Pulsed Electron Electron Double Resonance

(PELDOR oder DEER)

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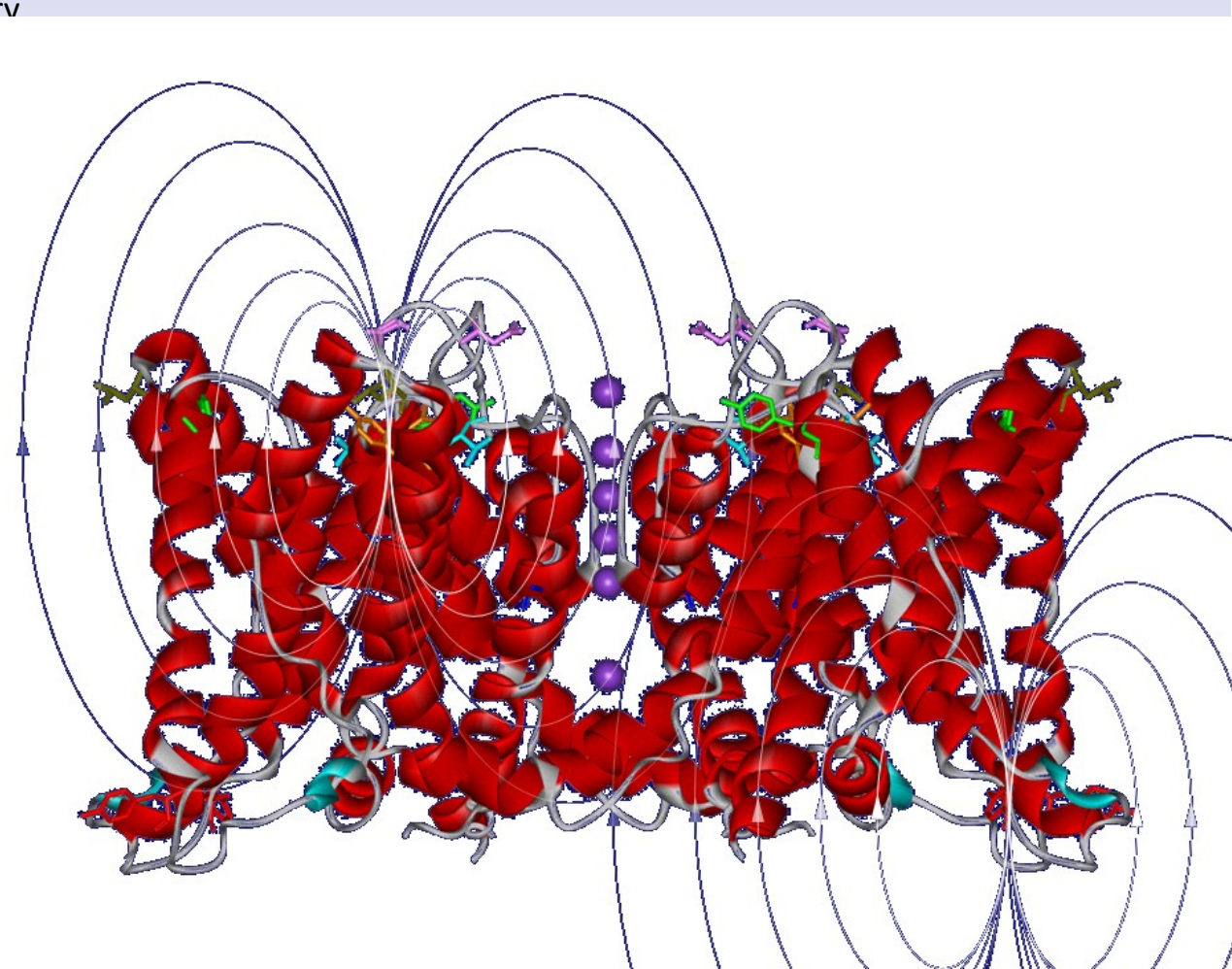
Center of Biological Magnetic Re

Goethe University Frankfurt



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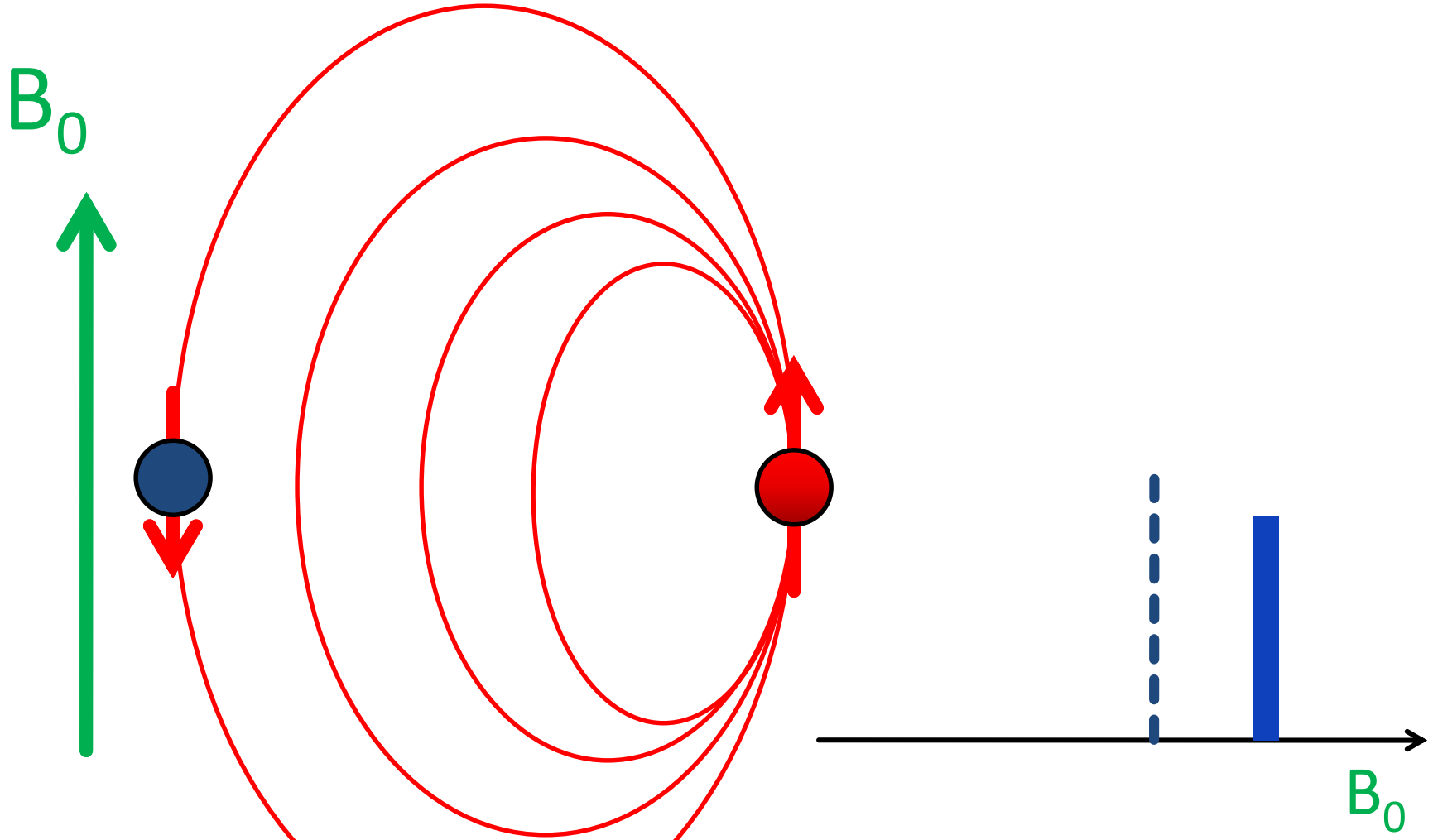
www.prisner.de

