

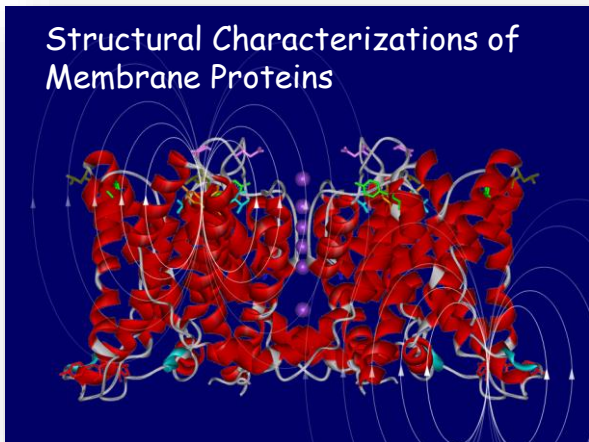
Flexibility of Nucleic Acid Structures

Investigated by Pulsed EPR

Thomas Prisner

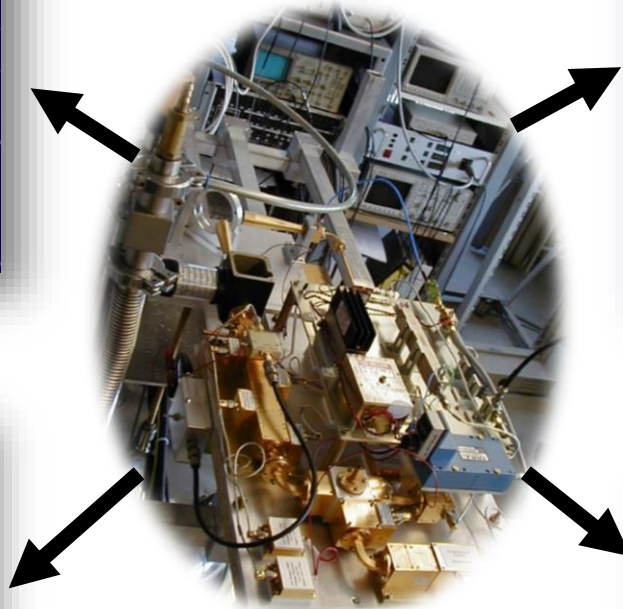


Structural Characterizations of Membrane Proteins

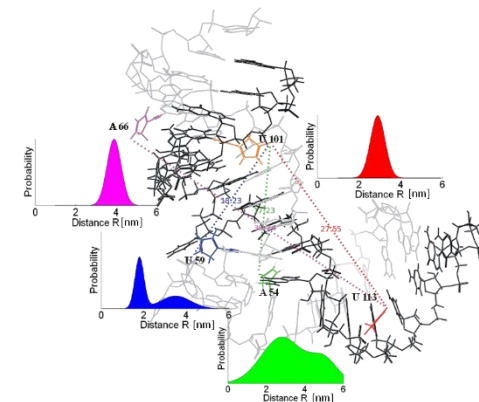


CRC 807

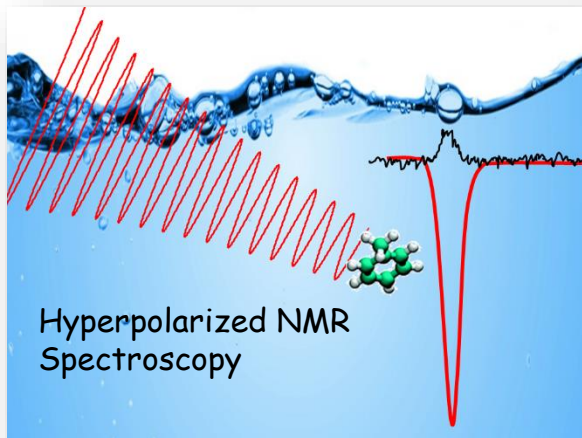
BMRZ



Conformational flexibility of NA



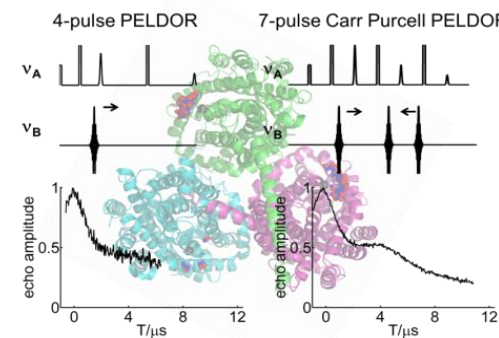
CRC 902



