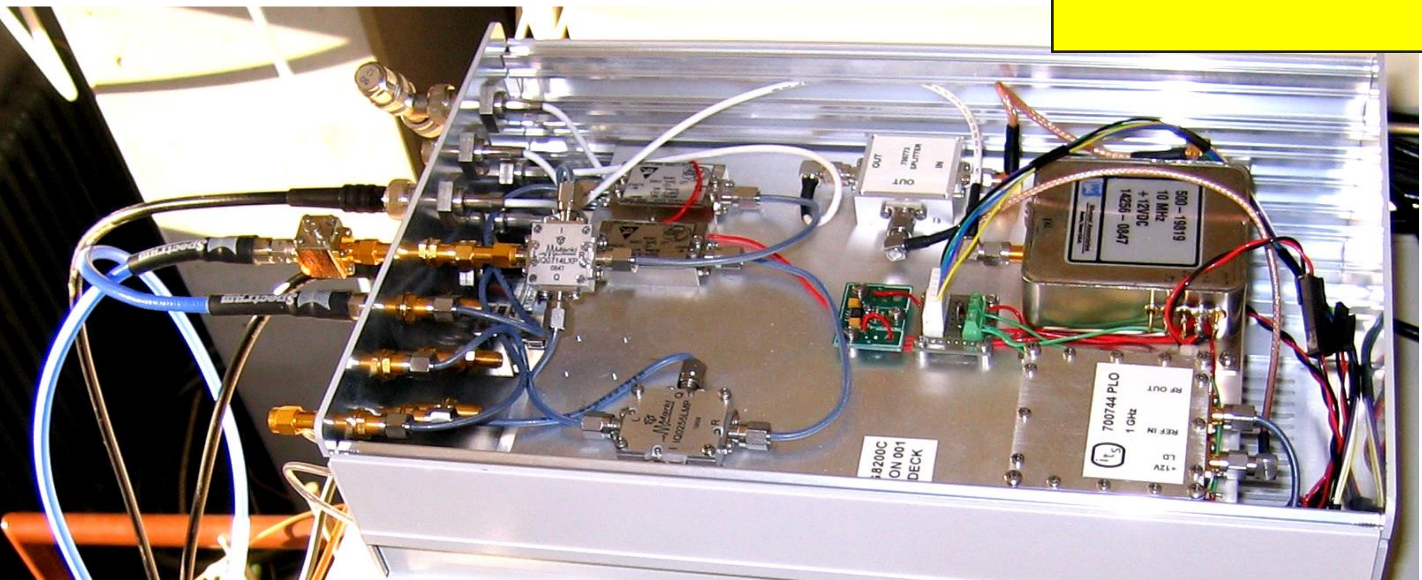
Arbitrary Waveform Generator

1 ns time resolution

14 bit resolution in amplitude and phase

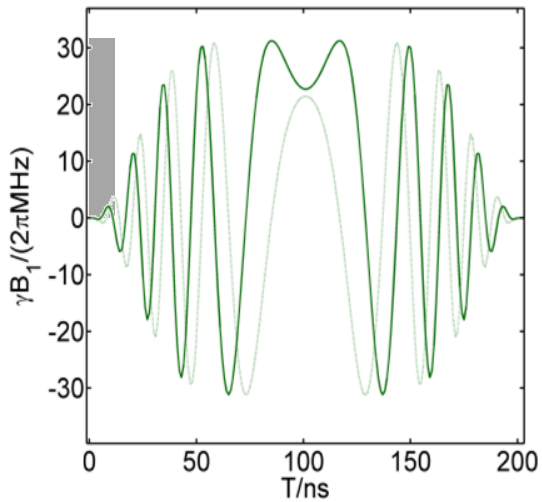
up to 100 μs long pulse shapes

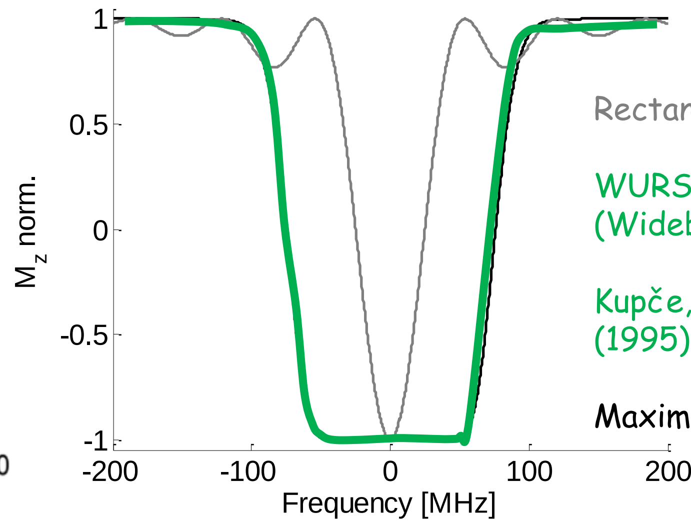




Amplitude / Phase modulated Pulses

