Pulsed EPR spectroscopy

with a focus on DEER (PELDOR)

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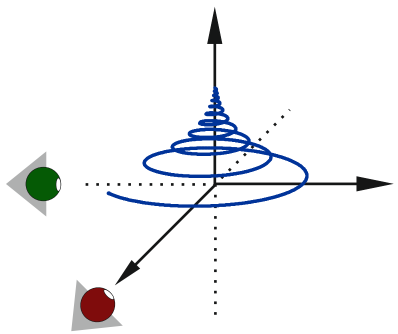
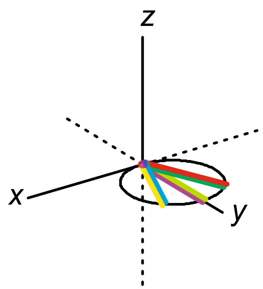
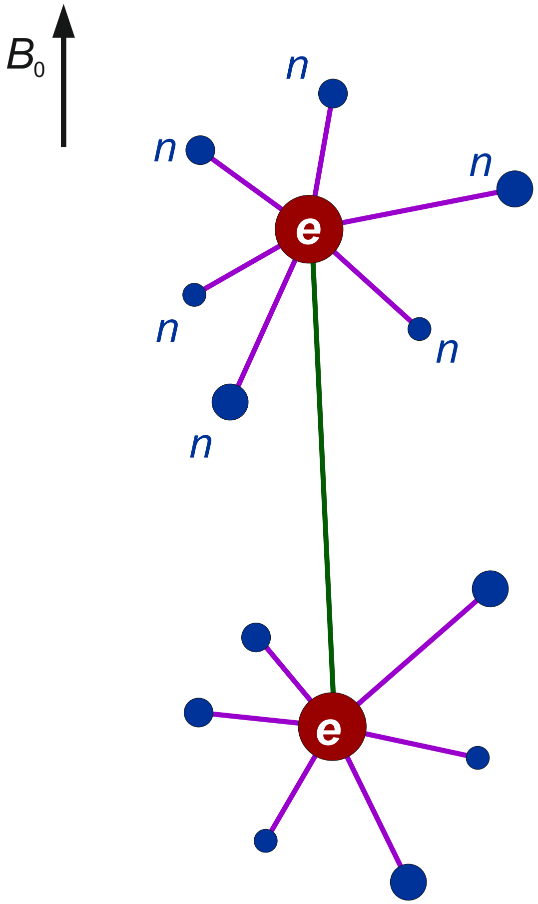
- Why pulsed EPR?
- How does it work? FID & echoes
- A few concepts and pulse sequences
- DEER: Basics and implementation
- DEER: Data analysis



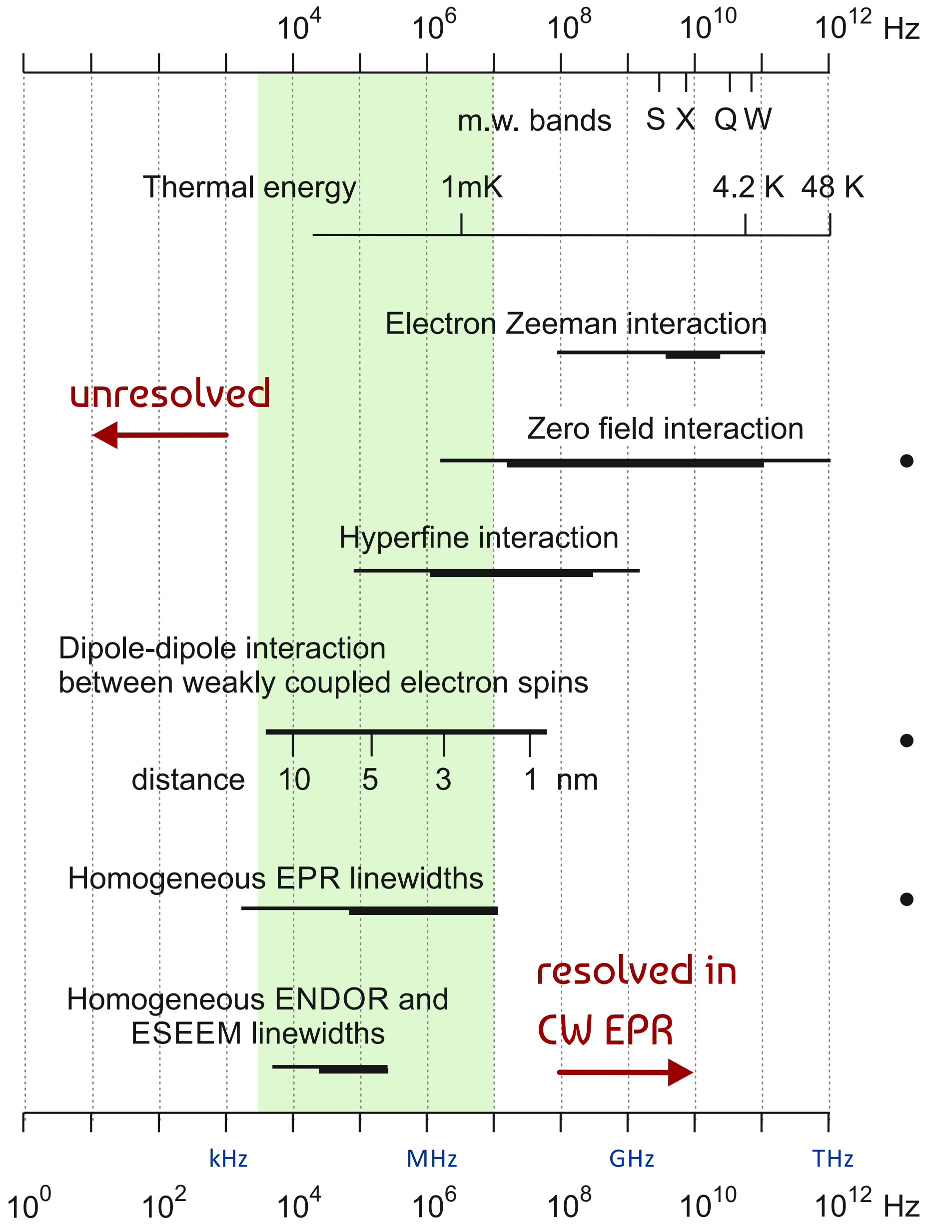


Interactions and the information that they provide



Name	Information
electron Zeeman	fingerprinting of radical type or metal coordination
hyperfine	distribution of the SOMO (reactivity) distance of protons from the center of spin density
nuclear Zeeman	identification of nuclei that give rise to hfi
nuclear quadrupole	binding situation of the nucleus for $I > 1/2$ (chemical shift is not available)
zero-field	fingerprinting of triplet type or metal coordination spin state for metal ions (low or high spin)
exchange	orbital overlap (important for electron transfer)
dipole-dipole	distances in the nanometer range (15 - 100 Å)

An overview of microwave bands and interactions



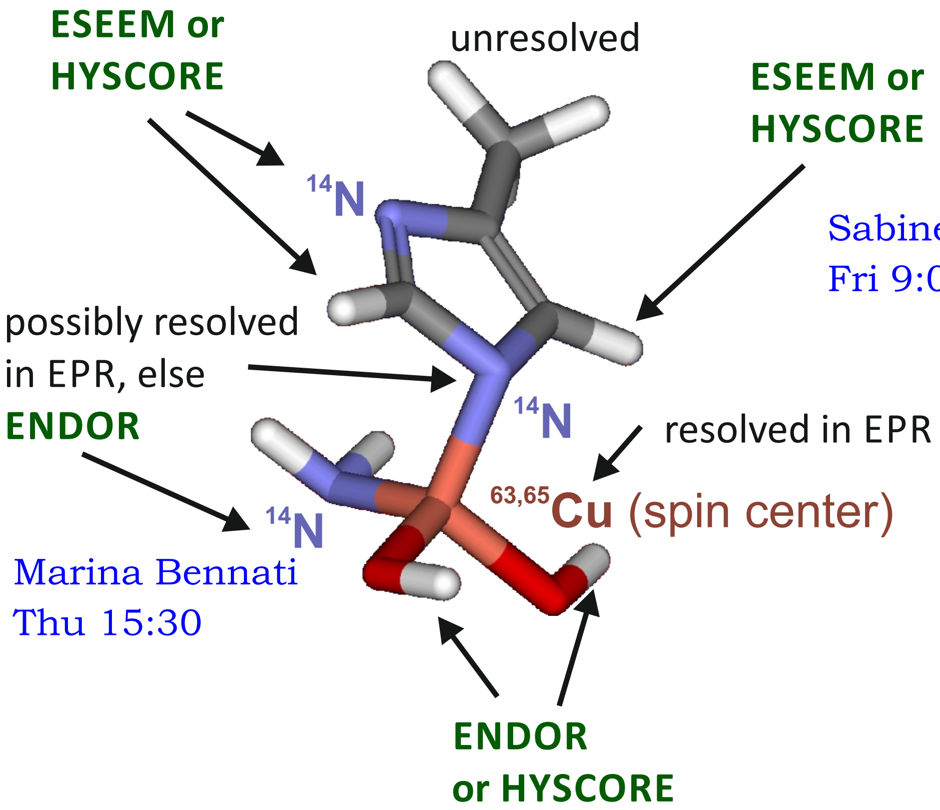
By separating interactions, pulsed EPR provides information lost by line broadening in CW EPR

- for electron group spin > 1/2 (more than one unpaired electron)
- most valuable source of EPR restraints on structure
- resolution limit depends on sample preparation

1 mT corresponds to 28 MHz

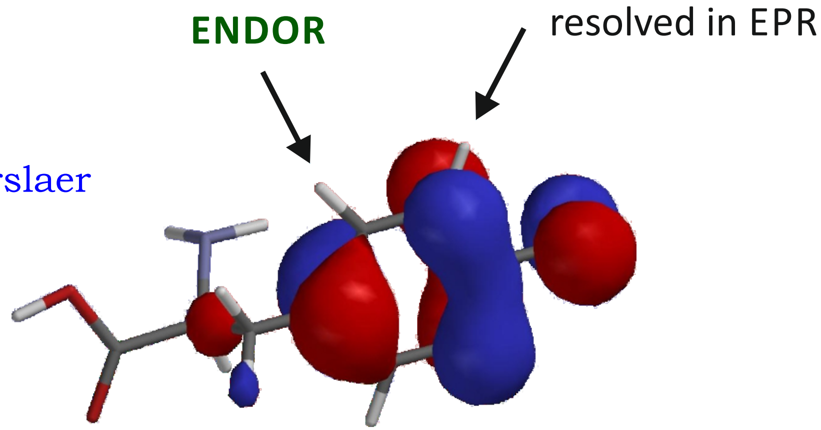
Example: Measuring hyperfine couplings

Histidine-coordinated Cu(II)



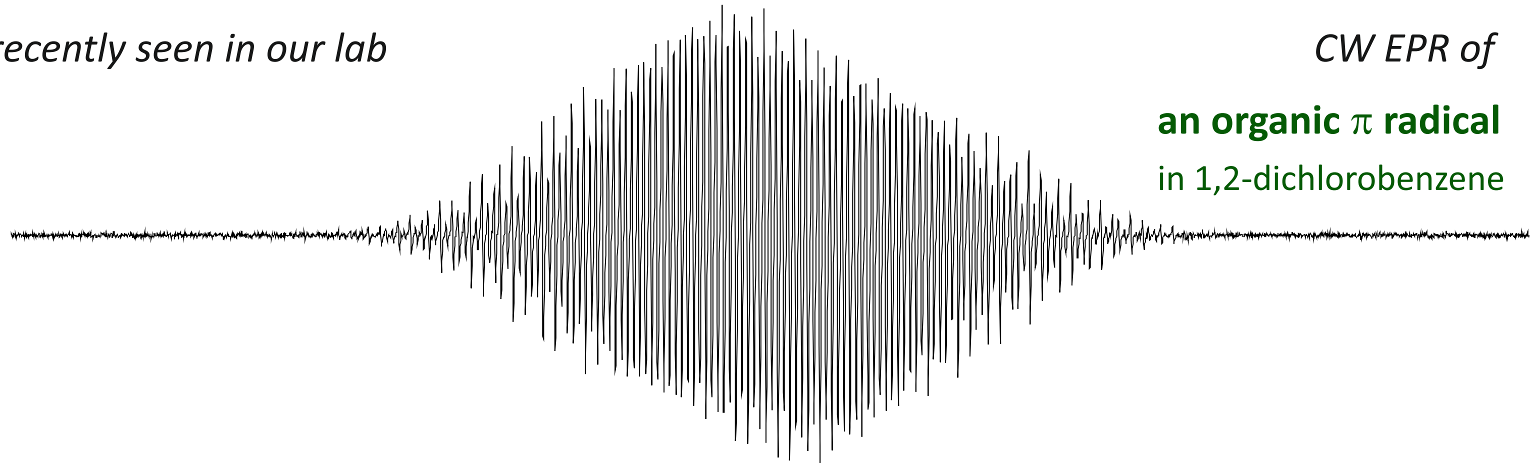
Sabine Van Doorslaer
Fri 9:00

Tyrosyl radical



Even if it's resolved, ...