Teaching / Biophysik-Kurs

Magnetic dipole-dipole interaction between two spin labels



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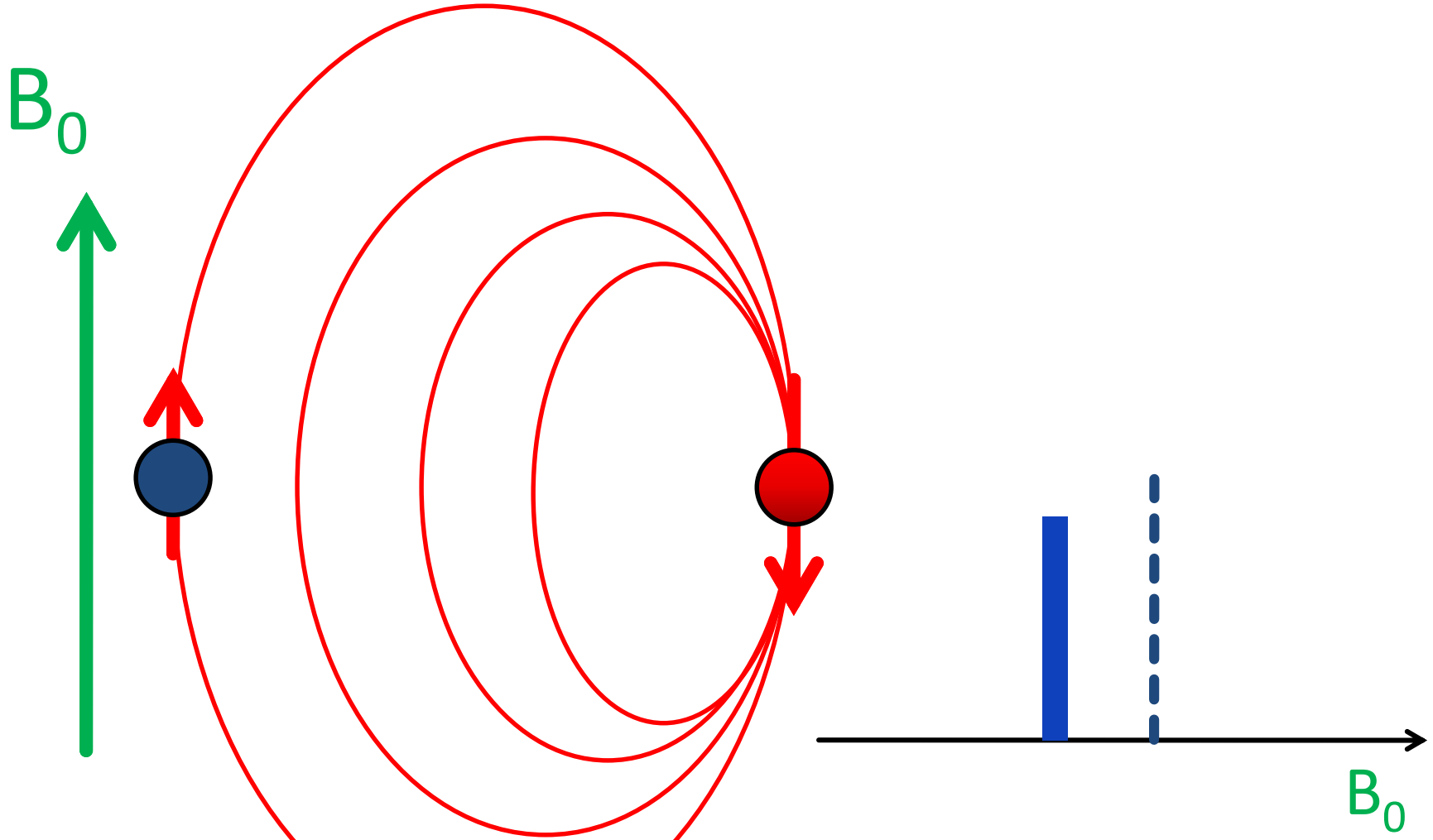

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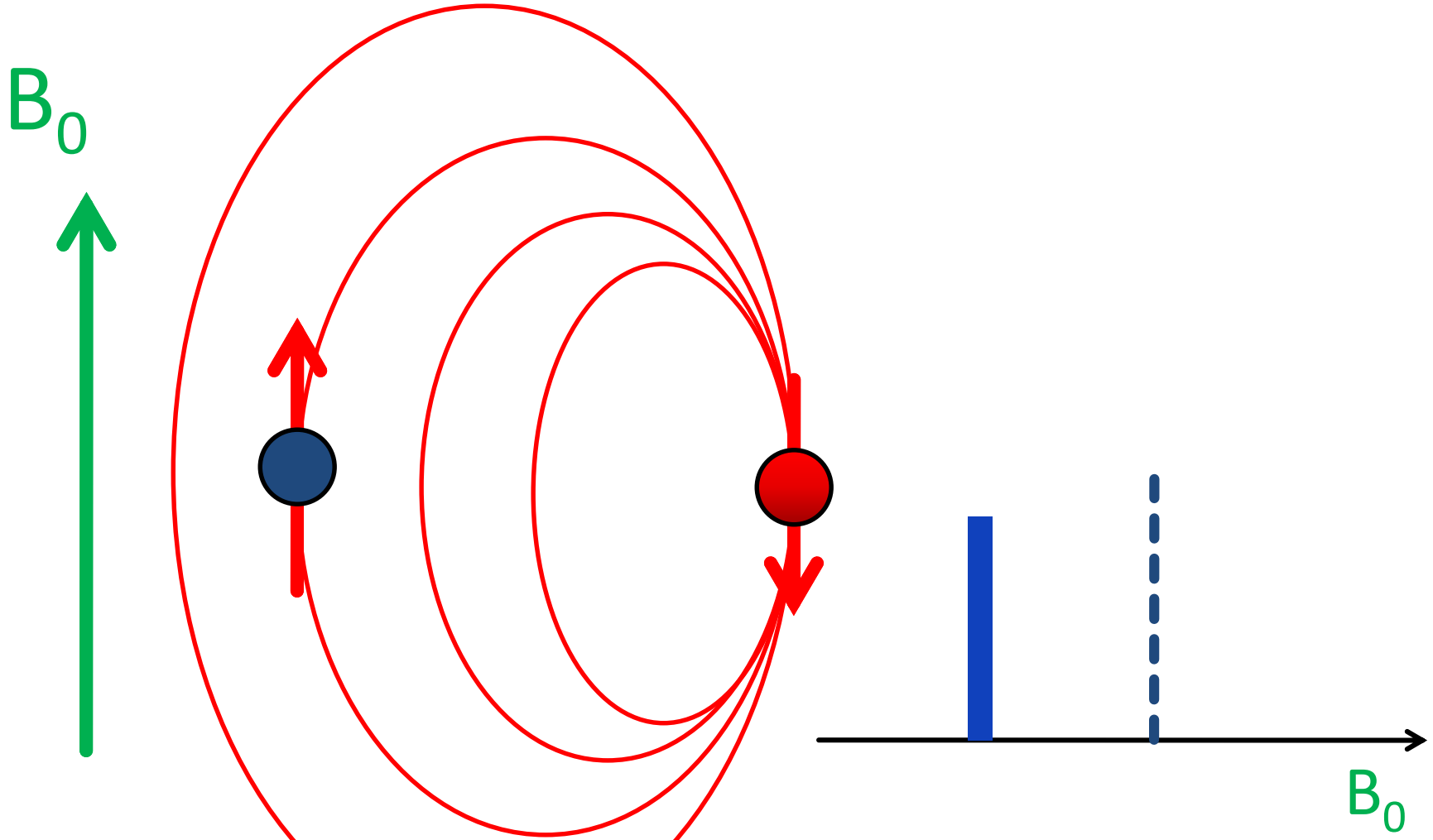
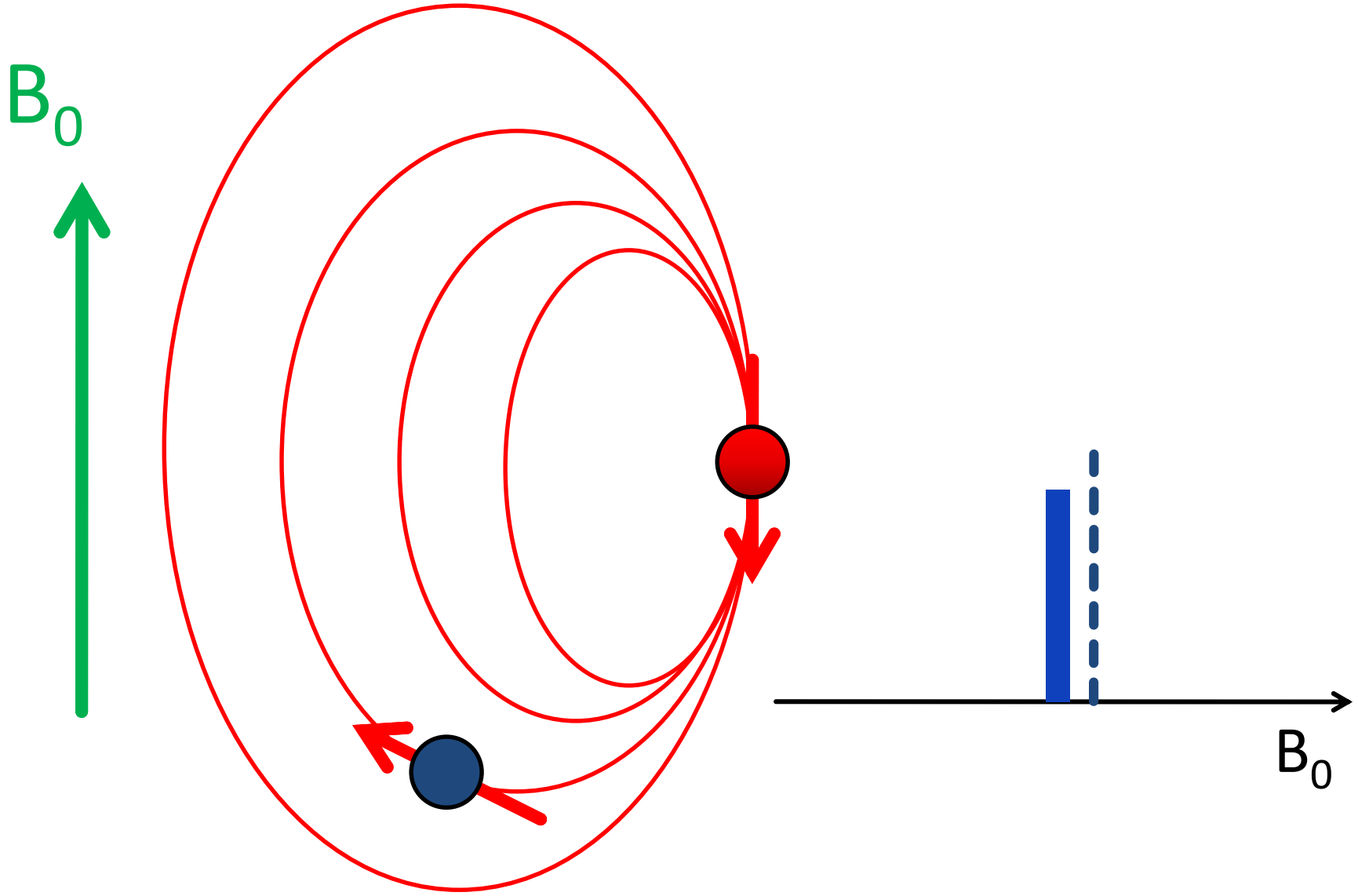
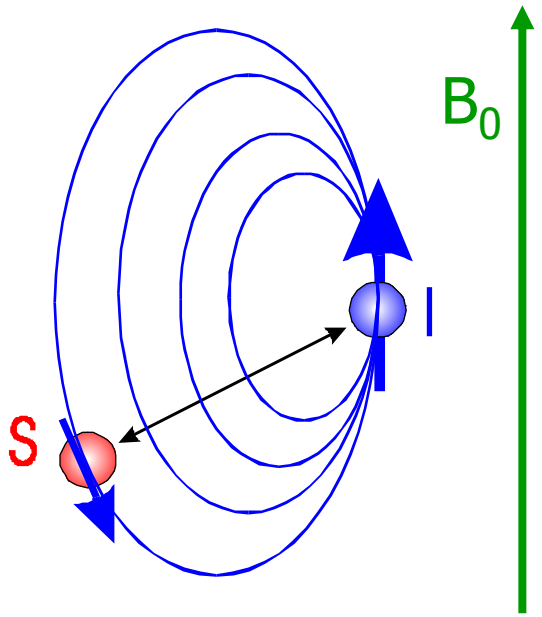
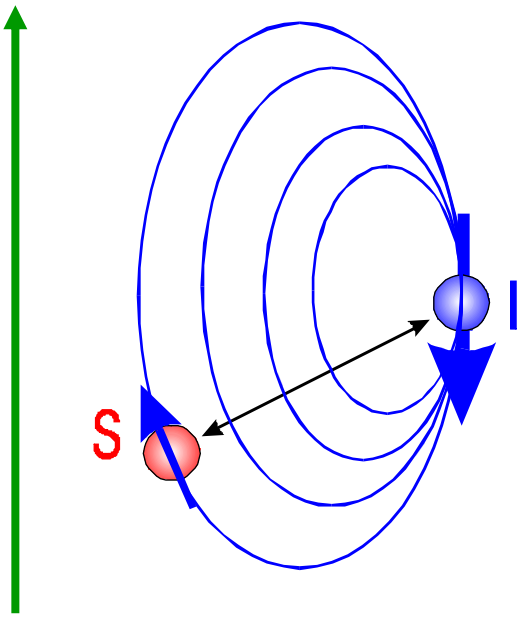