WURST π Pulse





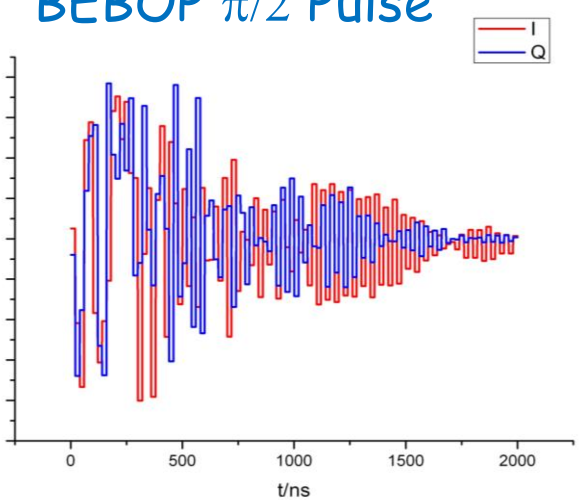
Rectangular inversion pulse (16 ns)

WURST inversion pulse (200 ns)
(Wideband, uniform rate, smooth truncation)

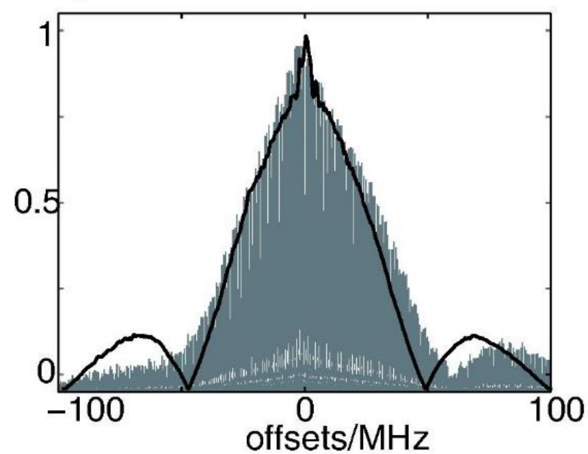
Kupče, E.; Freeman, R. *J. Magn. Reson.* **115**, 273 (1995)