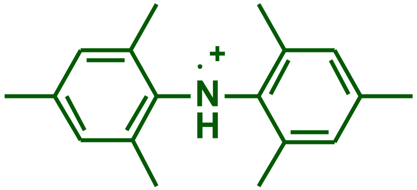
recently seen in our lab



CW EPR of

an organic π radical

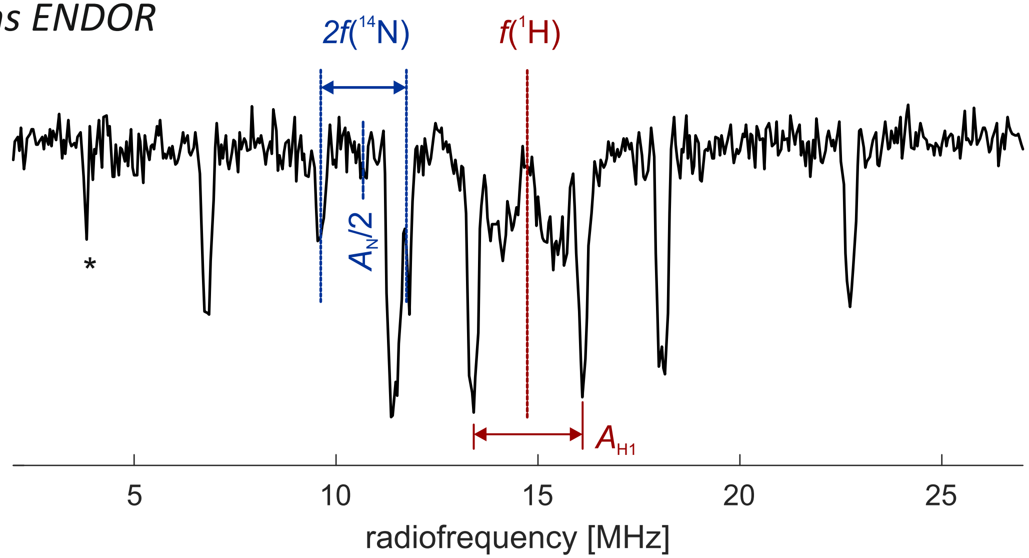
in 1,2-dichlorobenzene



sample courtesy
Agnes Kütt
University Tallin

a more complicated experiment can make it simpler

Mims ENDOR



⊕ identity of the nuclei (^{1}H, ^{14}N)

⊕ direct read-off of hyperfine couplings

Pulsed EPR is a zoo of techniques

EPR spectra

- Fourier-transform EPR
- field-swept echo-detected EPR

Electron spin relaxation

- Hahn echo decay (T_2/T_m)
- Inversion recovery (T_1)

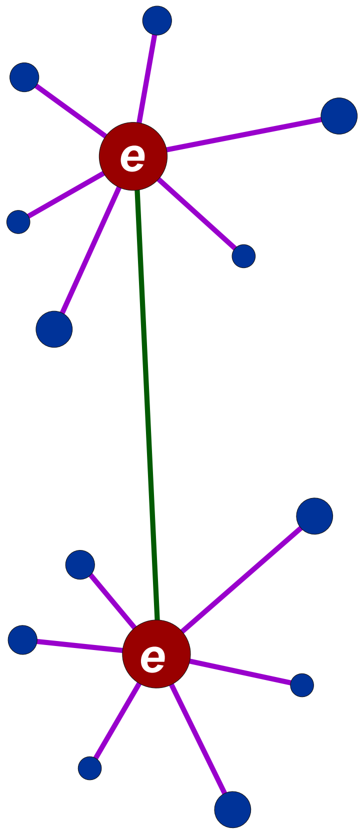
Hyperfine couplings
nuclear Zeeman &
nuclear quadrupole interactions

- ESEEM
- HYSCORE
- Davies ENDOR
- Mims ENDOR
- ELDOR-detected NMR

Sabine, Fri 9:00

Marina, Thu 15:30

Daniella, Fri 11:00

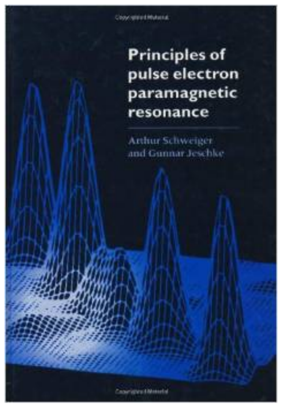


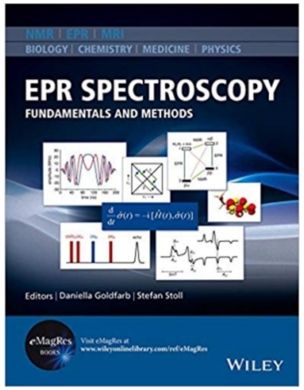
Electron-electron couplings

- DEER/PELDOR
- RIDME
- SIFTER
- DQC-EPR

Fine print

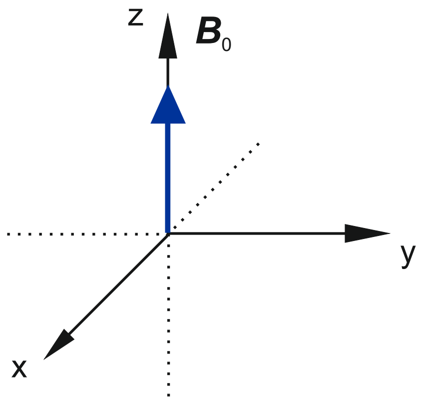
- more techniques exist
- new ones are still coming up
- almost all of them fit into one of the four boxes



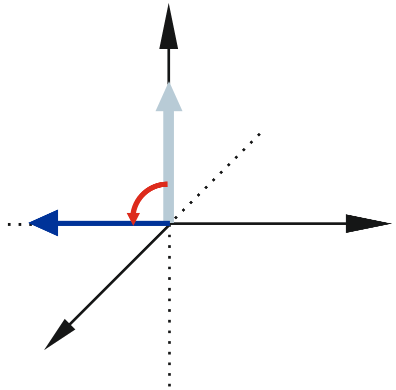


Free induction decay (FID) and Fourier transform EPR

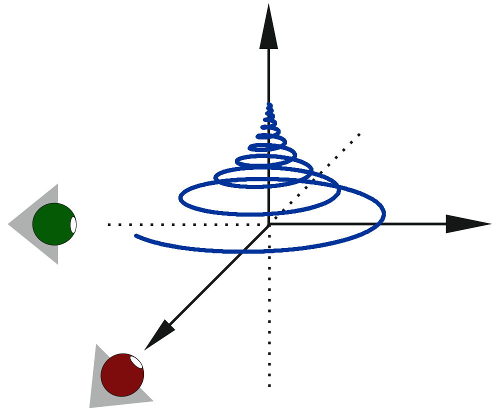
Thermal equilibrium



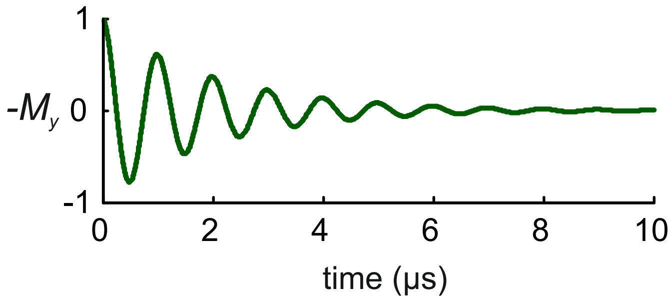
$\pi/2$ (90°) pulse

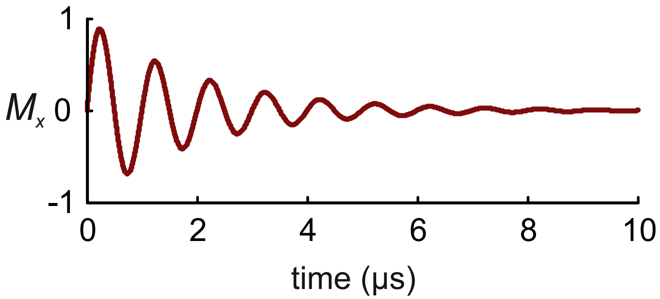


Free evolution (FID)

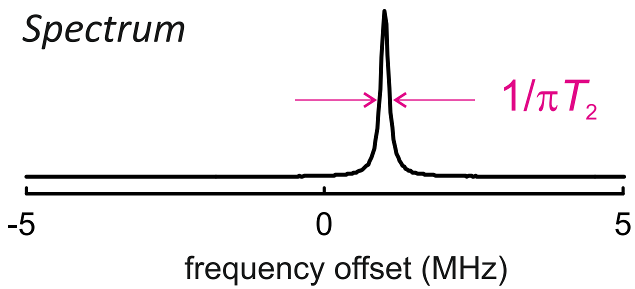


Complex (quadrature) signal: $-M_y + i\,M_x$





Fourier transform



Motion of the magnetization vector without relaxation

Classical equation of motion

spin has, both, angular momentum J and a magnetic moment μ

$$\frac{d\vec{J}}{dt} = \underbrace{\vec{\mu} \times \vec{B}(t)}_{\text{torque}}$$

$\vec{J}$ total angular momentum vector, $\vec{B}(t)$ includes static and oscillatory magnetic fields

as $\gamma \vec{J} = \vec{\mu}$ and $\vec{M} = \vec{\mu}/V$, we have

$$\frac{d\vec{M}}{dt} = \vec{M} \times \gamma \vec{B}(t)$$

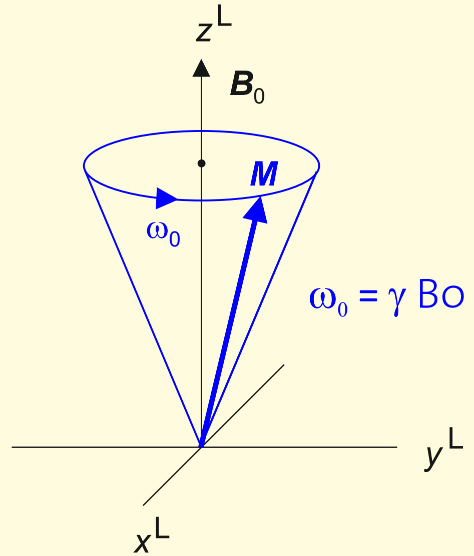
and by expanding the cross product

$$\dot{M}_x = -\gamma B_z(t)\, M_y + \gamma B_y(t)\, M_z$$

$$\dot{M}_y = \gamma B_z(t)\, M_x - \gamma B_x(t)\, M_z$$

$$\dot{M}_z = \gamma B_x(t)\, M_y - \gamma B_y(t)\, M_x$$

Precession in a static field B_0



$$\dot{M}_x = -\omega_0 M_y$$

$$\dot{M}_y = \omega_0 M_x$$

$$\dot{M}_z = 0$$

Relaxation in the magnetization vector model

Relaxation is due to flips of individual spins

⇒ only phenomenological description possible in a classical picture

⇒ length of $\vec{\boldsymbol{M}}$ is not a constant of motion

Two types of relaxation just a reminder, Thomas Prisner talked about this

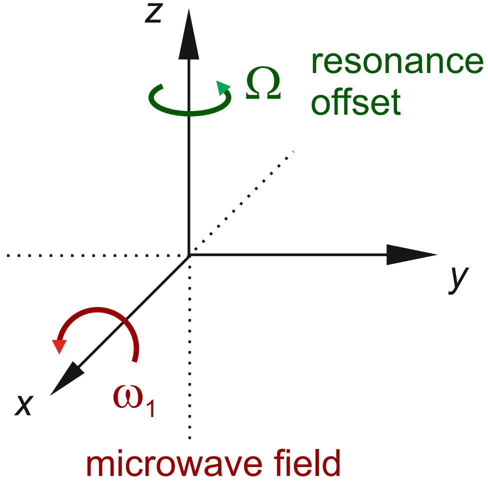
- longitudinal relaxation requires energy exchange with the environment, enthalpic, time constant T_1
- transverse relaxation reduces phase coherence of the ensemble, entropic, time constant T_2

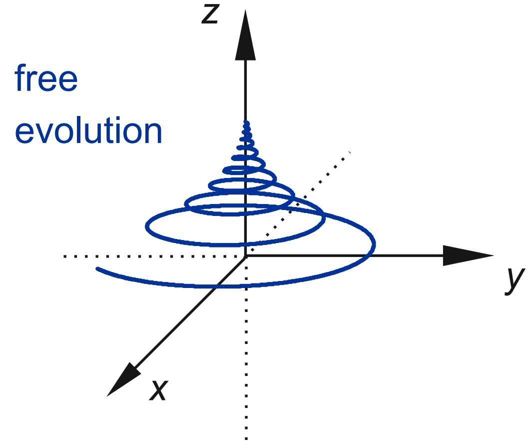
Bloch equations with relaxation rotating frame

$$\dot{M}_x = -\Omega\, M_y - \frac{M_x}{T_2}$$

$$\dot{M}_y = \Omega\, M_x - \omega_1\, M_z - \frac{M_x}{T_2}$$

$$\dot{M}_z = \omega_1\, M_y - \frac{M_z - M_0}{T_1}$$

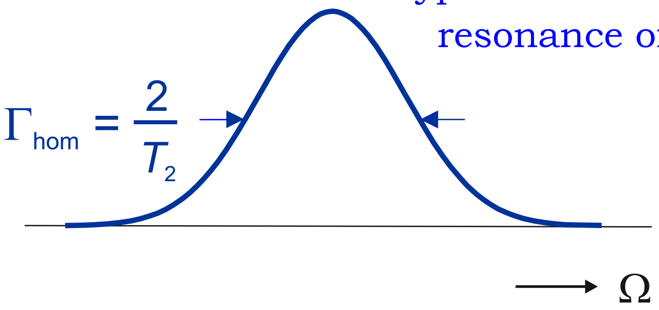




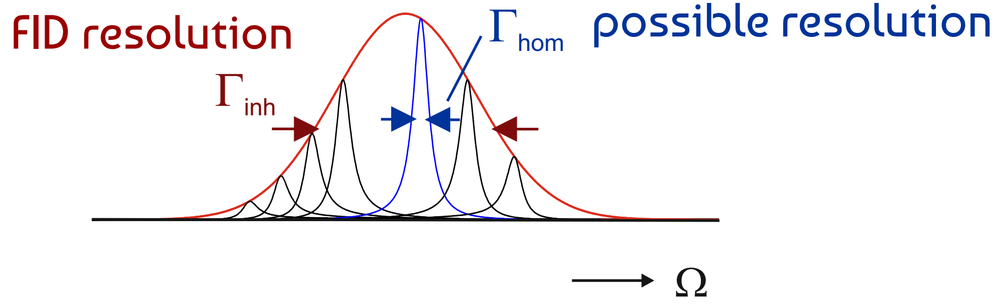
Homogeneous and inhomogeneous lines

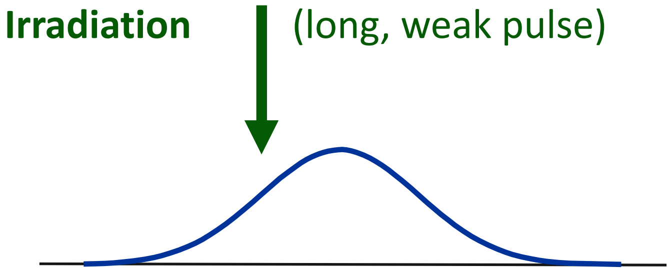
Homogeneous line

all spins of the same type with the same resonance offset

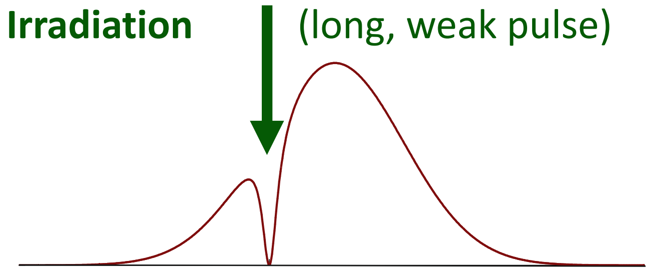


Inhomogeneous line





- saturation



only a spin packet is saturated

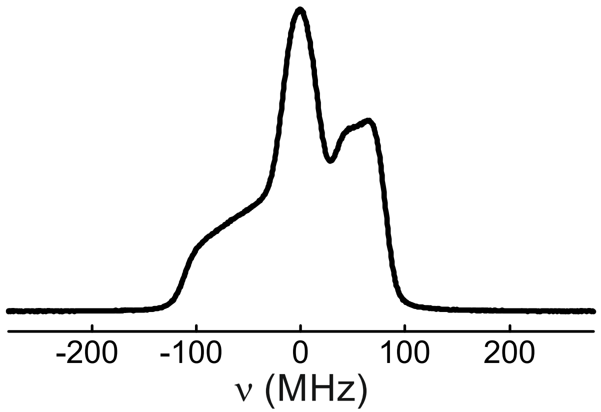
- spectral hole burning

  (used in Davies ENDOR, ELDOR-detected EPR)