Magnetic dipole-dipole interaction between two spin labels

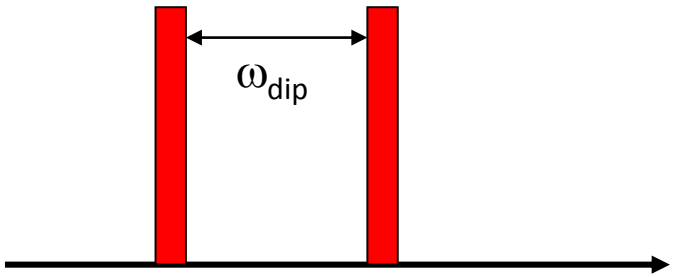


Magnetic Dipol-Dipol Interaction leads to splitting





Dipolar field of spin I at spin S leads to splitting of resonance line



$$\omega_{dip} = \frac{\gamma_s \mu_I}{r_{SI}^3}\left(1-3\cos^2(\theta)\right) \propto \frac{g_s^{eff} g_I^{eff}}{r_{SI}^3}\left(1-3\cos^2(\theta)\right)$$

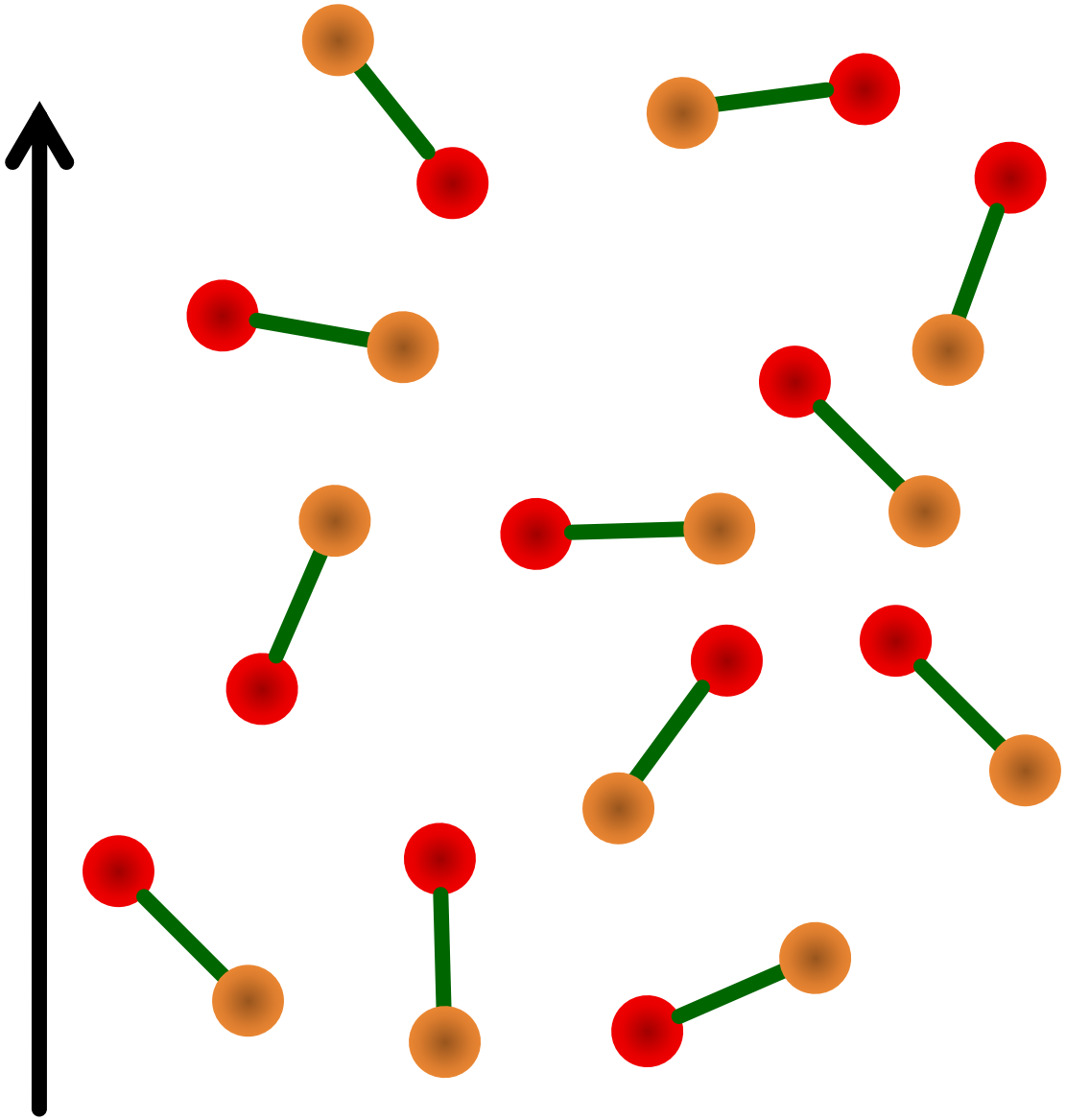
Dipolar spitting depends on:

g values

orientation θ

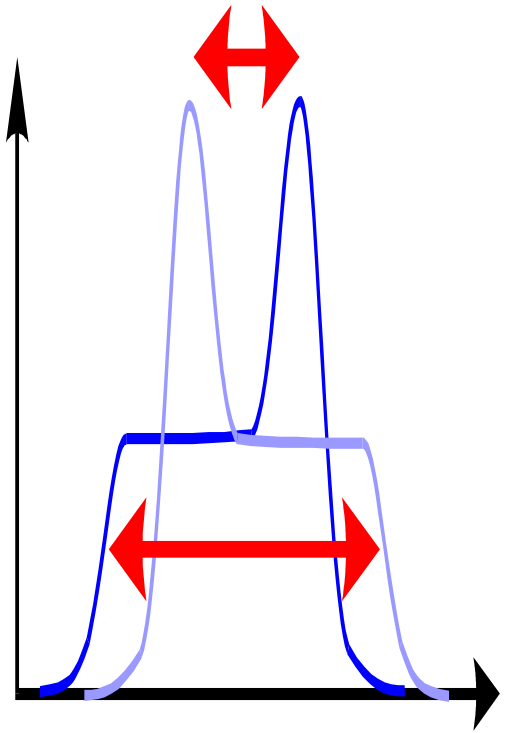
distance R

Frozen Sample with random orientation of molecules



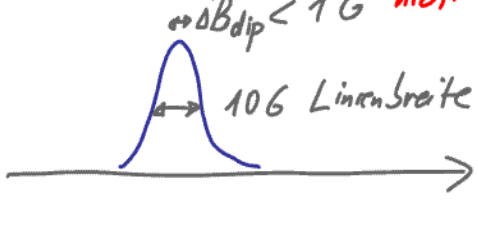
Dipolar Pake Pattern

Width < MHz

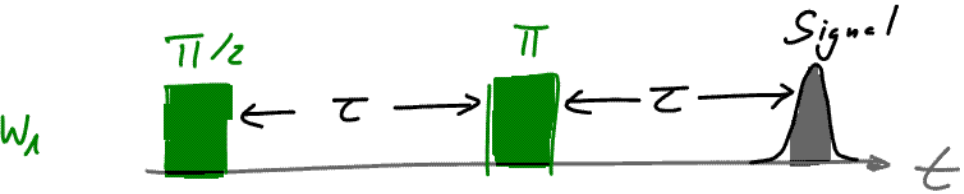


Bei $R = 3\,nm$:

$\Leftrightarrow \Delta B_{dip} < 1\,G$ nicht detektierbar !

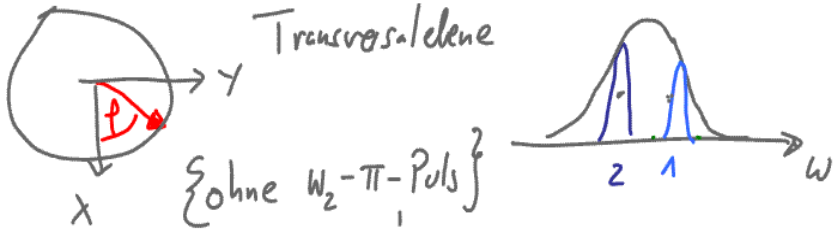


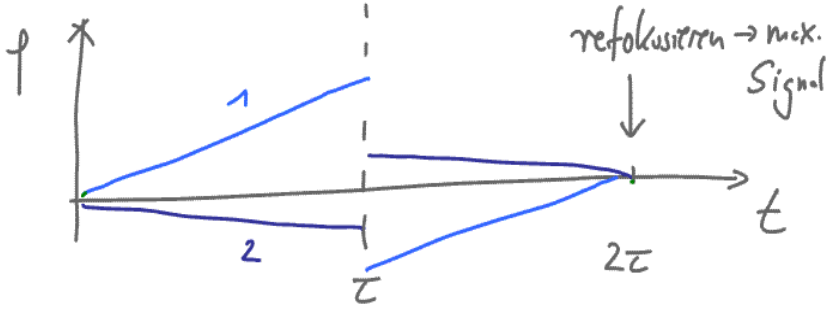
Idee 1: Benutze Pulsmethoden um inhomogene Linienbreite zu verschmälern !

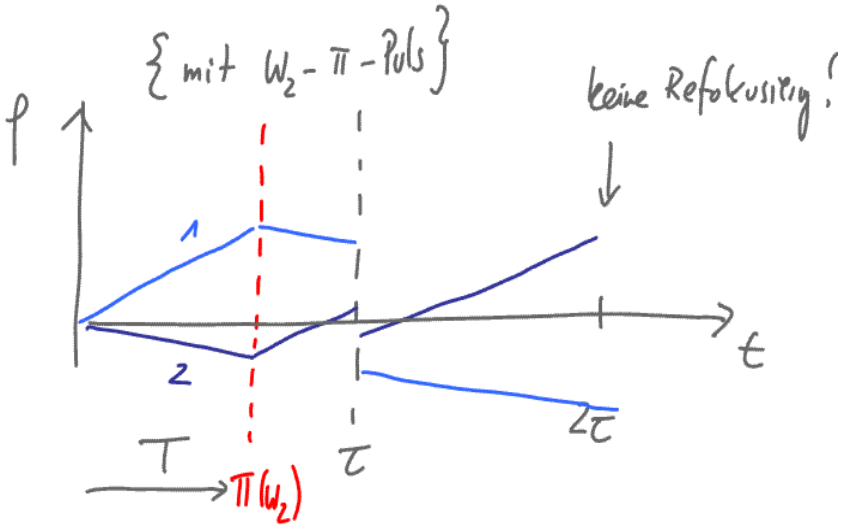


Problem: Refokussiert alle inhom. WW – also auch $\mathcal{H}_{dd}$!

Idee 2: Invertiere selektiv Spin 2 während dem Experiment → vermeidet refokussierung von dipolarer WW



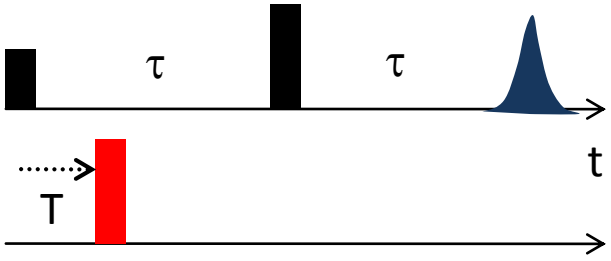


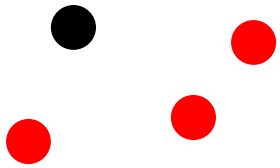


$$S(T) = S_{max} \cdot \cos(W_{dd} T)$$

Pulsed Electron Electron Double Resonance (PELDOR)

3-Pulse PELDOR Sequence



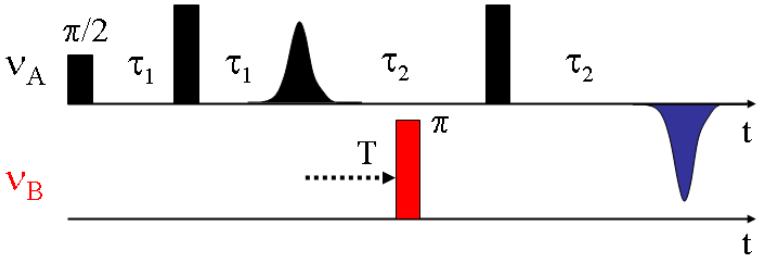


Milov, Salikov, Shirov, *Fiz.Tverd. Tela* 23, 975 (1981)
Milov, Ponomarev, Tsvetkov, *Chem. Phys. Lett*. 110, 67 (1984)

DEER (Double Electron-Electron Resonance)

Larsen, Singel, J. Chem. Phys. 98, 5134 460 (1993)