Maximal B_1 field strength of 11 G for both pulses

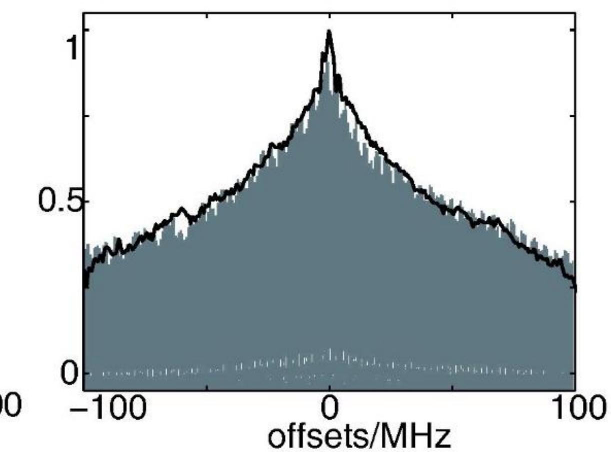
BEBOP π/2 Pulse



Rectangular



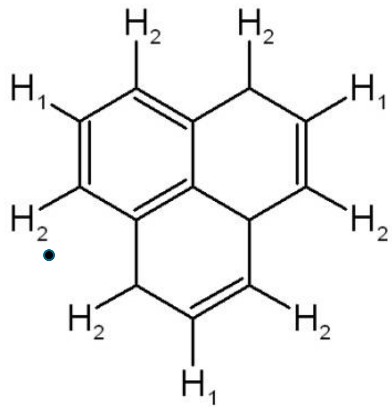
BEBOP OCT-Pulse

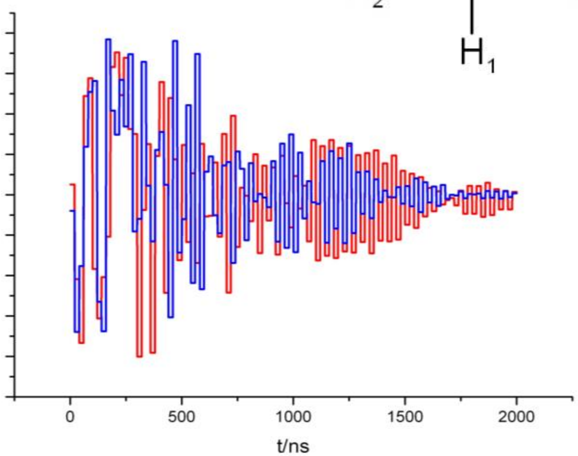


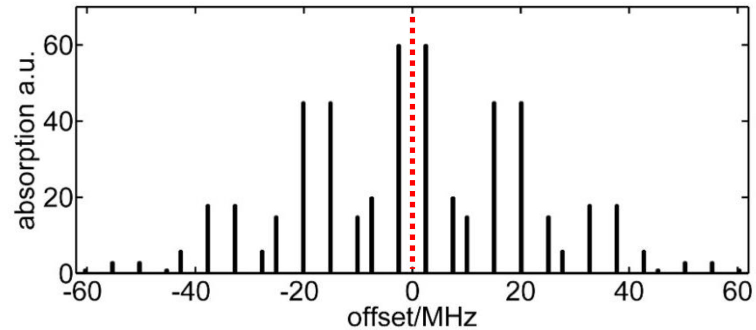
Broadband Excitation By Optimized Pulses
Skinner, Reiss, Luy, Khaneja, Glaser, J. Magn. Reson. 163, 8 (2003)

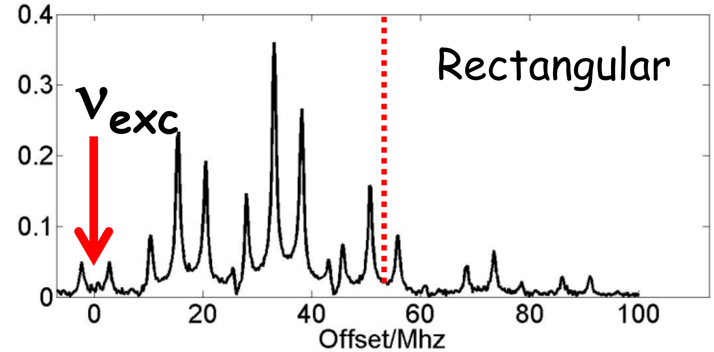
Spindler et al JMR (2012)