Hyperpolarized NMR Spectroscopy

EU, DIP, DFG

New Methods for EPR spectroscopy



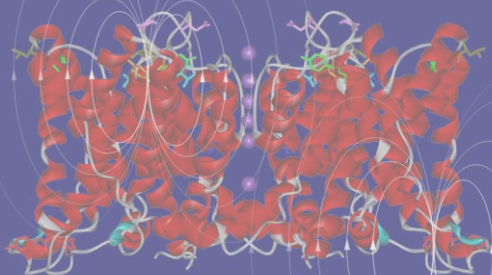
SPP 1601, DFG/ANR



DNP for MRI

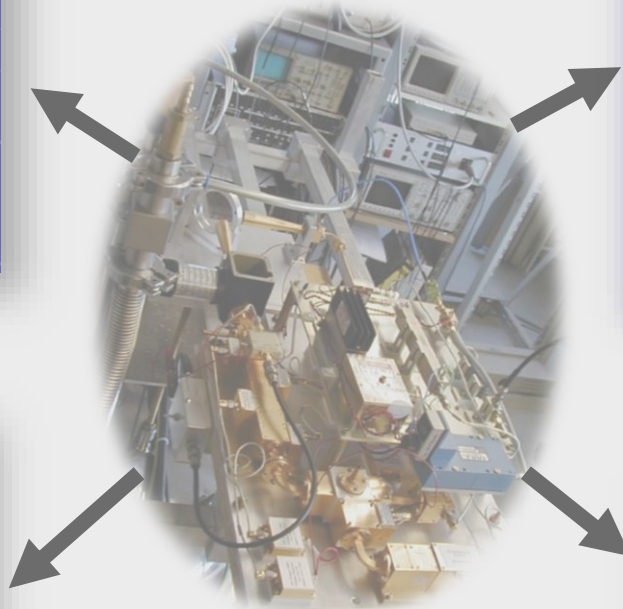
DFG

Structural Characterizations of Membrane Proteins

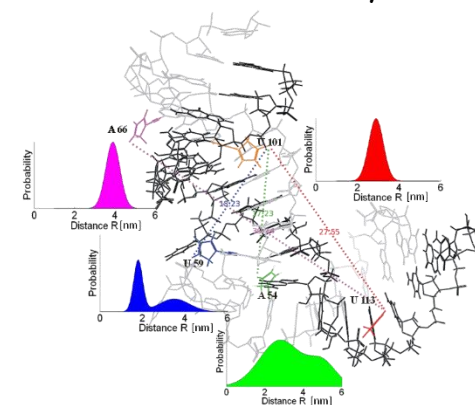


CRC 807

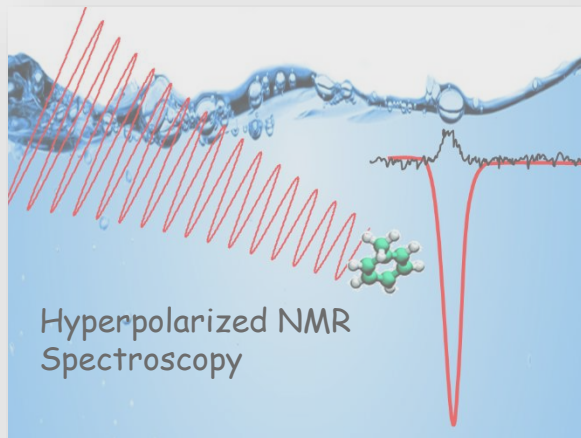
BMRZ



Conformational flexibility of NA