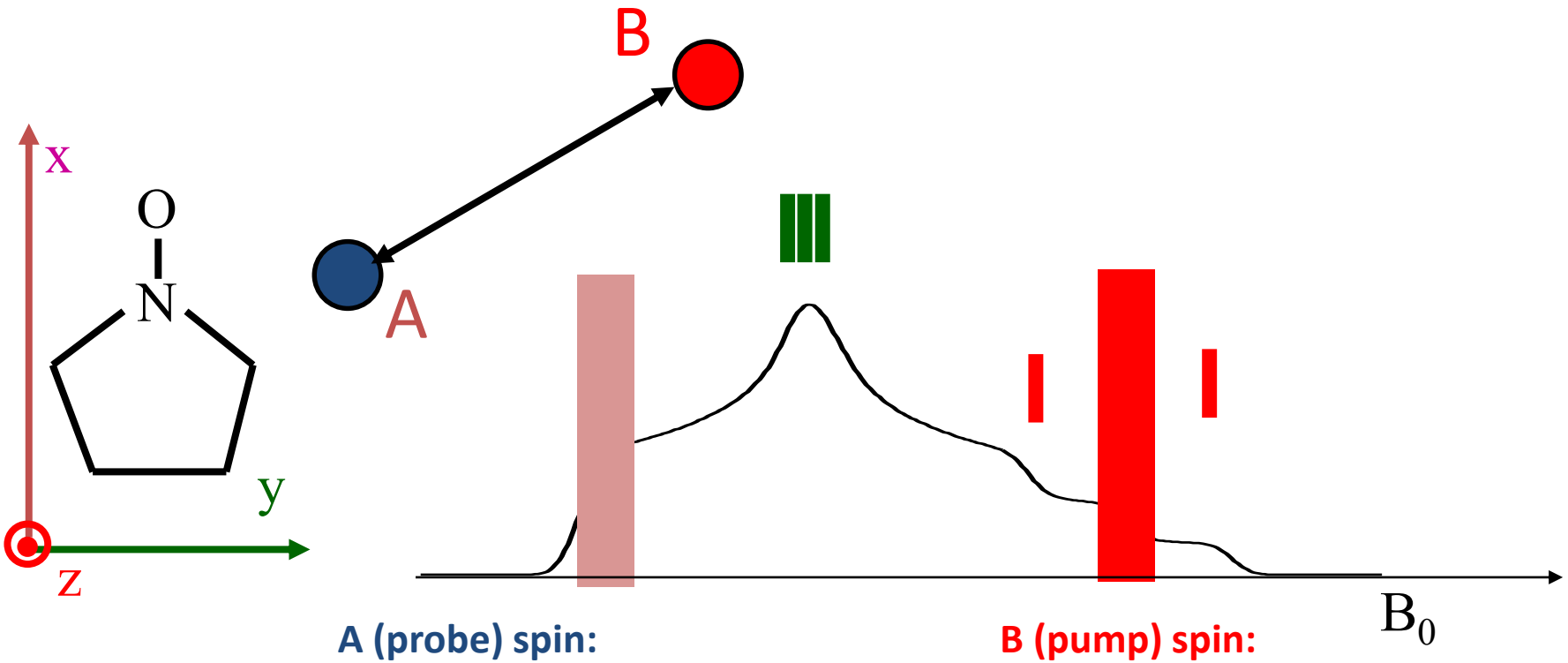
4-Pulse DEER Sequence



Martin, Pannier, Diederich, Gramlich, Hubrich, Spiess, *Angew. Chem*. 37, 2833 (1998)

Pannier, Veit, Godt, Jeschke, Spiess, *J. Magn. Reson*. 142, 331 (2000).

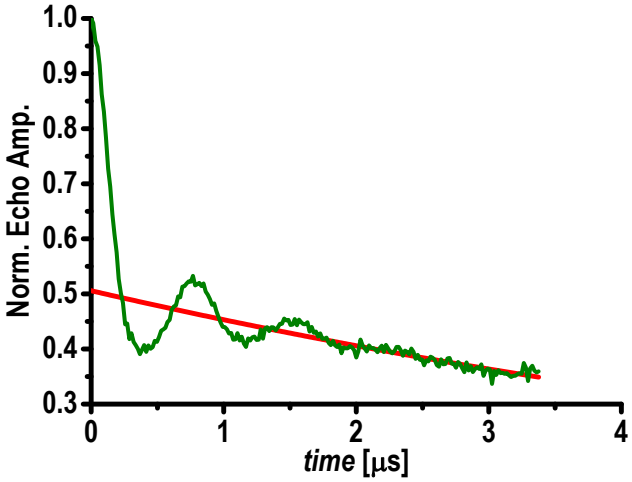
How to define A and B spins ?

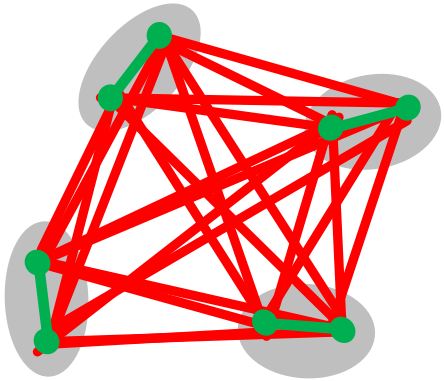


A (probe) spin:
X parallel to magnetic field

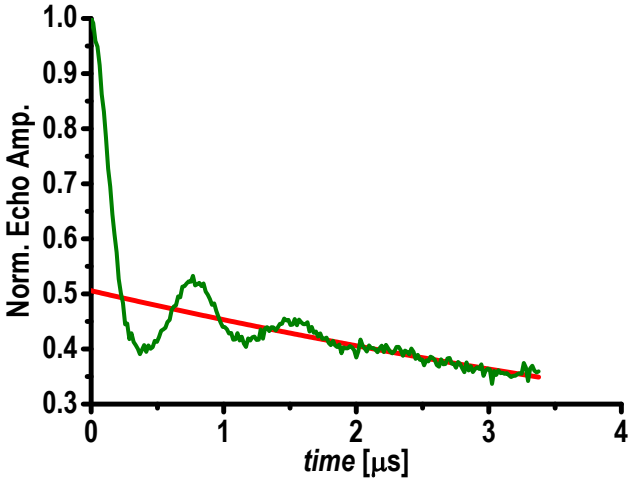
B (pump) spin:
Z parallel to magnetic field
and $m_I=0$