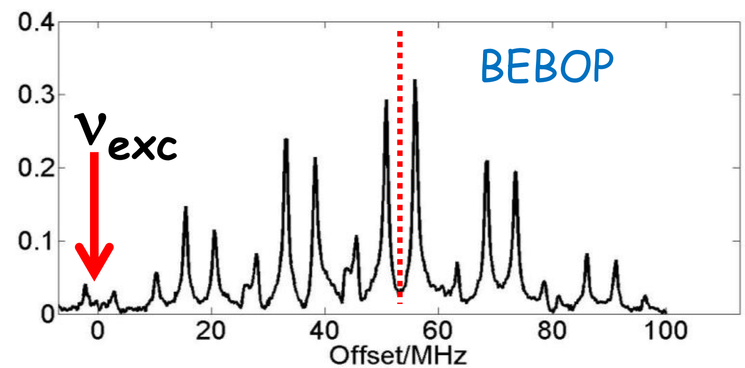
FT-EPR with BEBOP pulse









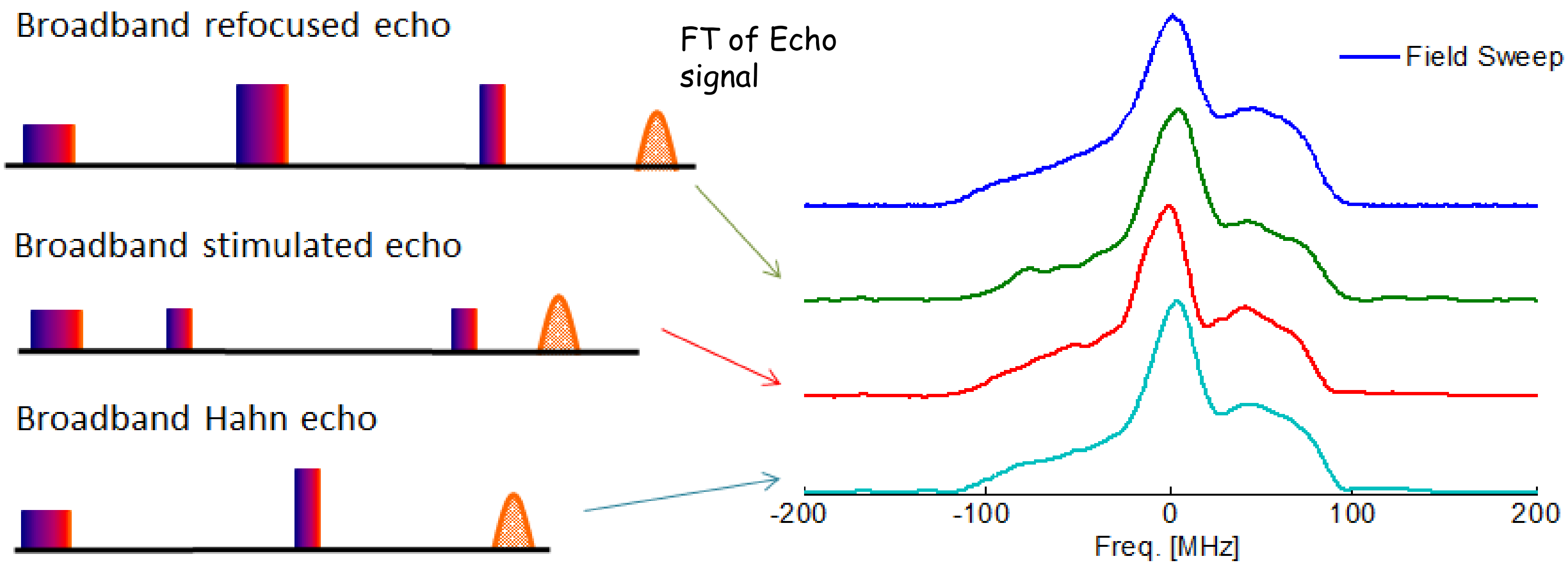


Much better intensity profiles obtained with excitation pulses derived by OC-Theory (BEBOP)

Collaboration with S. Glaser (TU Munich)

Spindler et al JMR (2012)