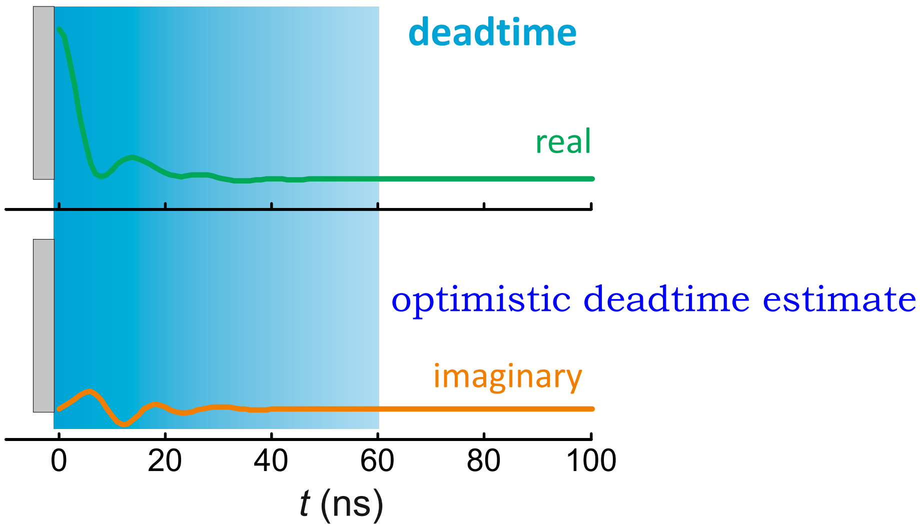
! in the solid state, inhomogeneous broadening by anisotropic interactions is often dominant

Why a single pulse is not sufficient for solids

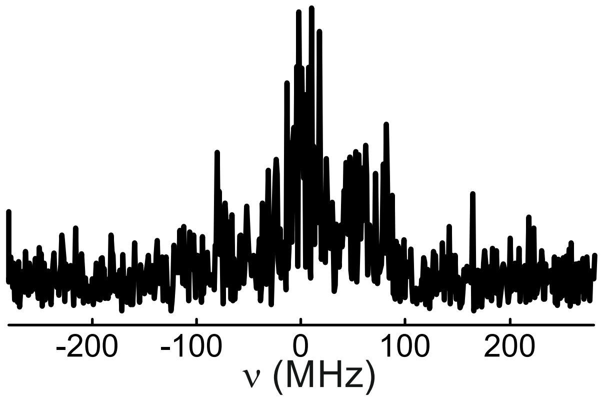
EPR absorption spectrum of a nitroxide
(X band)



... and the corresponding FID



FT of the accessible part of the FID



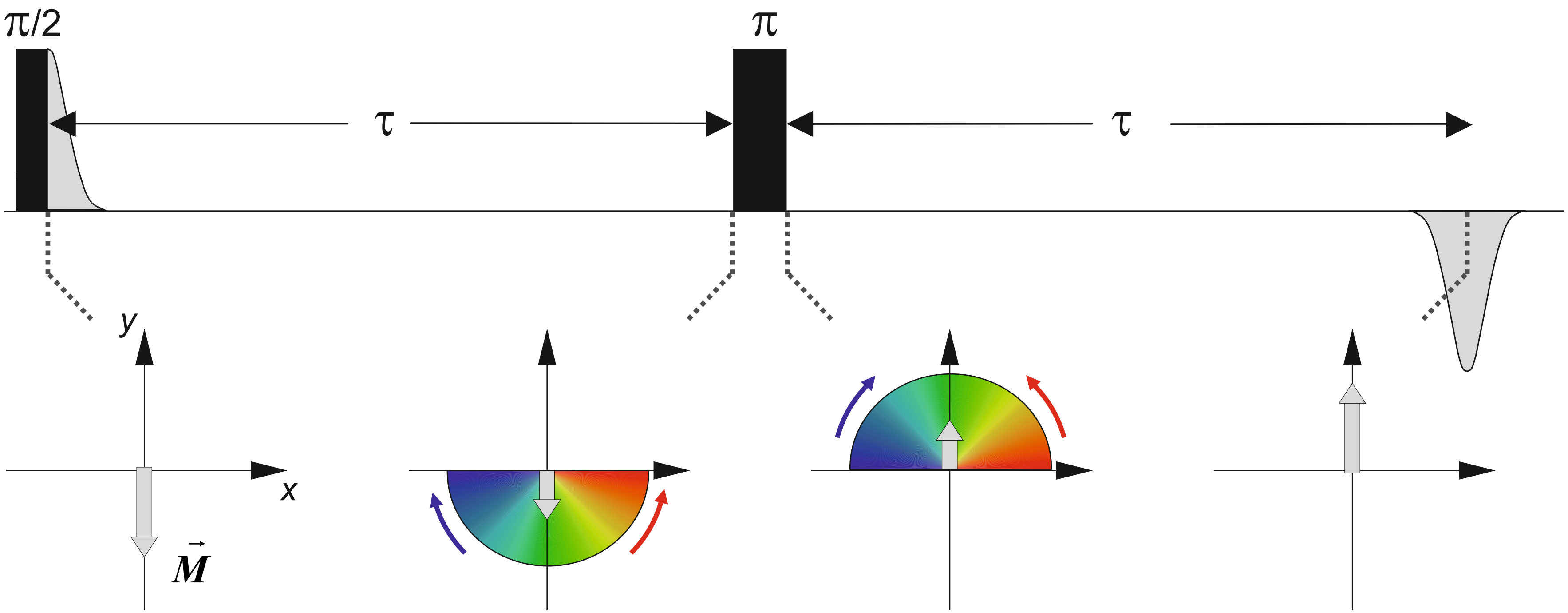
- destructive interference of spin packets with different resonance offset dampens FID within dead time

FT EPR by FID detection possible only for:

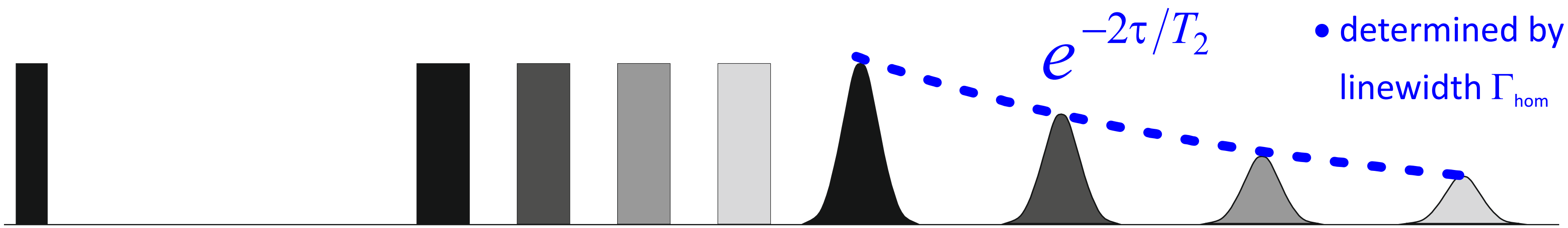
- spectral widths < 100 MHz*
- line widths <≈ 3 MHz

*with modern arbitrary wavefunction generators: up to 800 MHz

The primary echo (Hahn echo)

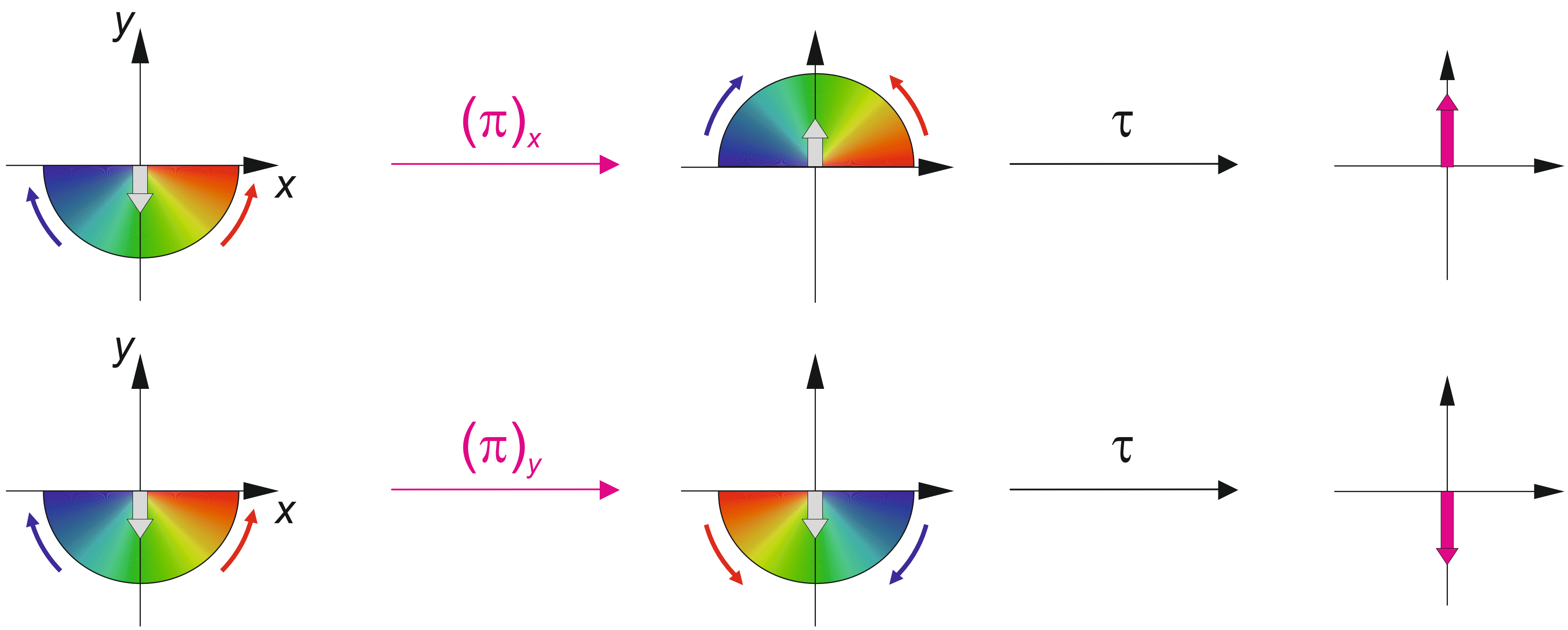


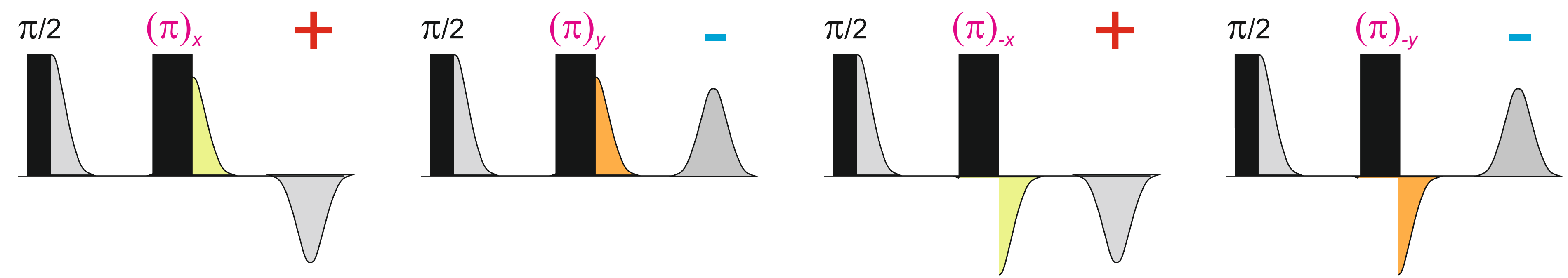
Echo decay



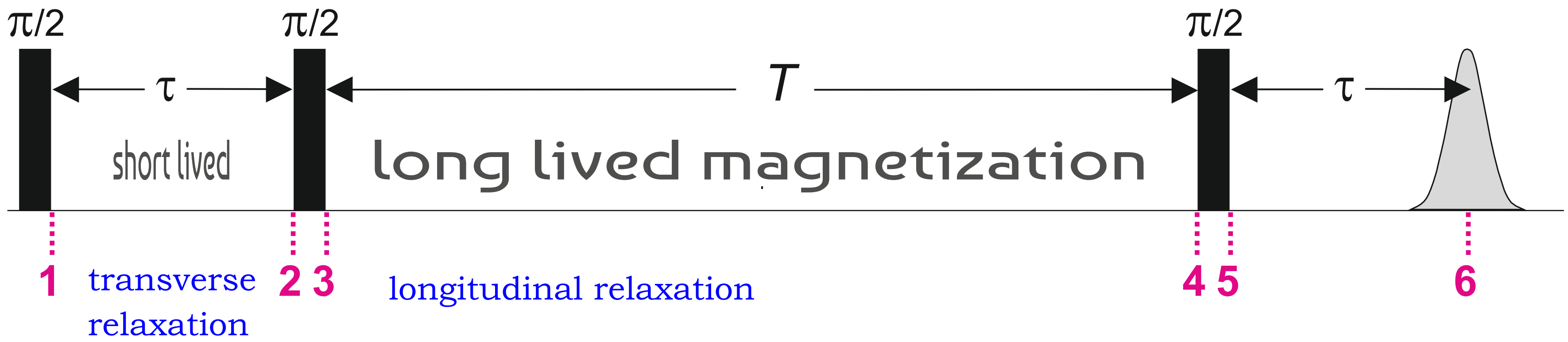
- determined by homogeneous linewidth Γ_{hom}