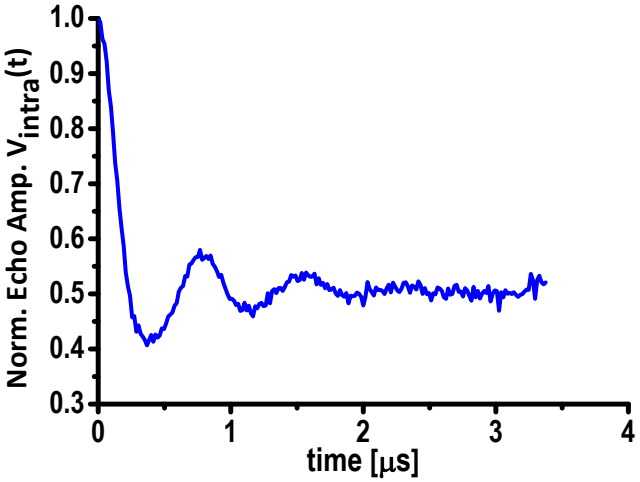
PELDOR – Data Analysis

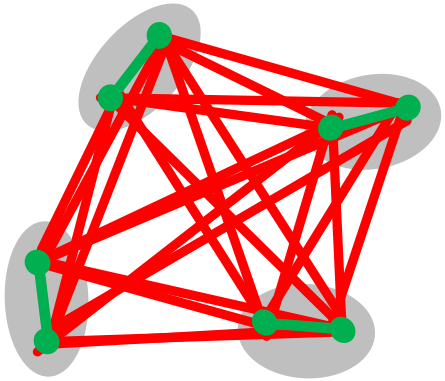


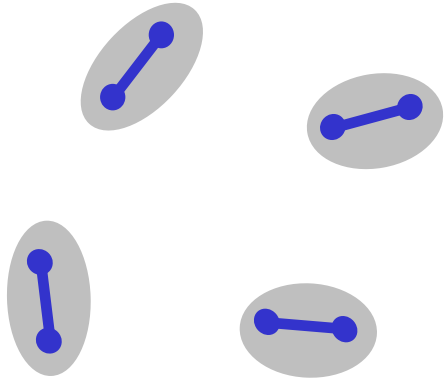


PELDOR – Data Analysis

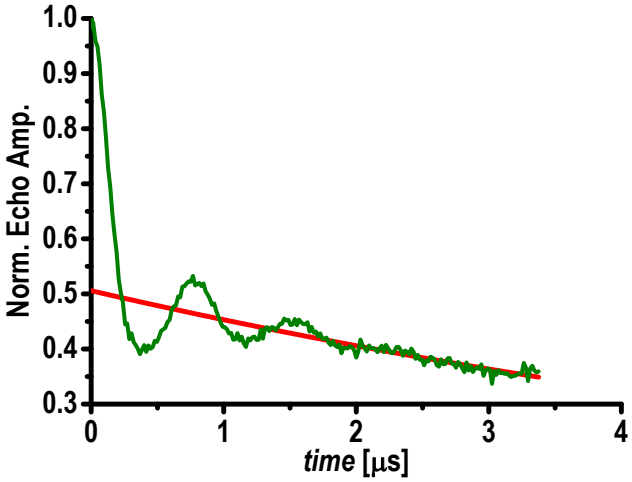


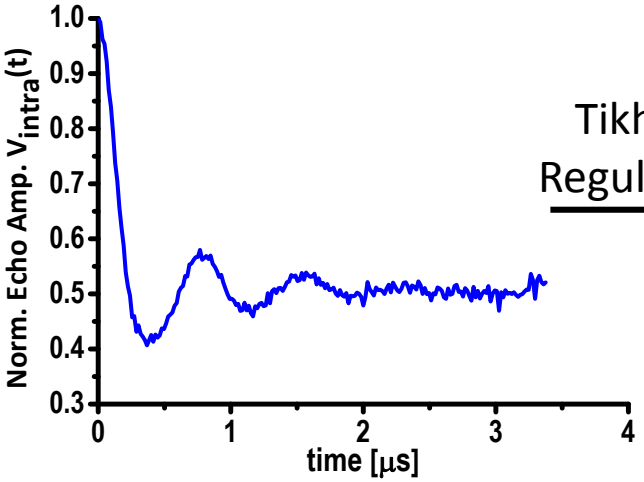




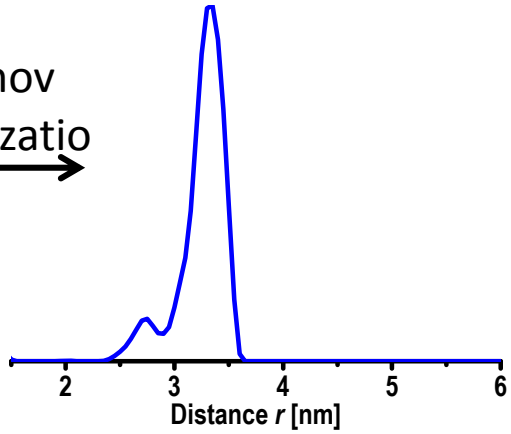


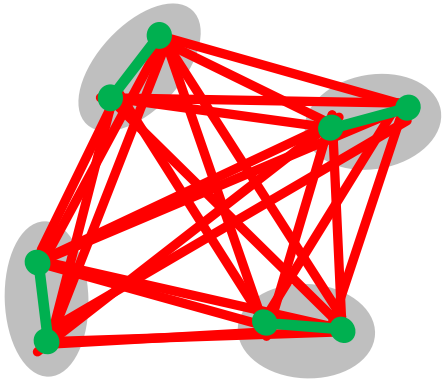
PELDOR – Data Analysis

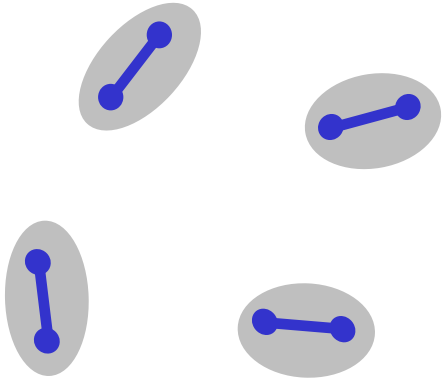




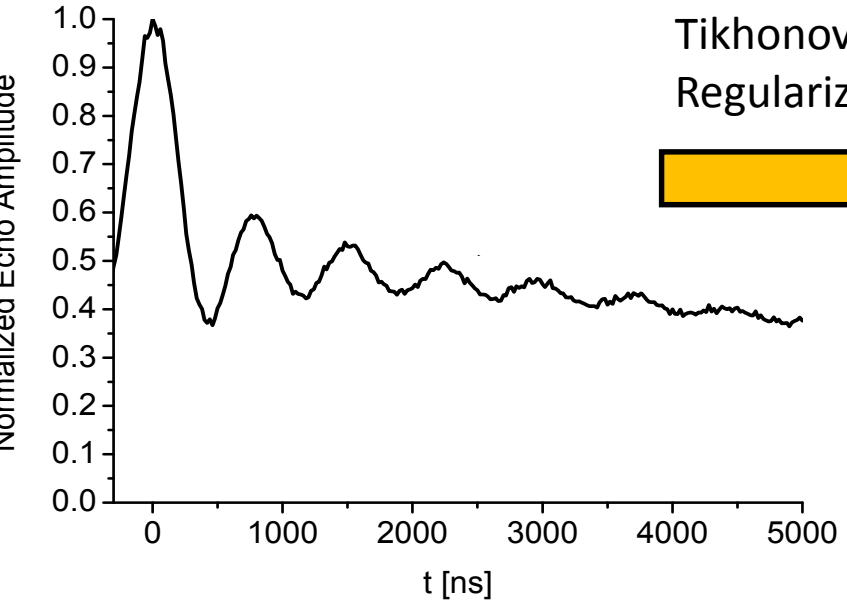
Tikhonov Regularization



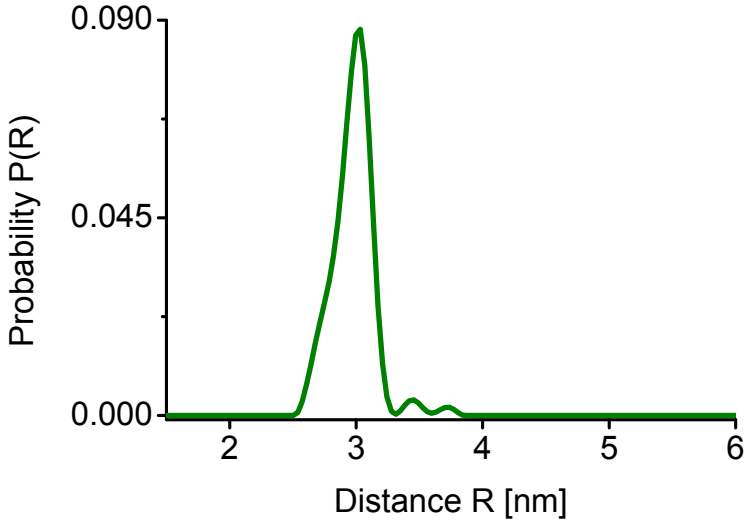




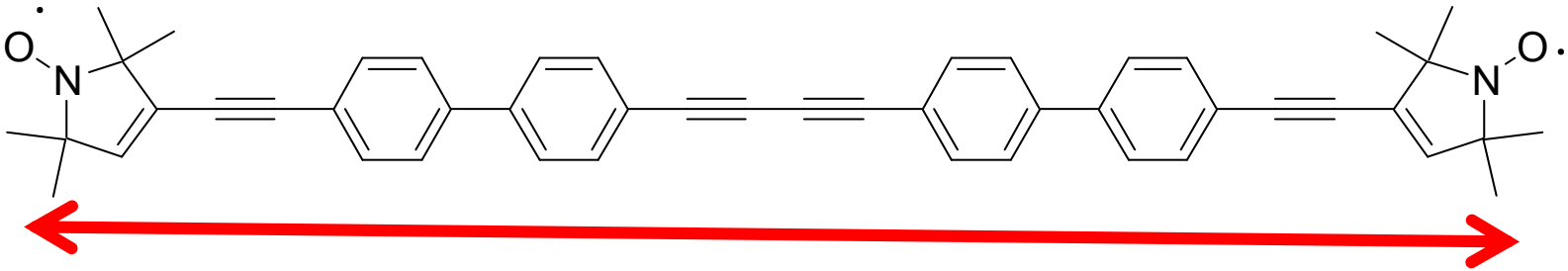
Extraction of Distance Distribution Function



Tikhonov Regularization



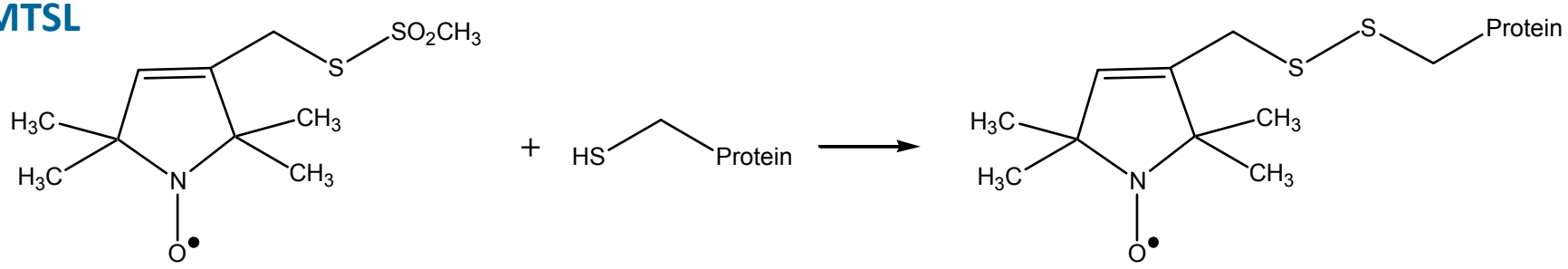
- Fit of Pake-Patterns to Time trace
- Assumes R ≥ 0 P(R)≥0, smooth function



Spin Labeling of Proteins

Methanthiosulfonate:

MTSL

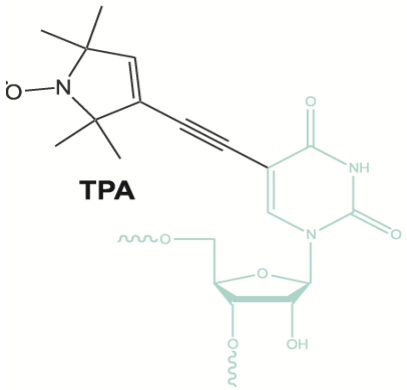


Auch:
Gd-Komplexe als Spin Labels (Säure-Resistent für in-cell Anwendungen)
Trityl-Radikale als Spin Label (eventuell für Experimente bei RT)

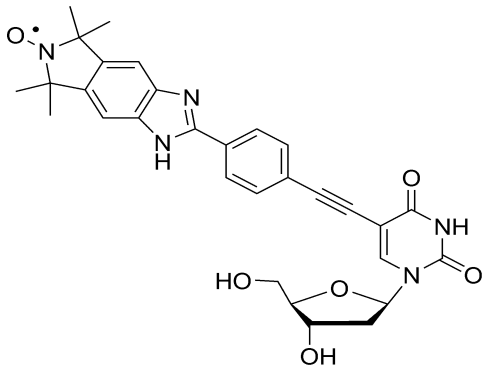
Oder:
Intrinsische Kofaktoren in Proteinen die sich reduzieren/oxidieren lassen oder per se paramagnetisch sind
(Ni, Cu, Mn, FeS, heme, aminosäure radikale, Flavine ....)