Echo sequences with WURST pulses

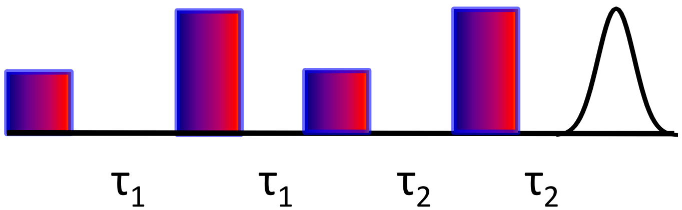


Bohlen, Ray, Bodenhausen JMR (1989)

Schoeps et al JMR (2015)

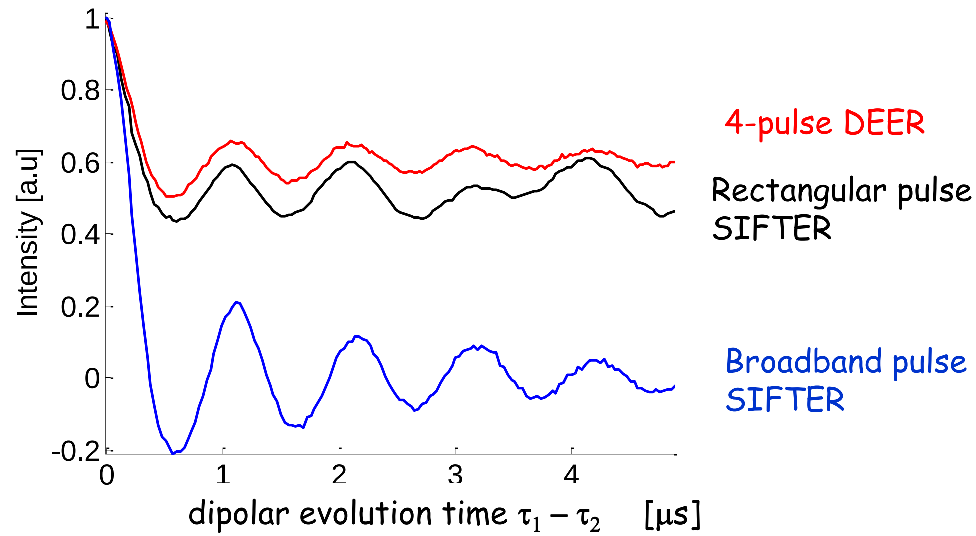
With broadband pulses full nitroxide lineshape can be excited!

SIFTER with WURST pulses



(Single Frequency Technique for Refocusing Dipolar Couplings)

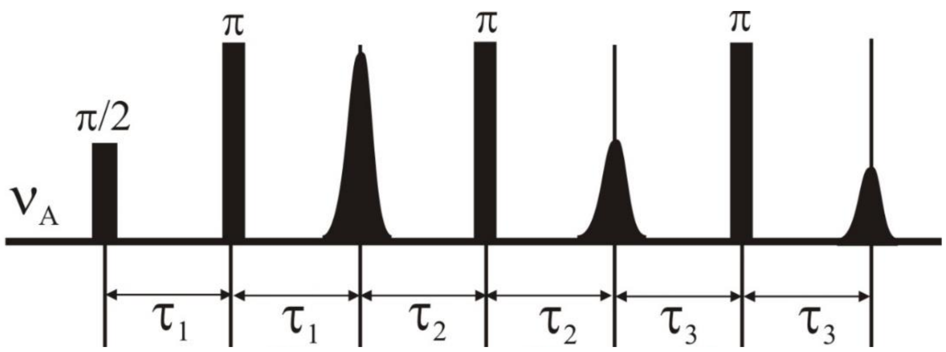
Jeschke, Pannier, Godt, Spiess
Chem. Phys. Lett. 331, 243 (2000)