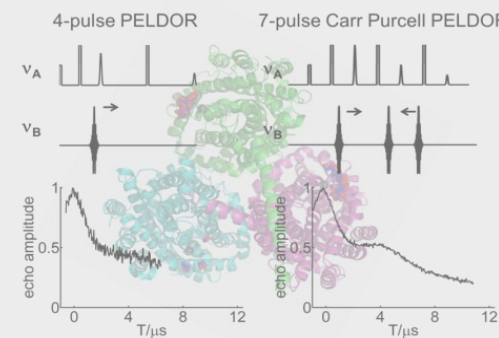
CRC 902



Hyperpolarized NMR Spectroscopy

EU, DIP, DFG

New Methods for EPR spectroscopy



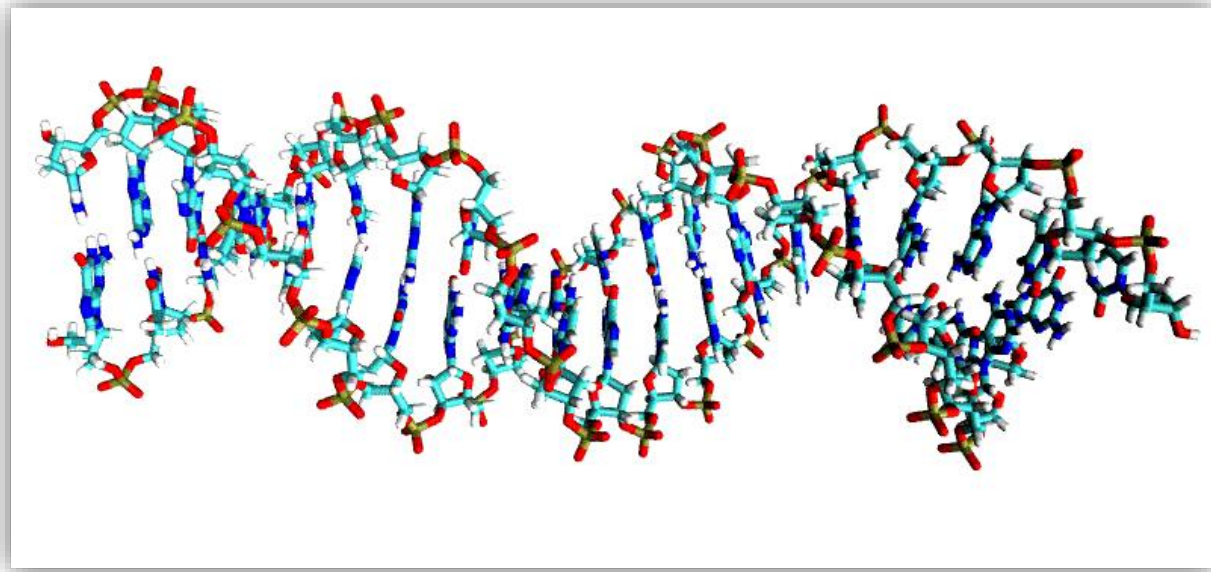
SPP 1601, DFG/ANR



DNP for MRI

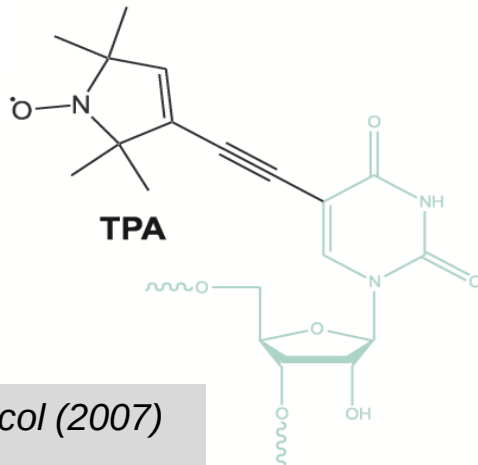
DFG

Structure and Flexibility of Nucleic Acids

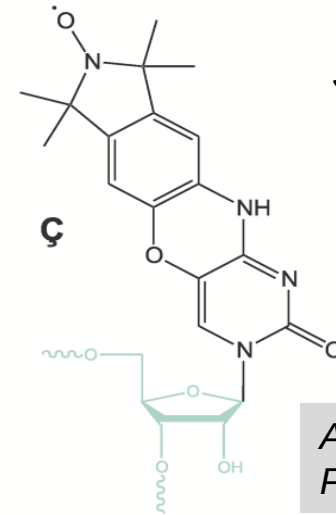


Structure and Flexibility of Nucleic Acids

Joachim Engels
(Uni Frankfurt)