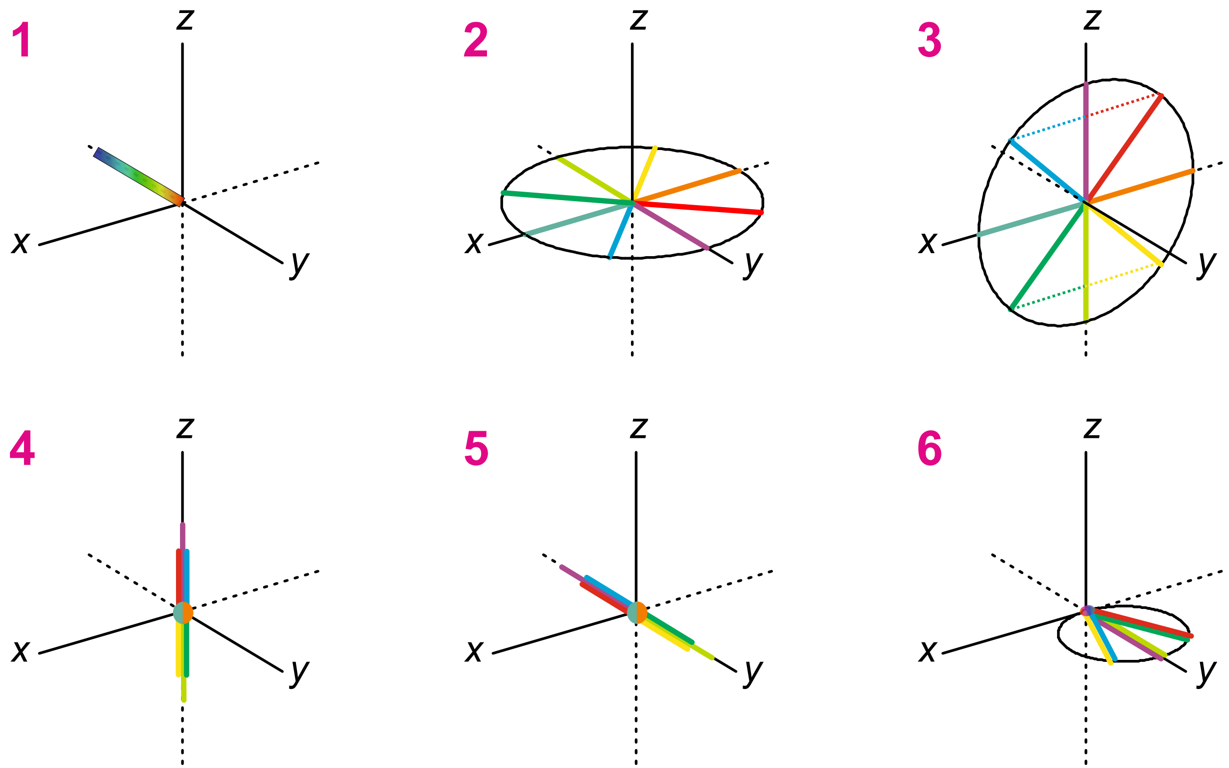
Basics of phase cycling





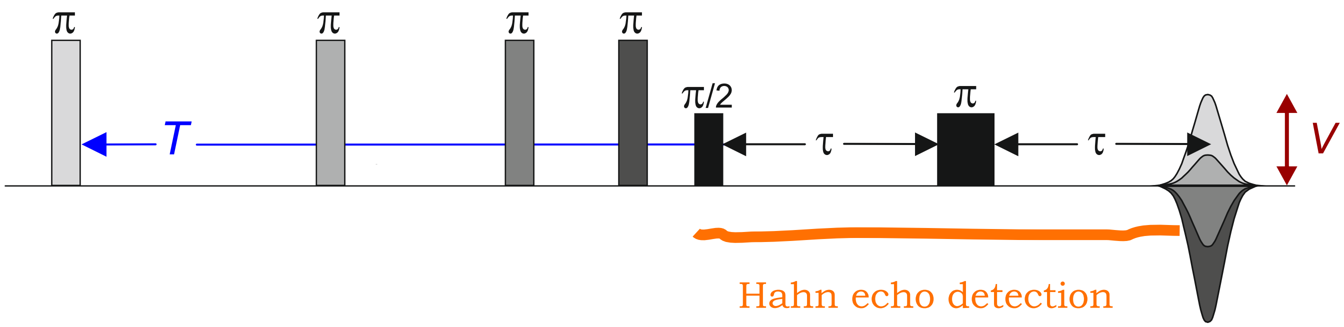
The stimulated echo experiment





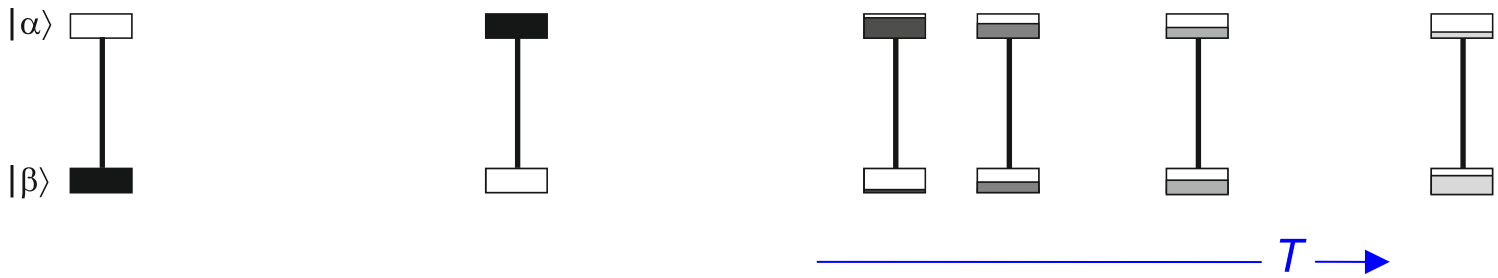
- half of the magnetization is lost by relaxation
- the other half decays *approximately* with $1/T_1$ during time T
- typically, some kind of magnetization transfer during time T is observed

The inversion recovery pulse sequence

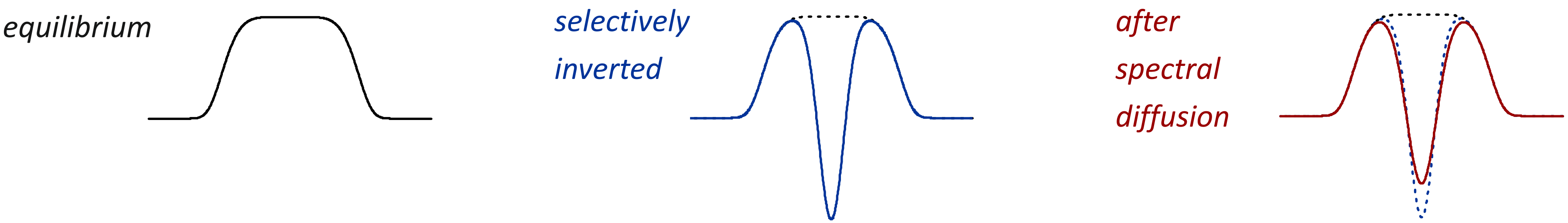


$V(T)/V(0) = 1 - 2\exp\{-T/T_1\}$

Thermal equilibrium **After inversion** **Longitudinal relaxation**



Beware of spectral diffusion spins change resonance frequency or exchange magnetization



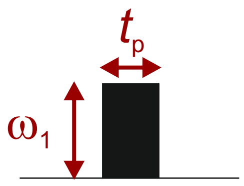
Nutation and excitation bandwidth

Rotating-frame Hamiltonian during irradiation

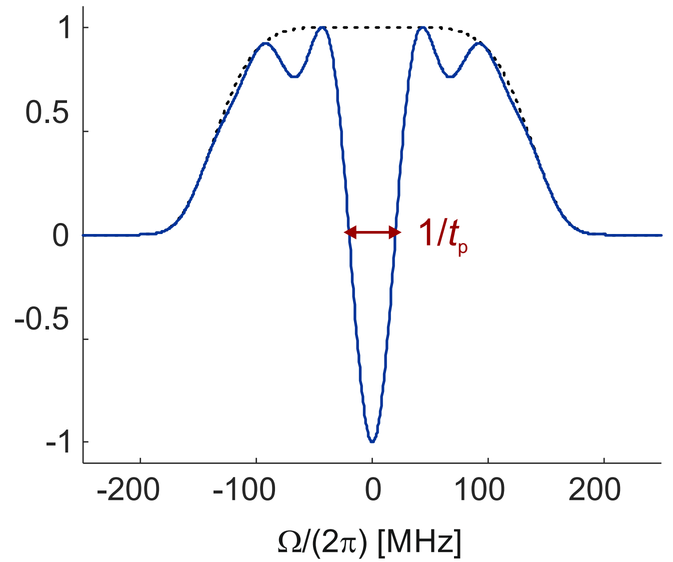
$$\mathcal{H} = \Omega\, S_z + \omega_1\, S_x$$

The flip angle is defined on resonance

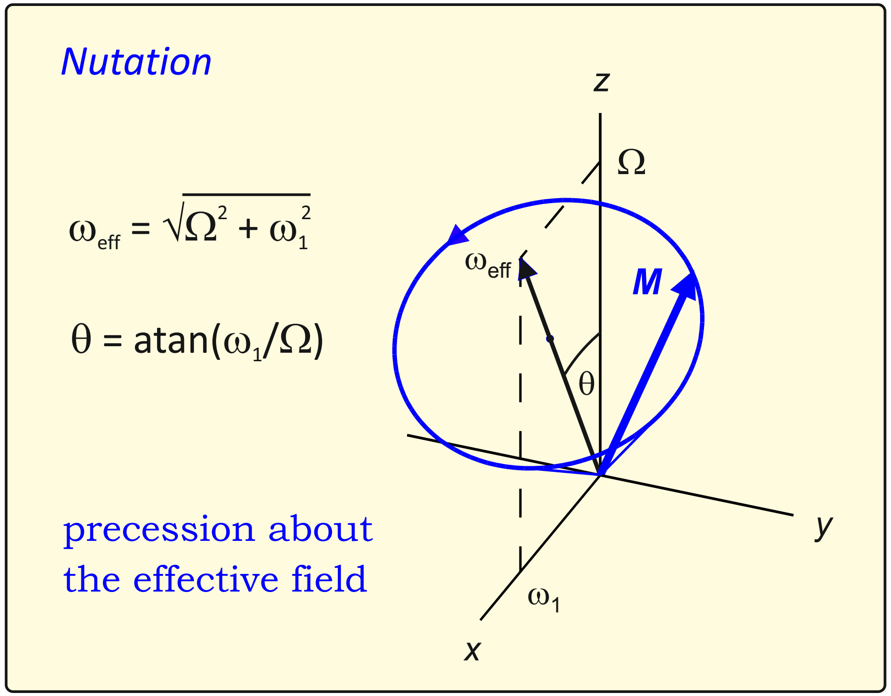
$$\beta = \omega_1\, t_p$$



Excitation profile



Simulation for
$\beta = \pi$
t_p = 20 ns



- the Fourier transform of a boxcar function approximates the excitation profile for a *small* flip angle:

$$P(\Omega) \approx \frac{\sin(\Omega\, t_p/2)}{\Omega\, t_p/2}$$

Overcoming the microwave power limit to bandwidth

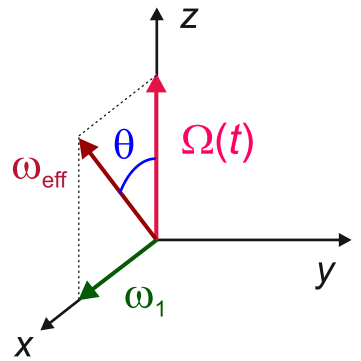
The power-bandwidth relation for rectangular monochromatic pulses

$\mathrm{BW} \approx 1/t_p \qquad t_p = \beta/\omega_1 \qquad \Rightarrow \mathrm{BW} \approx \omega_1/\beta = g_x\,\mu_B\,B_1/(\hbar\beta) \qquad \text{with} \quad B_1 \propto \sqrt{P_{mw}}$

! the time available for spin excitation is of the order of T_2 (microseconds) [coherent excitation] or T_1 (milliseconds) [saturation], whereas spectral width is of the order of 100 MHz … 10 GHz, corresponding to t_p = 10… 0.1 ns

Frequency-swept pulses

Accelerated frame picture

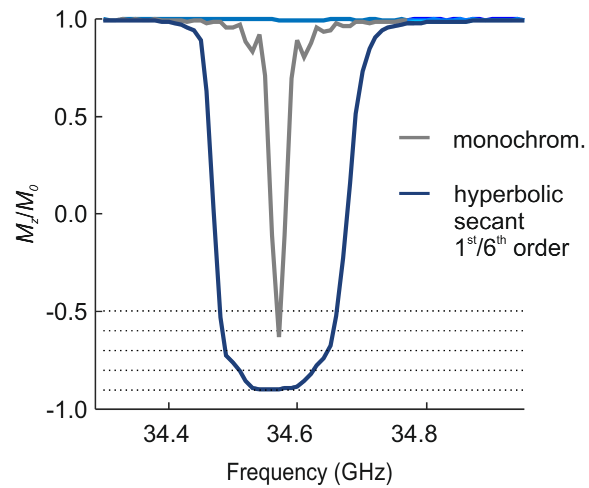


Adiabaticity

$Q = \omega_{eff}/|d\theta/dt|$

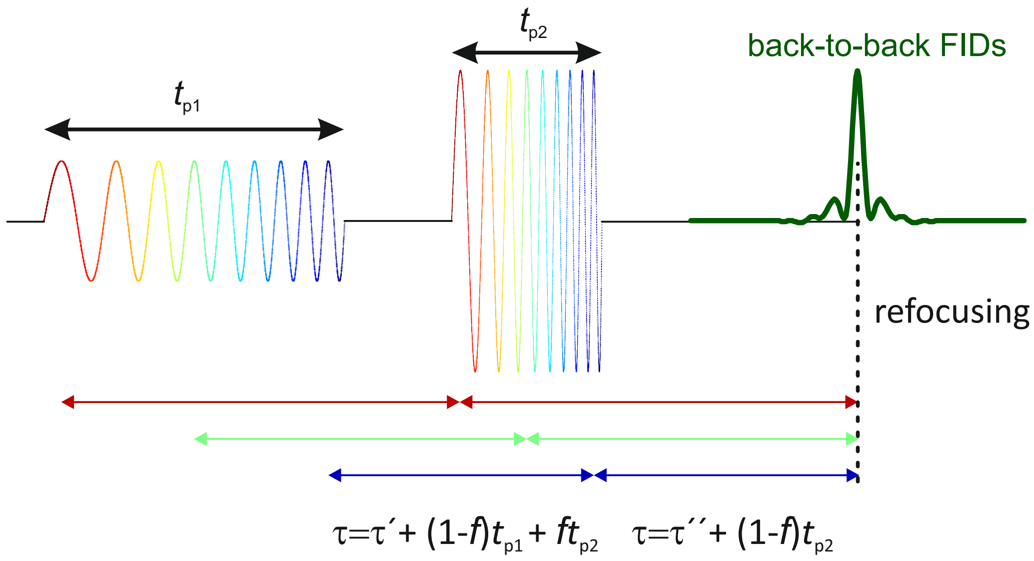
- for Q >> 1 the magnetization vector follows the effective field from +z to -z
  ⇒ ***perfect*** wideband inversion