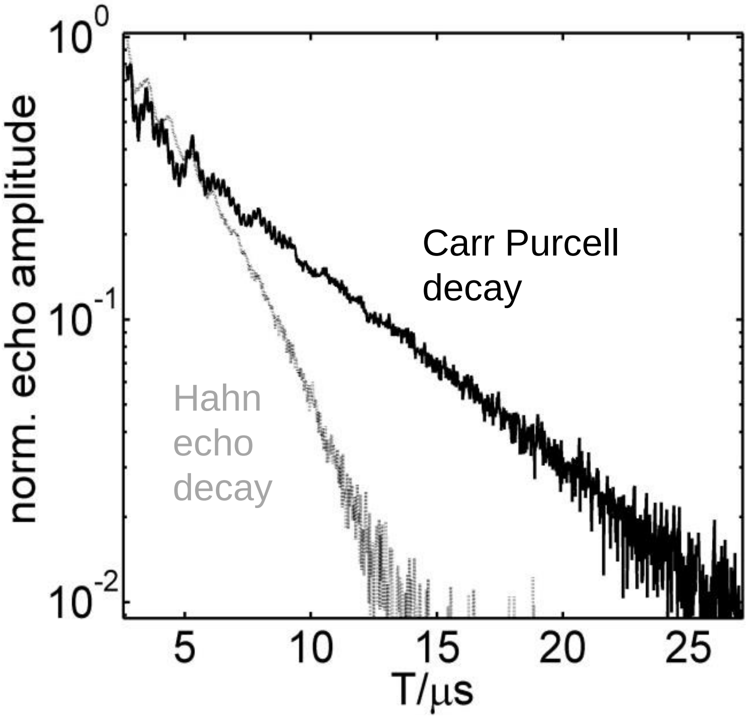
Schoeps et al JMR (2015)

With broadband pulses SIFTER without distortions with 100% modulation depth

Carr Purcell pulse sequence



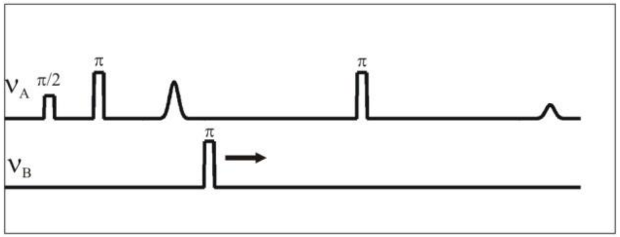
(Borbat, Freed J. Phys. Chem.Lett 4, 170 (2013)

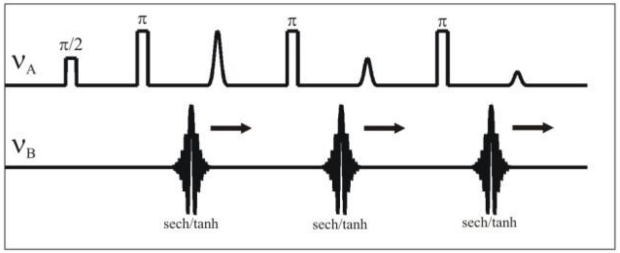


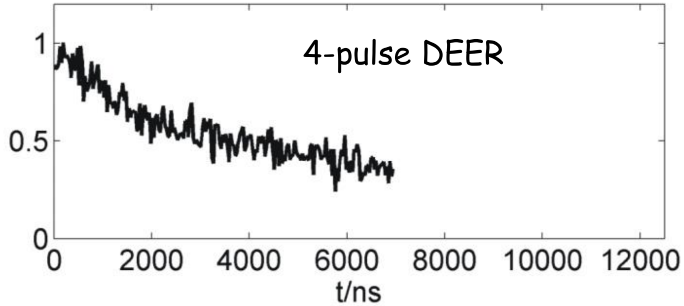
For nitroxide spinlabels detection time window can be prolonged by a Carr-Purcell refocusing