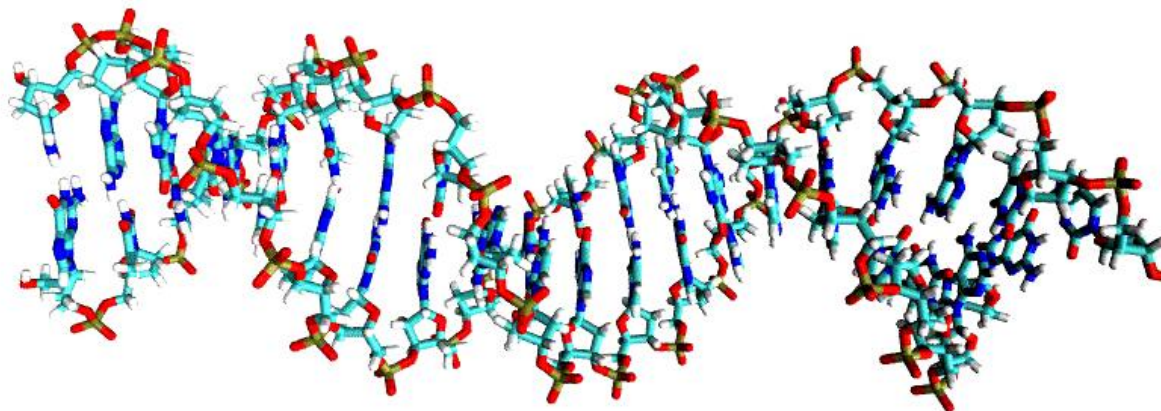


Nature Protocol (2007)
NAR (2007)

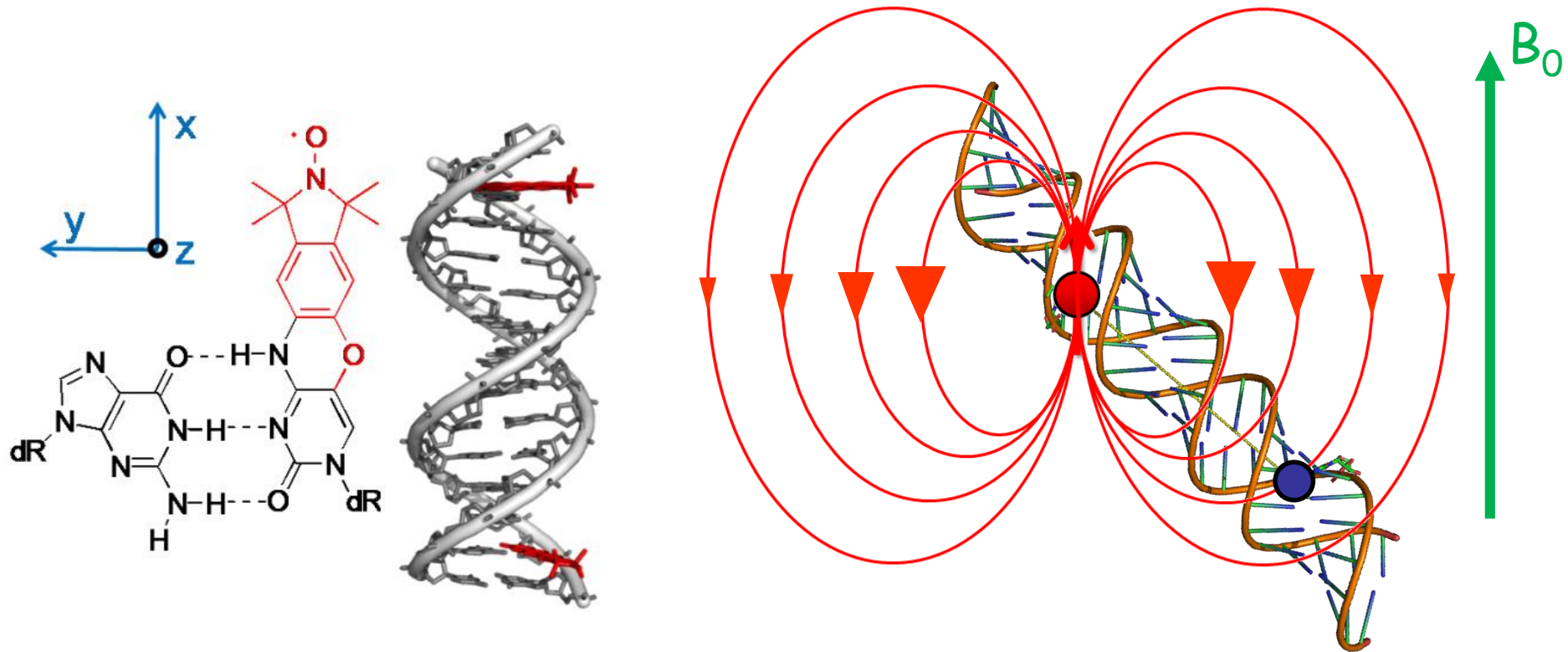


Snorri Sigurdsson
(Uni Iceland)