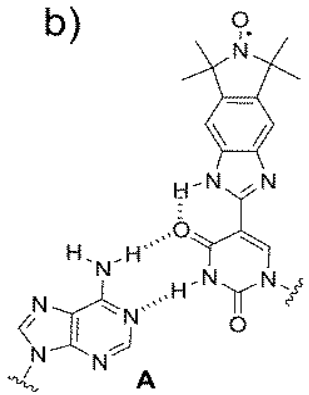
Spin labels for Nucleic Acids

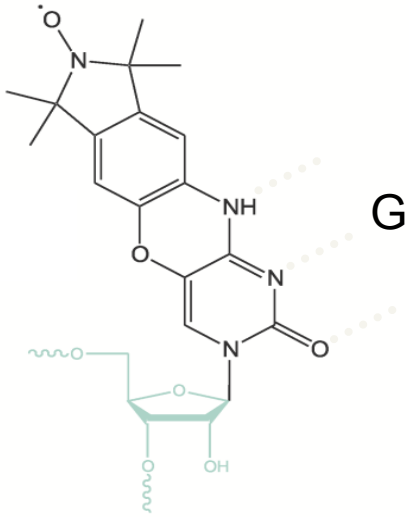
Joachim Engels (Uni Frankfurt)



Snorri Sigurdsson (University of Iceland)







Rotate around triple bond

Rigid in double stranded structures

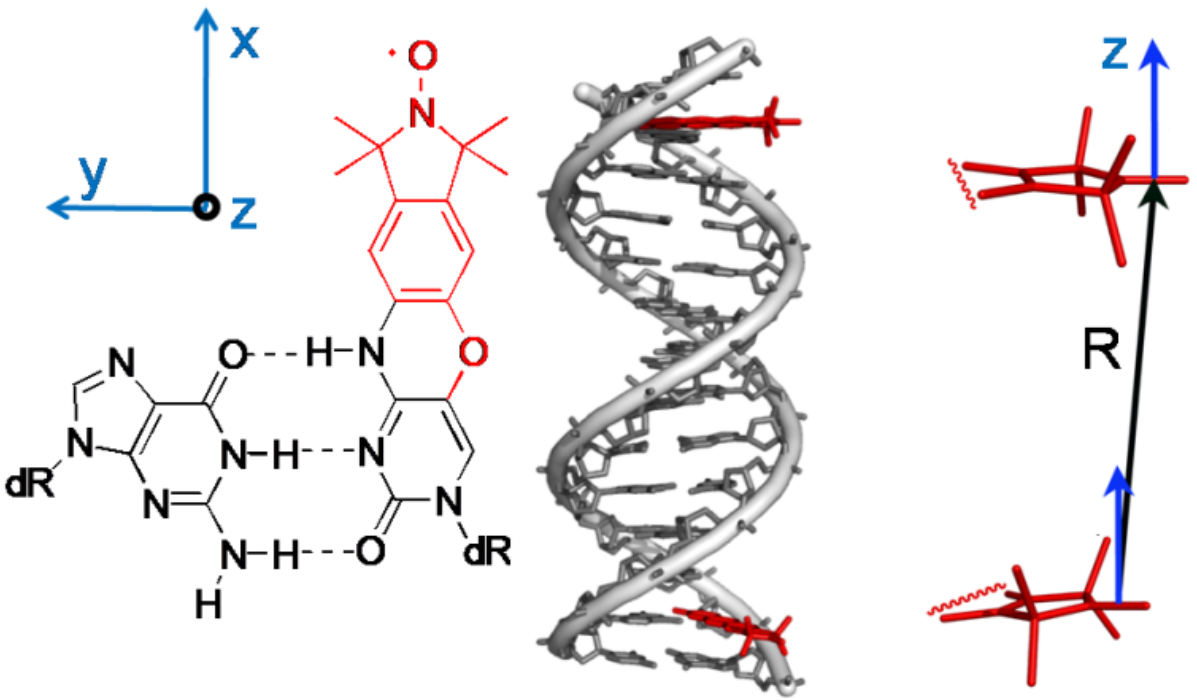
Nature Protocoll (2007)
NAR (2007)

Chem. Eur. J. (2014)

Angewandte (2009)
JACS (2011)

- All nucleotides in DNA and RNA can be spin-labeled
- Different degrees of rotational flexibility of spin label
- Different orientation with respect to nucleotide
- Synthesis with modified nucleobase

Incorporation of rigid spin label into NAs

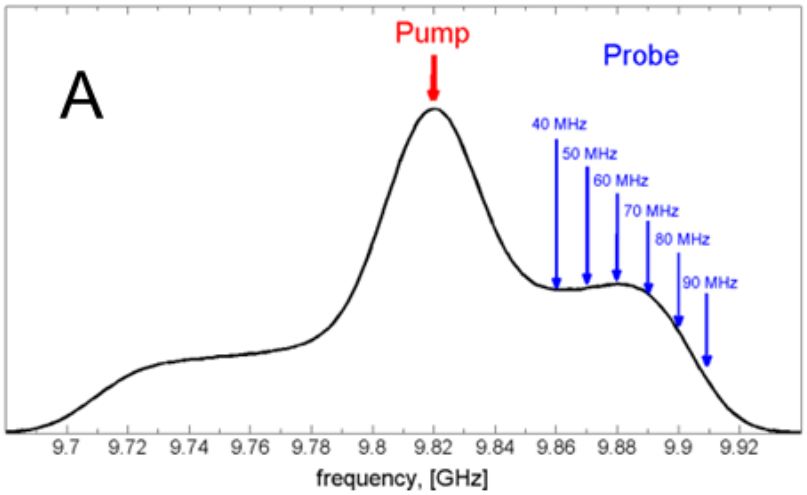


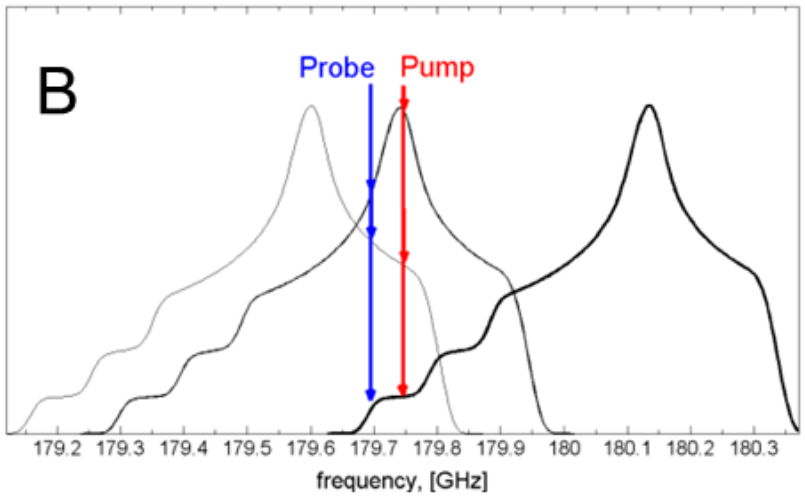
Snorri Sigurdsson (Rejkjavic)

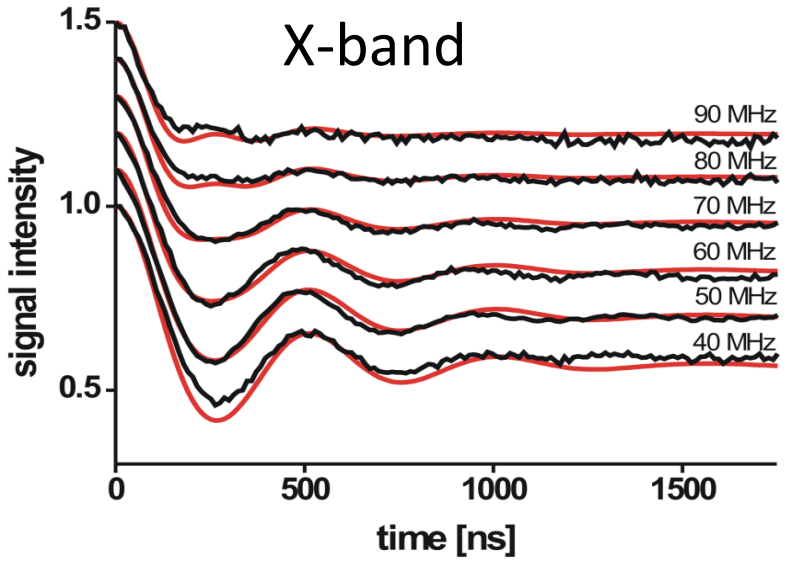
- Rigid in double stranded DNA and RNA
- Determination of orientation between two spin labels