Well, nobody is perfect



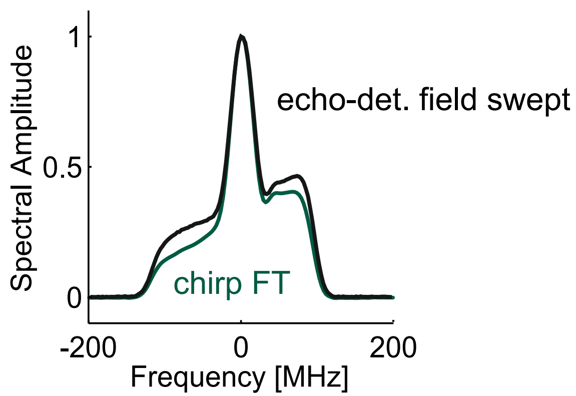
Chirp echoes

Primary chirp echo

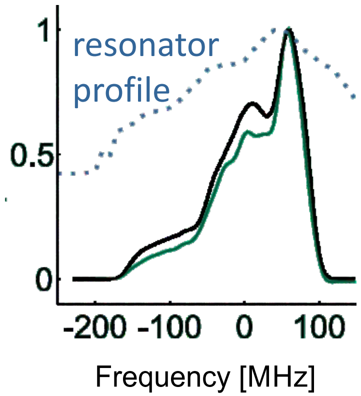


- a spin packet Ω is excited after time fraction $f = (\Omega - \omega_{start})/B$
- for all packets to refocus at the same time, we must have $\tau' + (1-f)t_{p1} + ft_{p2} = \tau'' + (1-f)t_{p2}$ for all f, which gives $t_{p1} = 2t_{p2}$
- Fourier transformation of the echo gives the frequency-domain EPR spectrum

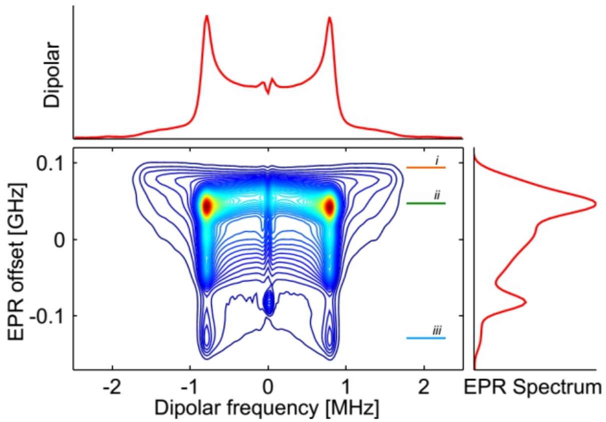
X-band chirp FT EPR



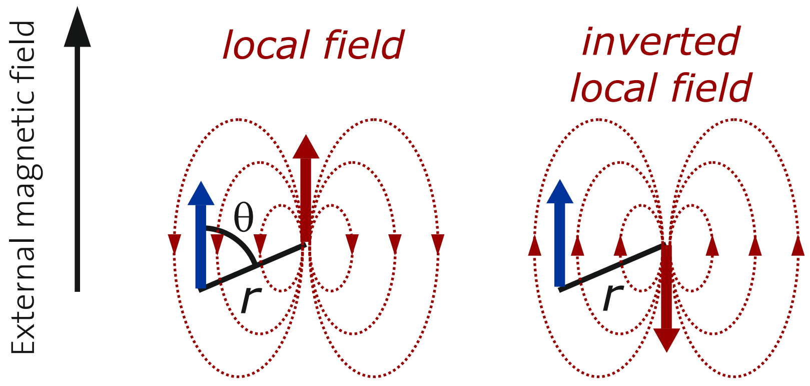
Q-band chirp FT EPR



Q-band SIFTER/FT EPR correlation



Dipole-dipole coupling



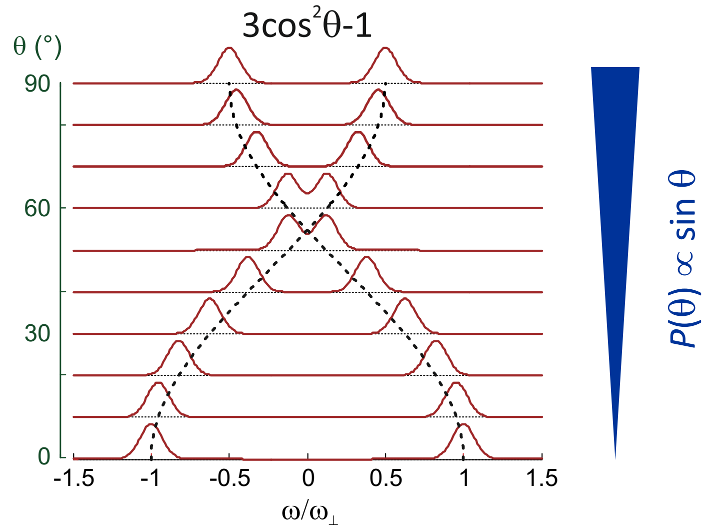
spin vector operators

$$\hat{H}_{\mathrm{dd}} = \frac{1}{r_{SI}^3}\frac{\mu_0\hbar}{4\pi}\gamma_S\gamma_I\left[\hat{\boldsymbol{S}}\cdot\hat{\boldsymbol{I}} - 3\frac{1}{r_{SI}^2}\left(\hat{\boldsymbol{S}}\cdot\boldsymbol{r}_{SI}\right)\left(\hat{\boldsymbol{I}}\cdot\boldsymbol{r}_{SI}\right)\right]$$

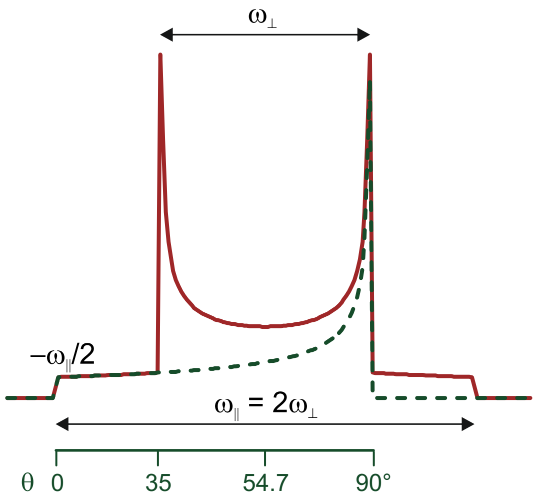
Relevant terms

$$\frac{1}{r_{SI}^3}\frac{\mu_0\hbar}{4\pi}\gamma_S\gamma_I\,(3\cos^2\theta-1)\,[\hat{S}_z\hat{I}_z - \tfrac{1}{2}(\hat{S}^+\hat{I}^- + \hat{S}^-\hat{I}^+)]$$

Orientation dependence



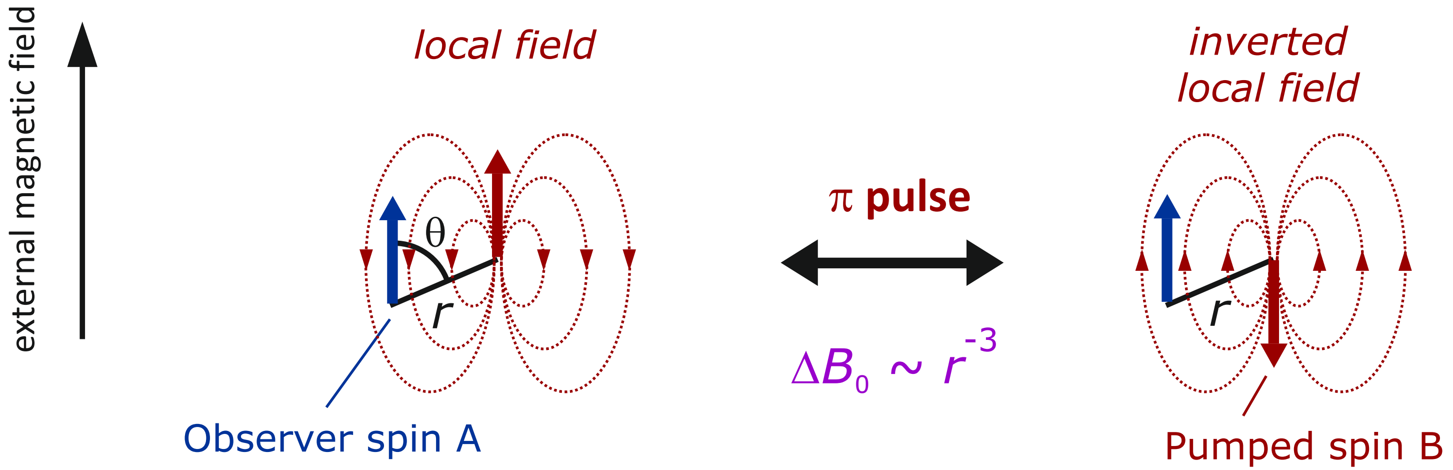
Pake pattern



To infer the distance r_{SI}, we must

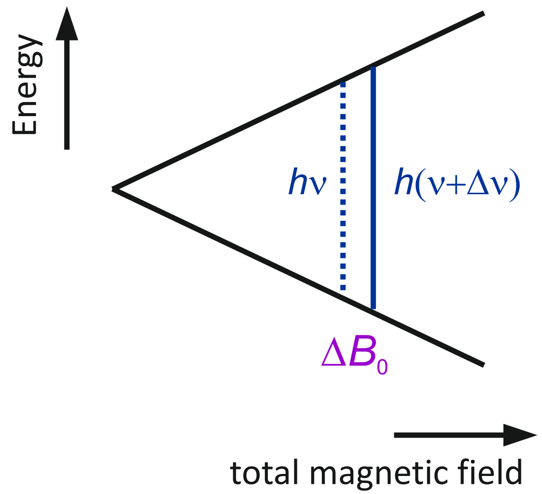
- separate dipole-dipole coupling from stronger interactions
- get rid of the orientation dependence

Separation of the electron-electron coupling from other interactions



1. Signal is labeled with resonance frequency $\nu = g\mu_B B_o/h$

2. Pumped spin is inverted, field shifts



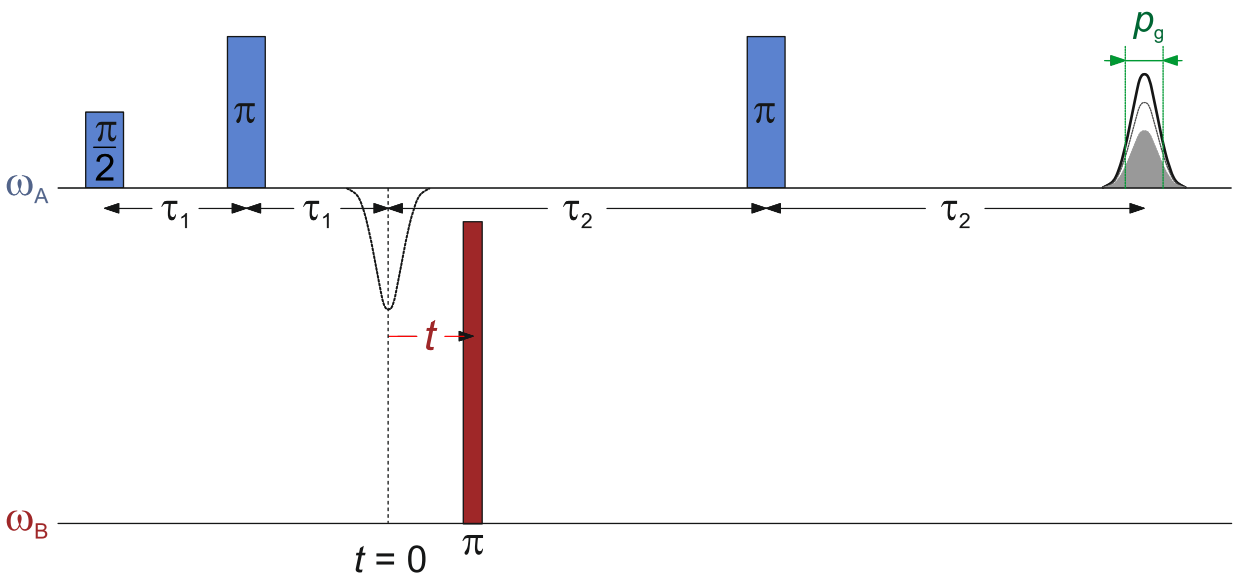
3. Field shift ΔB_0 is measured via change $\Delta\nu$ of resonance frequency

4. Distance r is computed from $\Delta\nu$

$$r^3 = (52.04\ \text{MHz}/\Delta\nu)\ \text{nm}$$

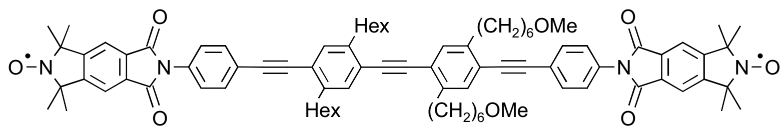
What is DEER?