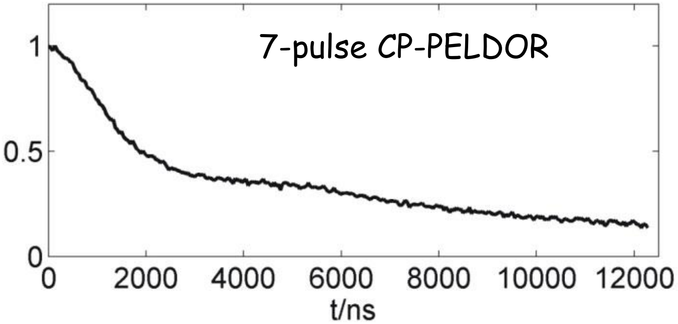
Carr Purcell PELDOR on BetP

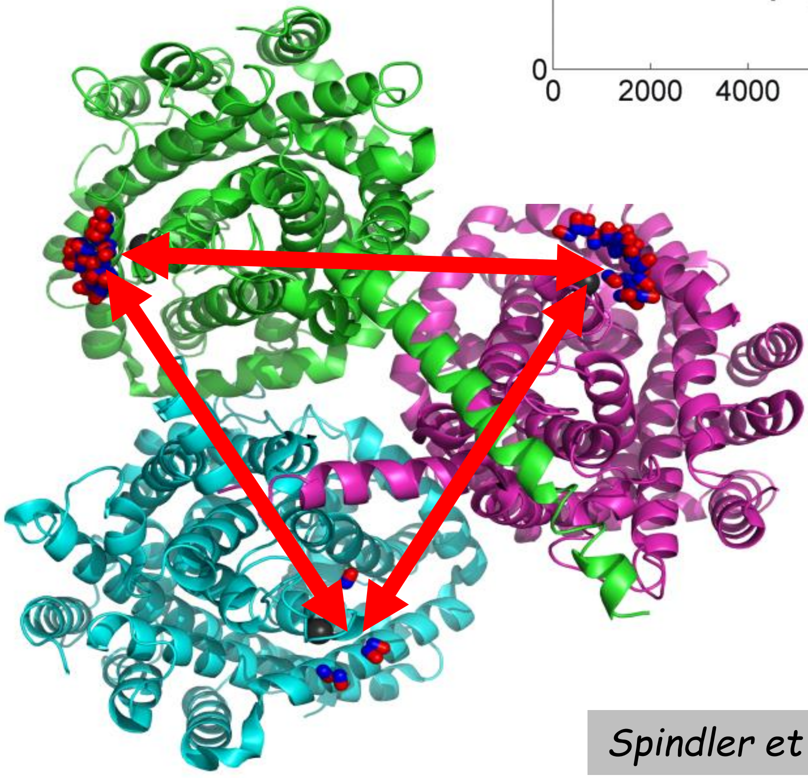
Collaboration with
C. Ziegler
(Uni Regensburg)



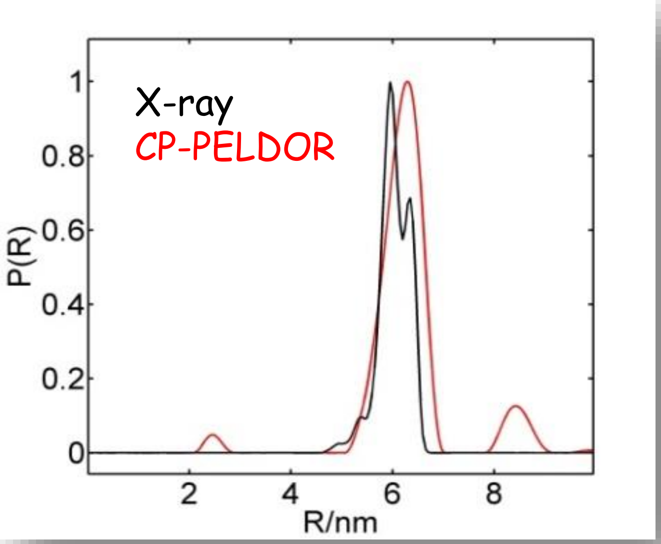








Strongly improved observation time window and S/N



Spindler et al JPC Lett. 2015

Carr Purcell SIFTER on BetP