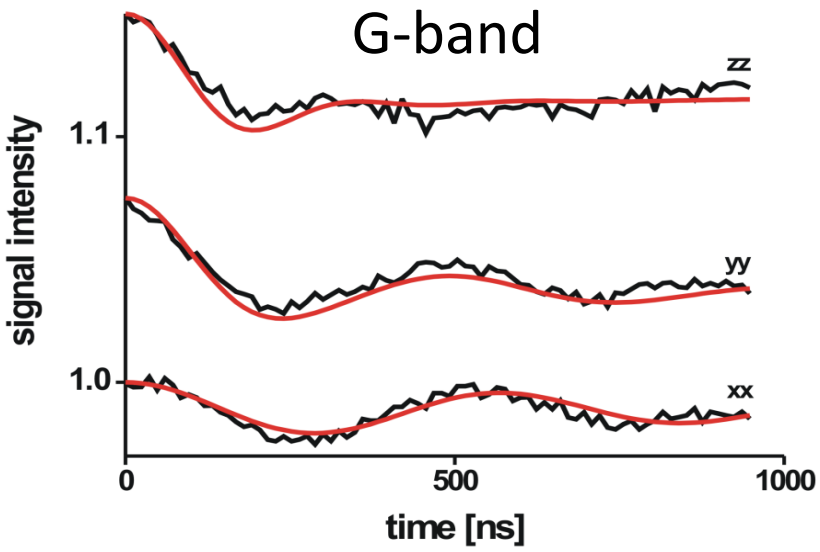
Angewandte 2010

Multi-Frequency / Multi-Field PELDOR





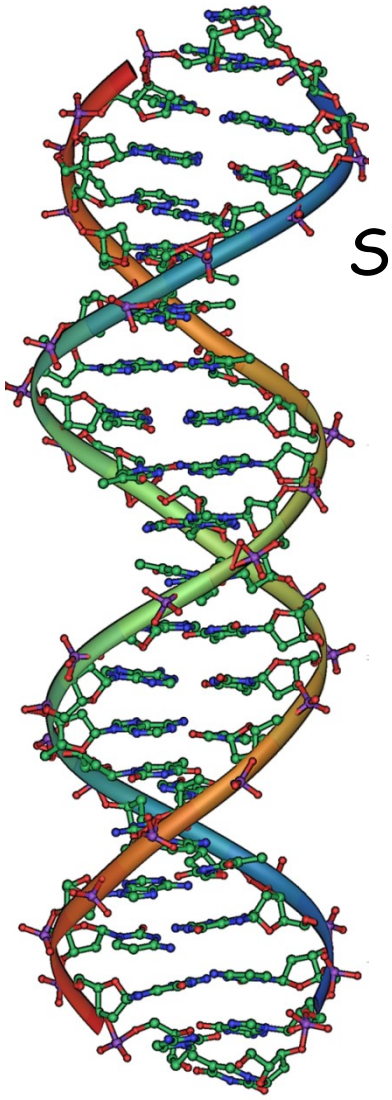




Mol. Phys. 2008, PCCP 2010

Simultaneous fit of all PELDOR traces

Investigation of dynamics of dsDNA



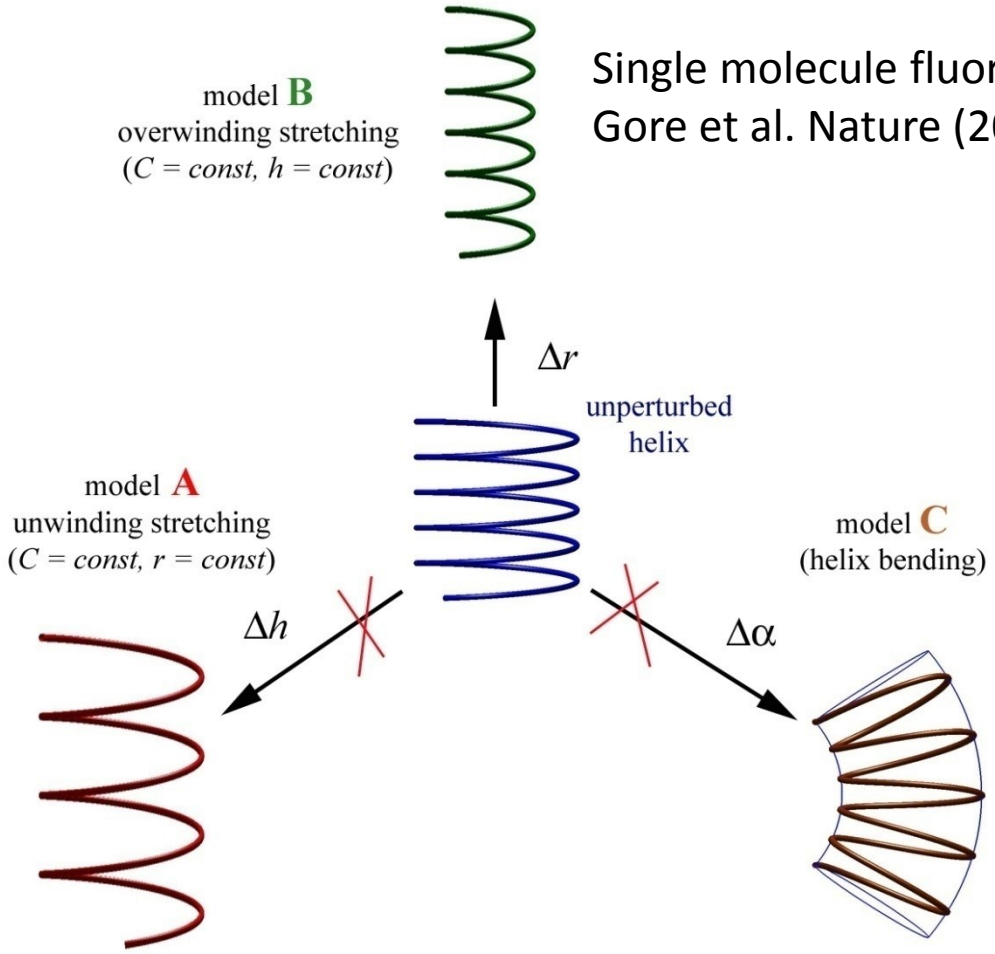
Stretching

$\Delta x^2 \sim L$

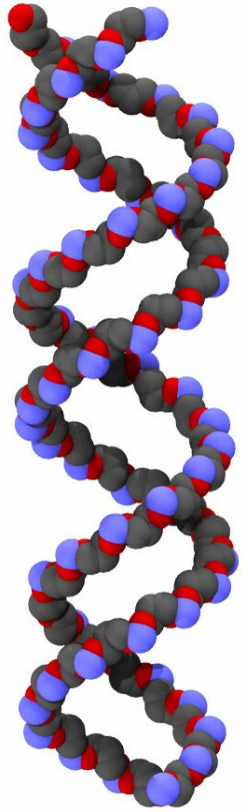
Bending

Twisting

Models for conformational dynamics of dsDNA



Single molecule fluoreszenz
Gore et al. Nature (2006)



PCA Grubmüller
Bouvier & Grubmüller
Biophys. J 93, 770 (2007)

SAXSI
Mathew-Fenn et al. Science (2008)

Modeling
Becker et al. Science (2009)

Übersichtsartikel zu EPR:

Vorlesungsskript Gunnar Jeschke (ETH Zürich / MPI Mainz)

Jeschke/Schweiger Principles of Pulse EPR Oxford University Press

Lund/Shiotani/Shimada Principles and Applicatons of EPR Spectroscopy Springer

Übersichtsartikel zu PELDOR /DEER:

Schiemann, O. and Prisner, T.F. (2007) Long-range distance determinations in biomacromolecules by EPR spectroscopy. *Quarterly Review in Biophysics*, **40**, 1-53.

Jeschke, G. (2012) *Annual Review of Physical Chemistry,* **63**, 419-446.

Übersichtsartikel zu PELDOR an RNA/DNA:

EPR Spectroscopy (Topics in Current Chemistry 321, 2012)
Springer Verlag

RNA Structure and Folding (Biophysical Techniques and Prediction Methods) 2013
De Gruyter Verlag