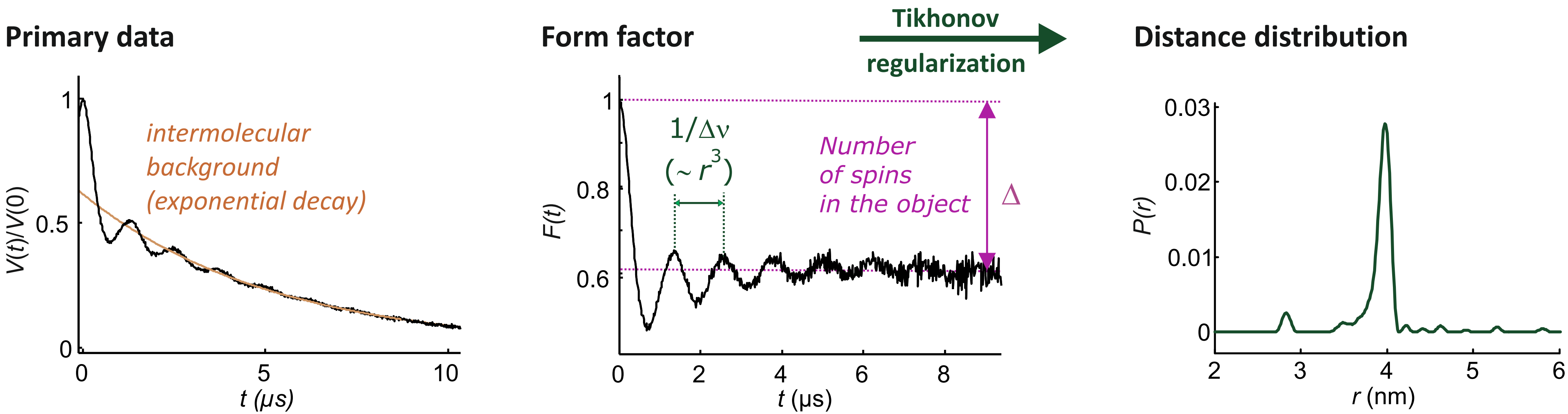
a proud animal that also accepts to be named PELDOR



The **echo amplitude** is observed as a function of ***time t***

Model compound





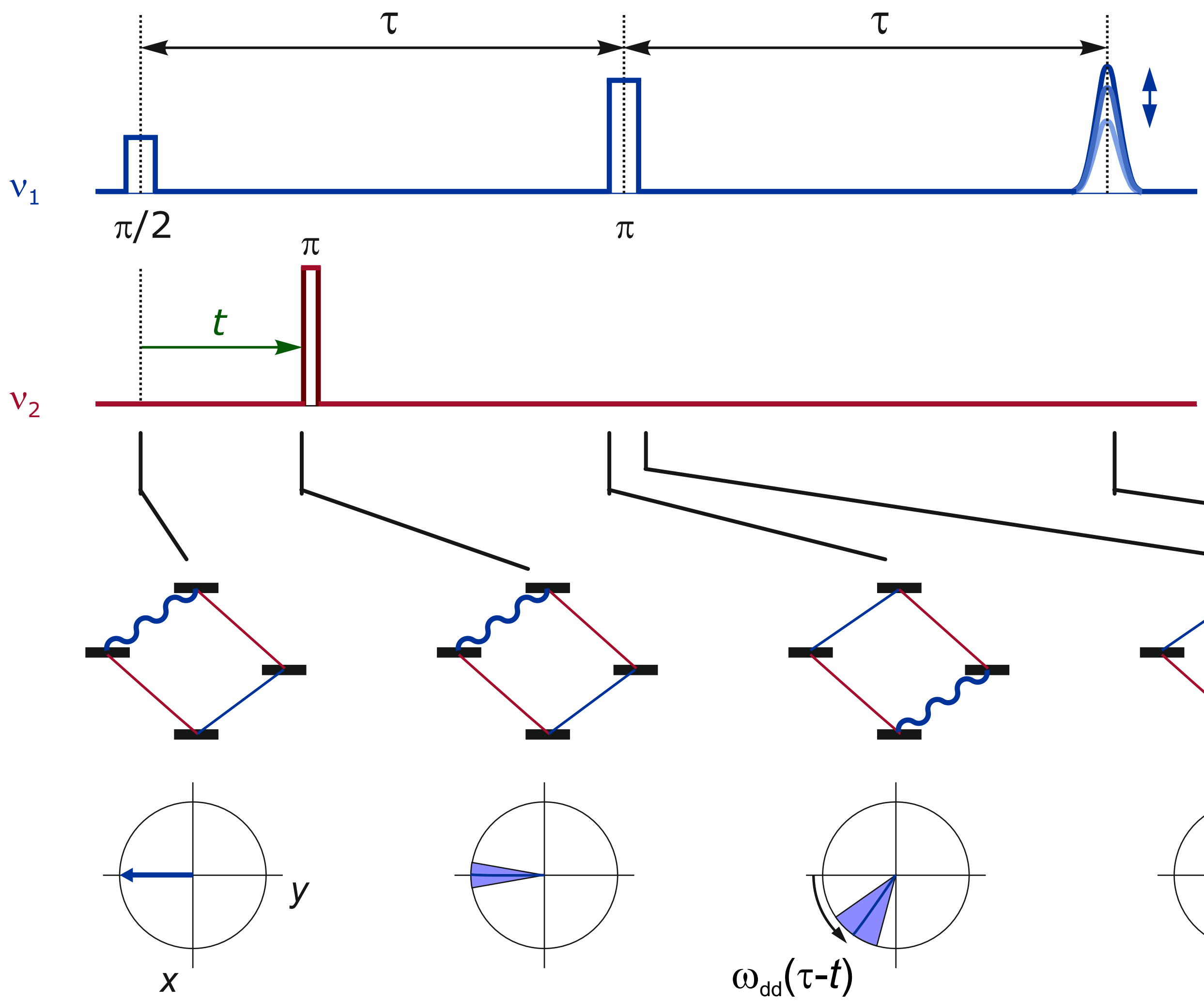
MARTIN RE et al., *Angew. Chem. Int. Ed.* **1998**, *37*, 2834

PANNIER M, VEIT S, GODT A, JESCHKE G, SPIESS HW, *J. Magn. Reson.* **2000**, *142*, 331

JESCHKE G et al. *J. Magn. Reson.* **2002**, *155*, 72

JESCHKE G et al. *Appl. Magn. Reson.* **2006**, *30*, 473

Separating the dipole-dipole interaction by a spin flip

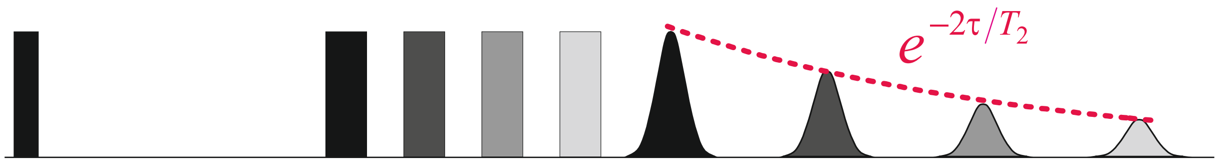


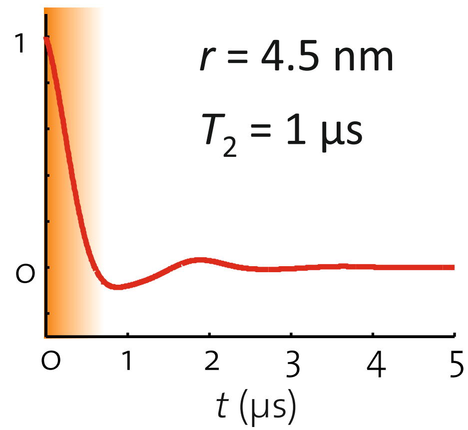
- constant delay τ:
  no echo variation
  due to relaxation
- variable delay t:
  resonance frequency
  is changed at different times

original experiment by Salikhov, Milov, Shirov, and Tsvetkov

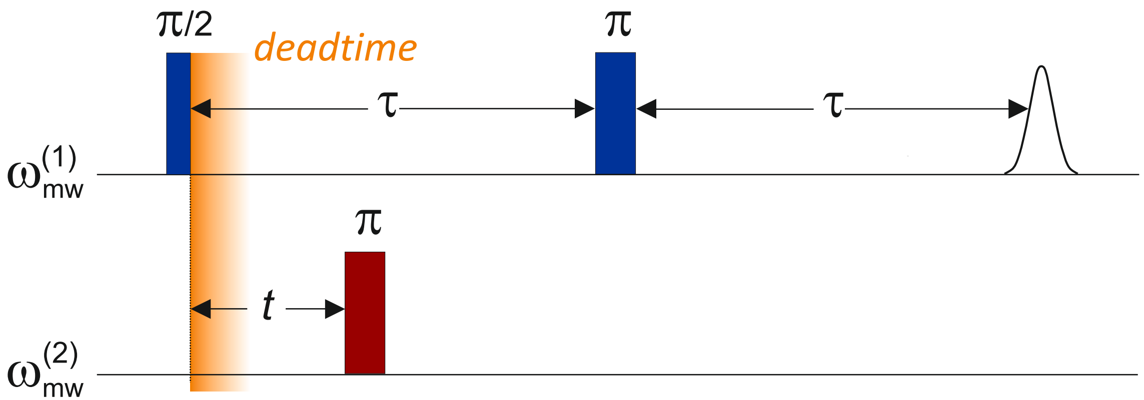
Ultimate resolution by a constant-time experiment

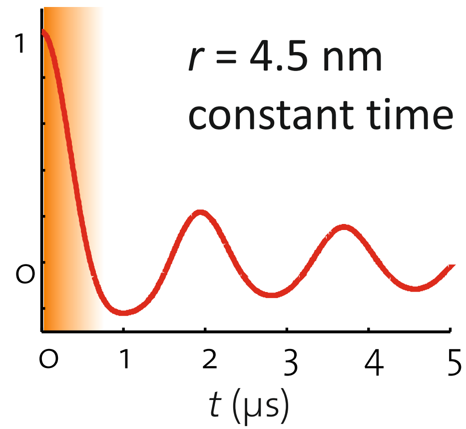
- if the total length of the pulse sequence changes, dipolar evolution is damped by relaxation
- for distances larger than ~3-4 nm, dipolar modulation is overdamped





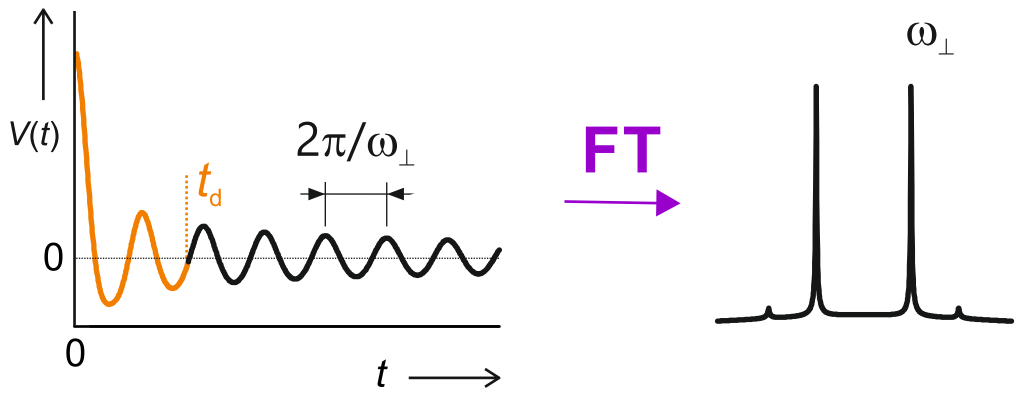
Keep τ fixed, vary only *t*, pulse sequence has constant length





We trade sensitivity for resolution

Eliminating dead-time by the four-pulse DEER experiment

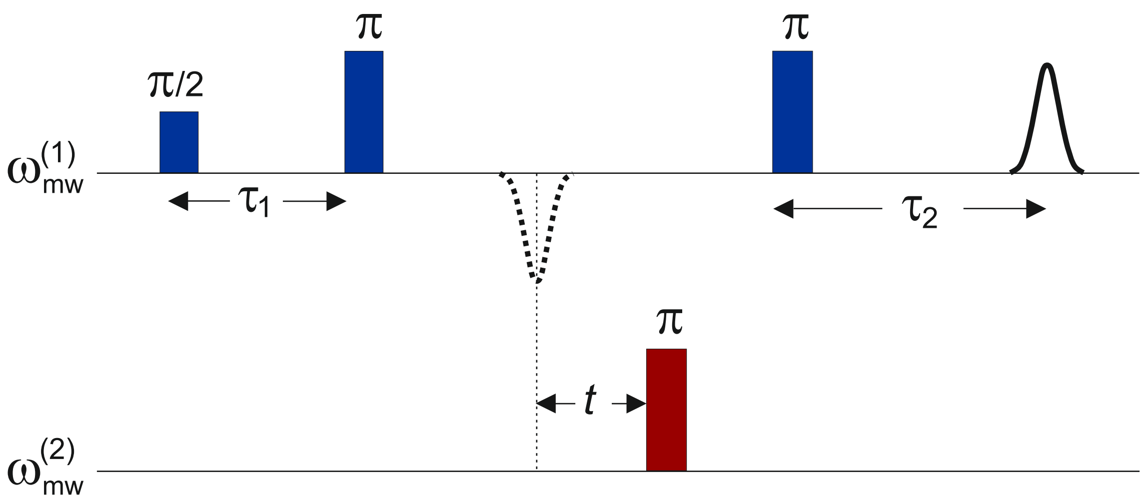


unreliable results due to deadtime for

- distributions with r < 2.2 nm
- direct extraction of distance distributions

sensitivity loss for small r

Remedy: Refocusing of the echo $\Rightarrow$ accessible t: $-\tau_1+t_d < t < \tau_2-t_d$



Typical values (nitroxides):

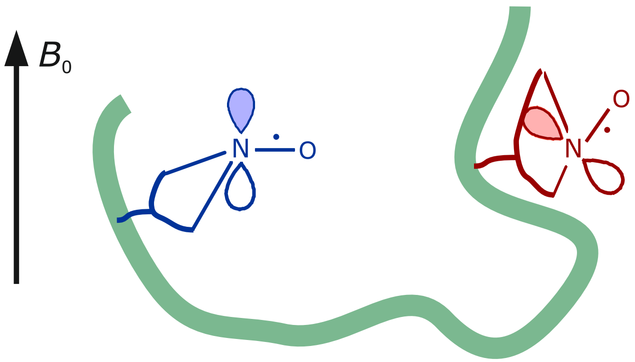
$\tau_1 \approx$ 120...200 ns, 400 ns with deuterated matrix

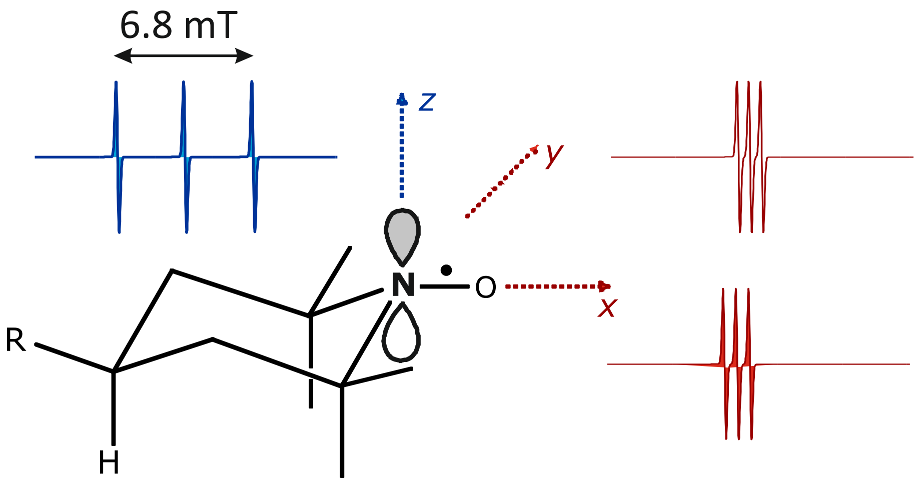
$\tau_2 \approx$ 1 µs (2 nm)... 6 µs (5-8 nm)

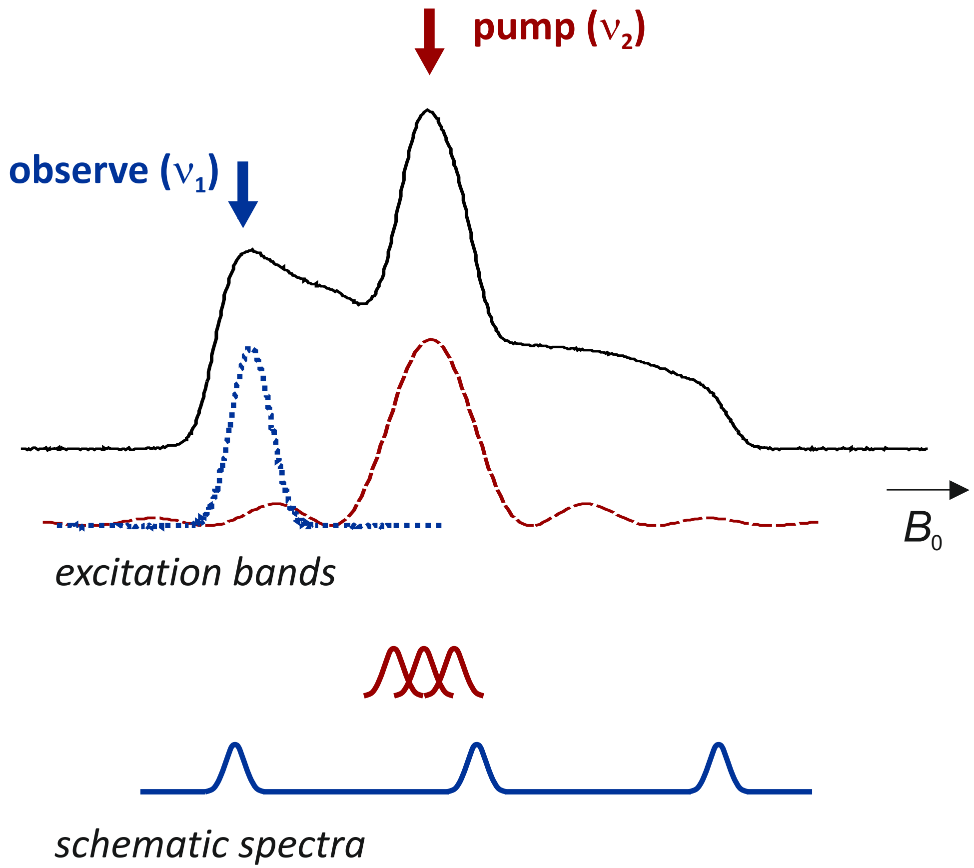
$t_p(1)$ = 32 ns

$t_p(2)$ = 12 ns (opt. sensitivity)

Why can we excite two nitroxide labels with two frequencies?



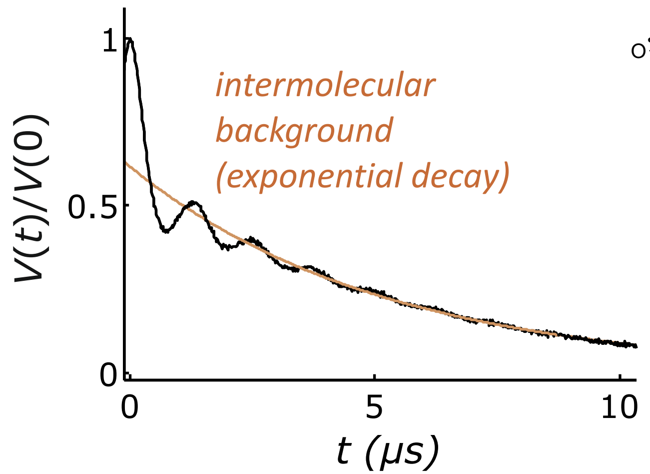


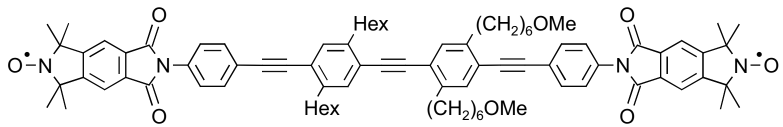


- only for a fraction λ of all spin pairs of the observed spin A the coupled spin B is indeed excited (pumped)

Information content of the DEER signal

Primary experimental data

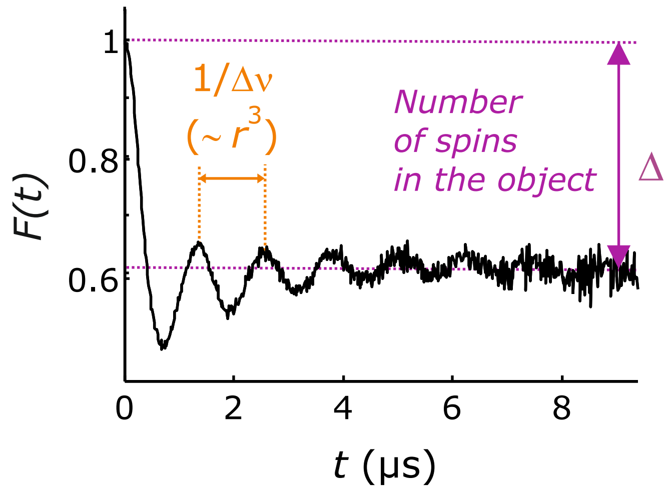




background

correction

Form factor (intramolecular)



- decay envelope depends on distance distribution: fast decay of modulation ⇒ broad distribution

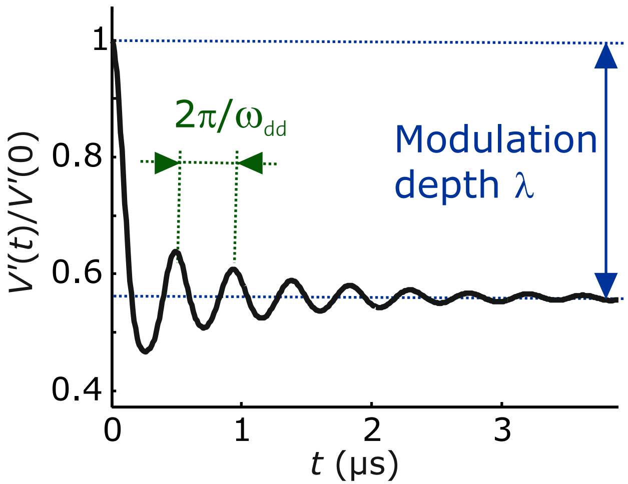
Dipole-dipole interaction $$\omega_{dd} = \frac{1}{r^3}\frac{\mu_0}{4\pi h} g_1 g_2 \mu_B^2$$

Dipolar frequency $$d(\theta) = \left(1 - 3\cos^2\theta\right)\omega_{dd}$$

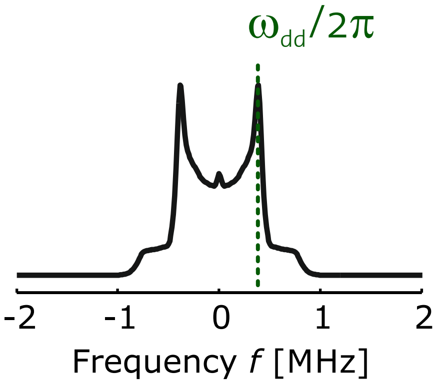
Form factor
of an isolated spin pair $$F(r,t) = \frac{V(t)}{V(0)} = 1 - \int_0^{\pi/2} \lambda(\theta)\left\{1 - \cos\left[2\pi\nu_{dd}(\theta)\right]\right\}\sin\theta\, d\theta$$

Data analysis by Fourier transformation

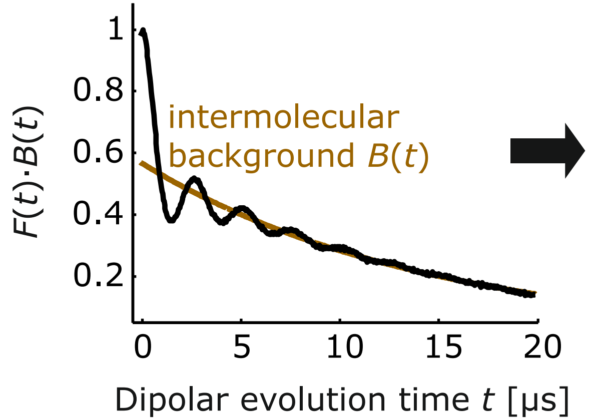
Dipolar evolution function (form factor)

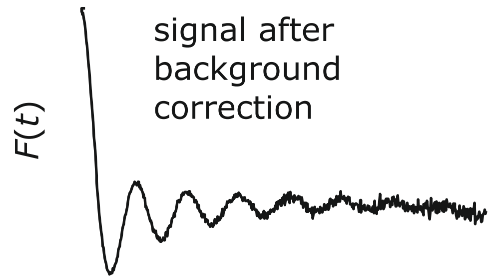


Fourier transformation

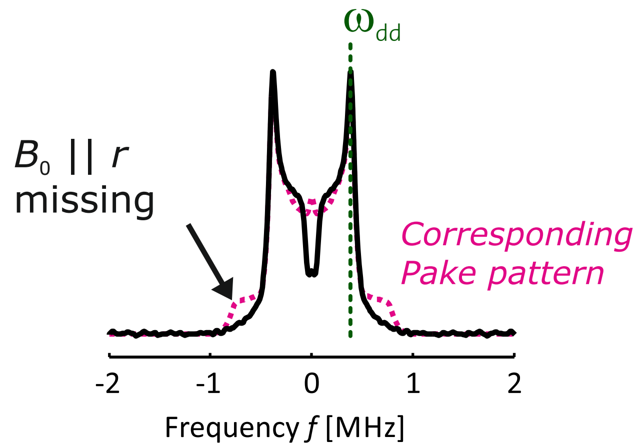


Normalized echo modulation



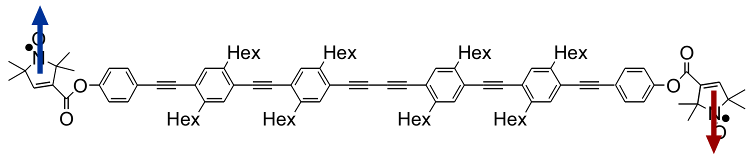


FT



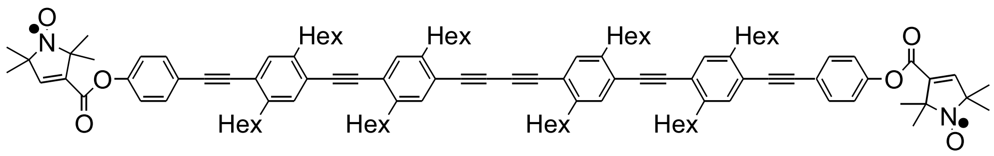
$\omega_{dd}/2\pi$ = 380 kHz

$\Rightarrow r$ = 5.05 nm



Average distance is not the full information

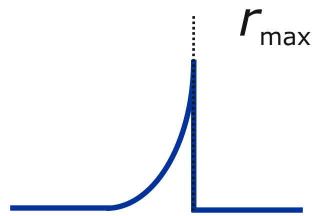
Molecular dynamics causes distribution of *r*



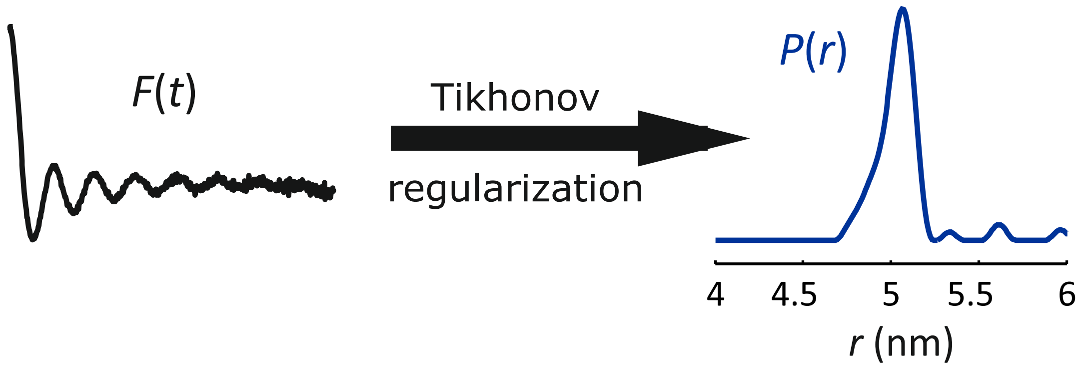
bent, higher energy, lower population, smaller *r*

stretched, min. energy, max. population, max. *r*

Expectation:



Experimental distance distribution



Basic mathematics

fit simulated dipolar evolution function $S(t)=K\,P(r)$

Minimize

$$G_\alpha(P) = \left\| KP(r) - F(t) \right\|^2 + \alpha \left\| \frac{d^2}{dr^2} P(r) \right\|^2$$

mean square deviation

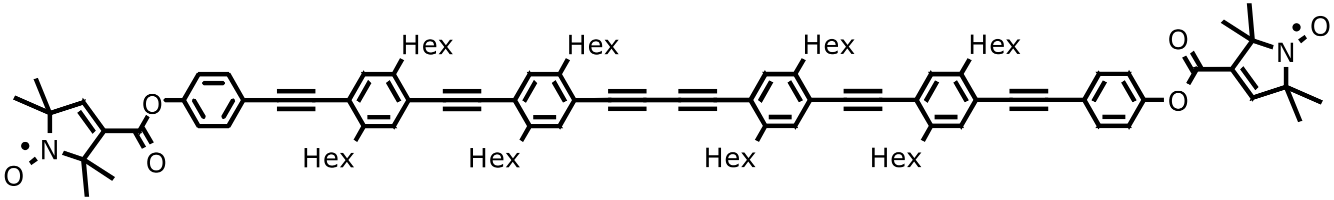
roughness of 2nd derivative

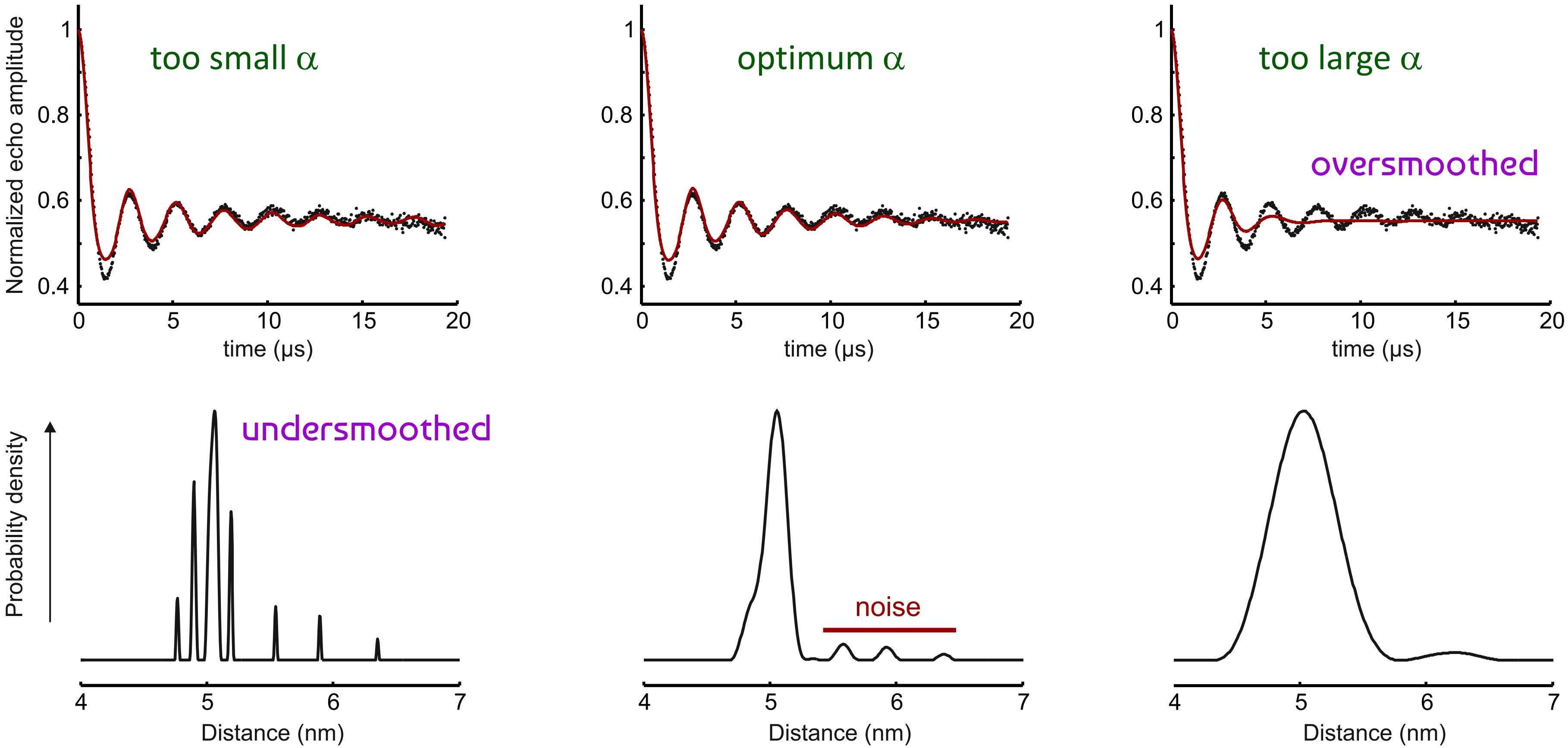
regularization parameter α

G. Jeschke, A. Koch, U. Jonas, A. Godt A, *J. Magn. Reson.* 155, 72-82 (2002)

G. Jeschke et al. *Appl. Magn. Reson.* 30, 473-498 (2006)

Why regularization is required and how to do it



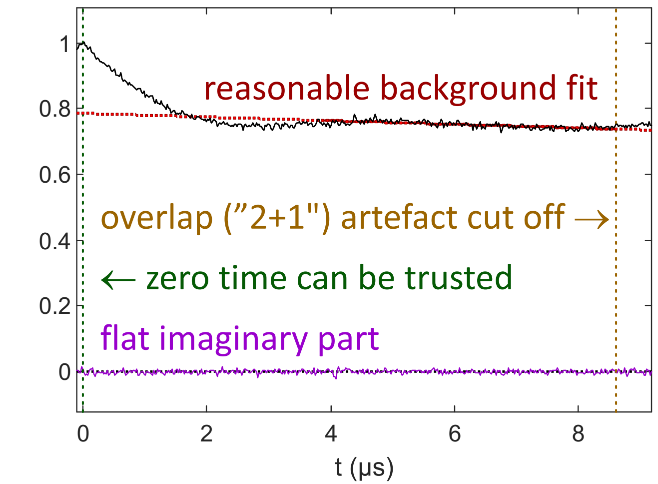


Don’t forget to validate your data

Don’t forget to validate your data

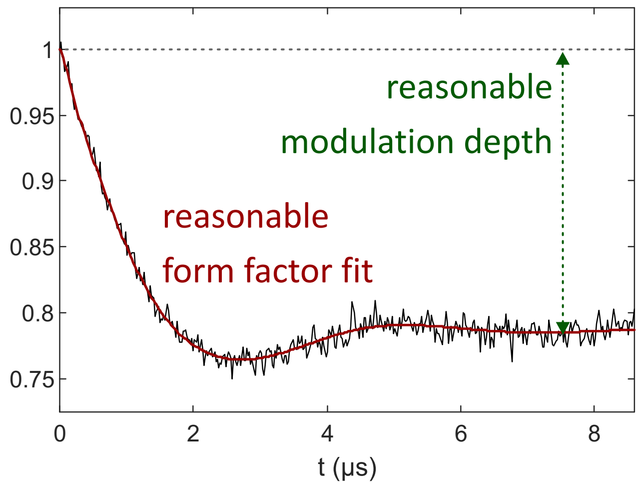
PTBP1/EMCV-IRES(D-F) 85 kDa protein-RNA complex, protein double mutant 235R1/388R1

Primary data

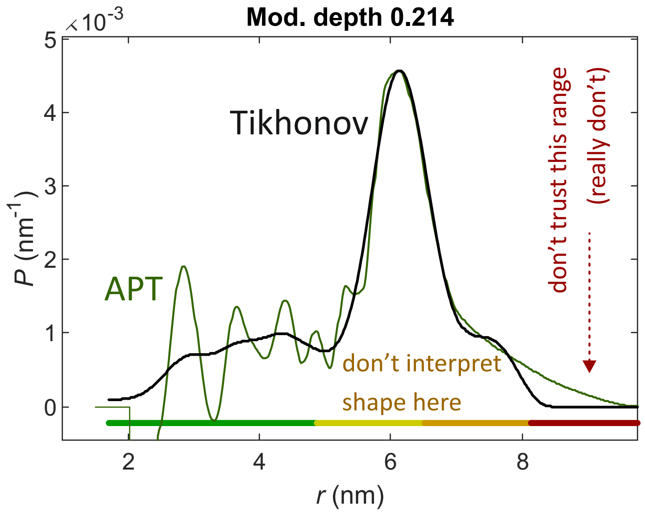


Form factor

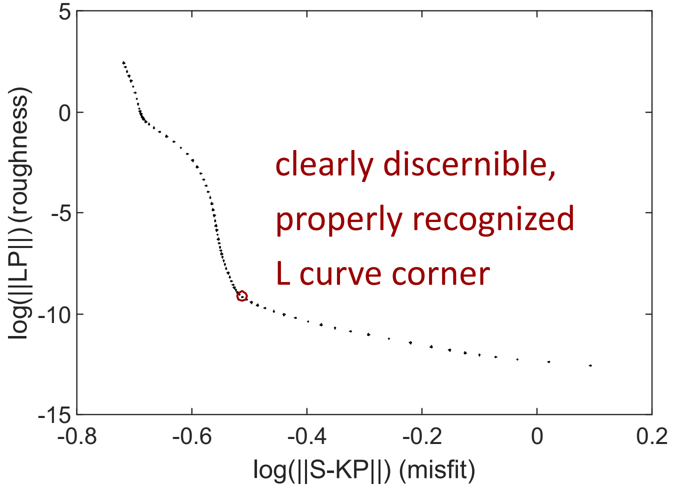
(with reasonable signal-to-noise ratio)



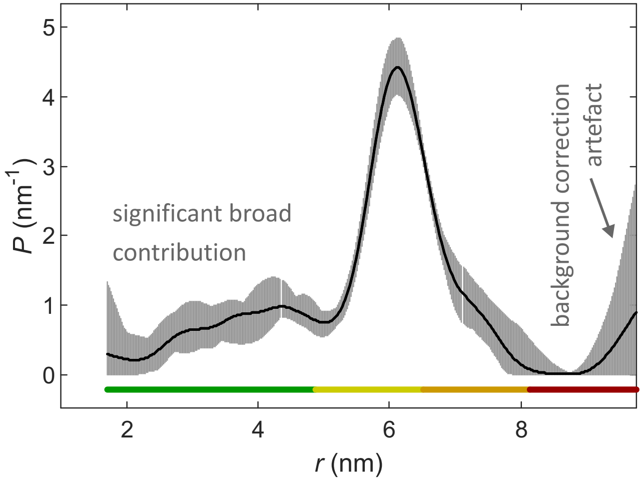
Distance distribution



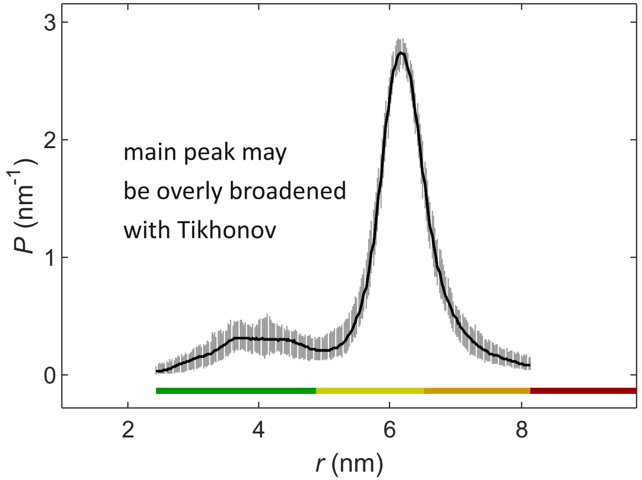
L curve



Validation



Second opinion (DEERNet)



Further reading & useful stuff on pulsed EPR

Product operator formalism and phase cycling

R.R. Ernst, G. Bodenhausen, A. Wokaun, *Principles of Nuclear Magnetic Resonance in One and Two Dimensions*, Oxford University Press, Oxford, 1987.

O. W. Sørensen, *Progr. Nucl. Magn. Reson. Spectro*sc. 21, 503-570 (1989).

Topics specific to pulse EPR

A. Schweiger, G. Jeschke, *Principles of Pulse Electron Paramagnetic Resonance*, Oxford University Press, Oxford, 2001.

Adiabatic & fast passage

J. Baum, R. Tycko, A. Pines, *Phys.Rev. A* 32, 3435–3447 (1985).

A. Doll, G. Jeschke, *J. Magn. Reson*. 280, 46-62 (2017)

Numerical computations

EasySpin, Spinach

Product operator computations & beyond

SpinDynamica

Signal processing

G. Jeschke, *Lecture notes, ETH Zürich*

epr.ethz.ch/education/messtechnik.html

(Pulsed) EPR lecture notes

G. Jeschke, *Lecture notes, ETH Zürich*

epr.ethz.ch/education.html

Further reading & useful stuff on DEER & related

General overview on techniques for distance distribution measurements

P. P. Borbat, J. H. Freed, eMagRes, Wiley, Vol. 5, 465–494 (2017). *Dipolar Spectroscopy – Single-Resonance Methods*

G. Jeschke, eMagRes, Wiley, Vol. 5, 1459-1475 (2016). *Dipolar Spectroscopy – Double-Resonance Methods*

Biological applications

G. Jeschke, Annu. Rev. Phys. Chem. 63, 419-446 (2012), *DEER distance measurements on proteins*

B. Endeward, A. Marko, V. P. Denysenkov, S. T. Sigurdsson, T. F. Prisner, Methods in Enzymology 564, 403-425 (2015) *Advanced EPR Methods for Studying Conformational Dynamics of Nucleic Acids*

G. Jeschke, Emerging Topics in Life Science, 2, 9-18, (2018) *The contribution of modern EPR to structural biology*

Structure modelling based on distance distributions

G. Jeschke, Proteins 84, 544-560 (2016)

G. Hagelueken, D. Abdullin, O. Schiemann, Methods in Enzymology, 563, 595-622 (2015) *mtsslSuite: Probing Biomolecular Conformation by Spin-Labeling Studies*

G. Jeschke, Protein. Sci. 27, 76-85 (2018)
MMM - A toolbox for integrative structure modelling

DeerAnalysis & MMM

epr.ethz.ch/software.html

deeranalysis.org

epr @eth