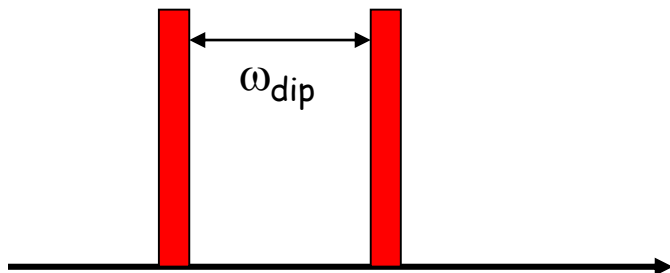
Angewandte (2009)
Phys. Rev. E (2009)



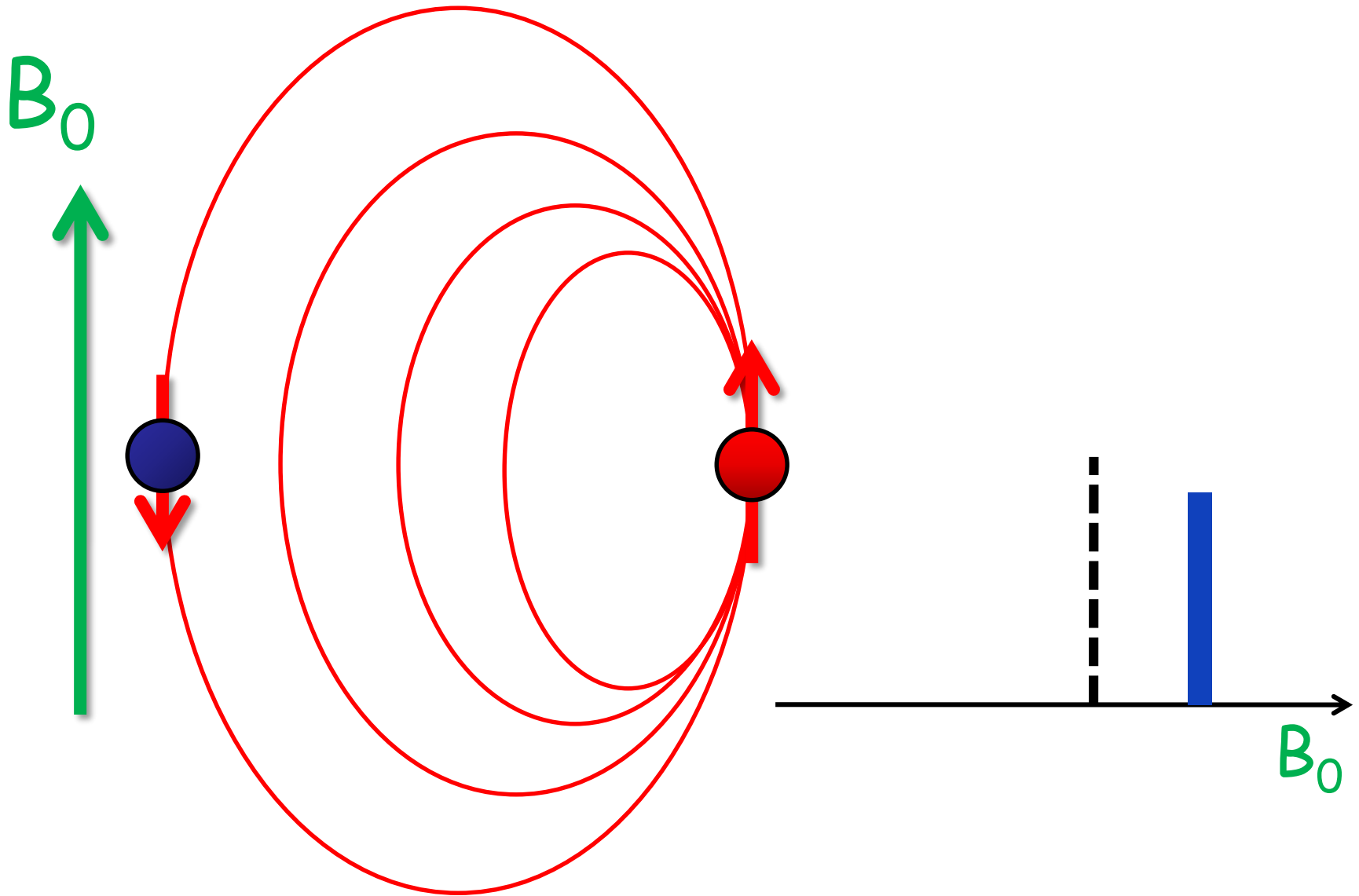
Magnetic Dipol-Dipol Interaction between spin-labels



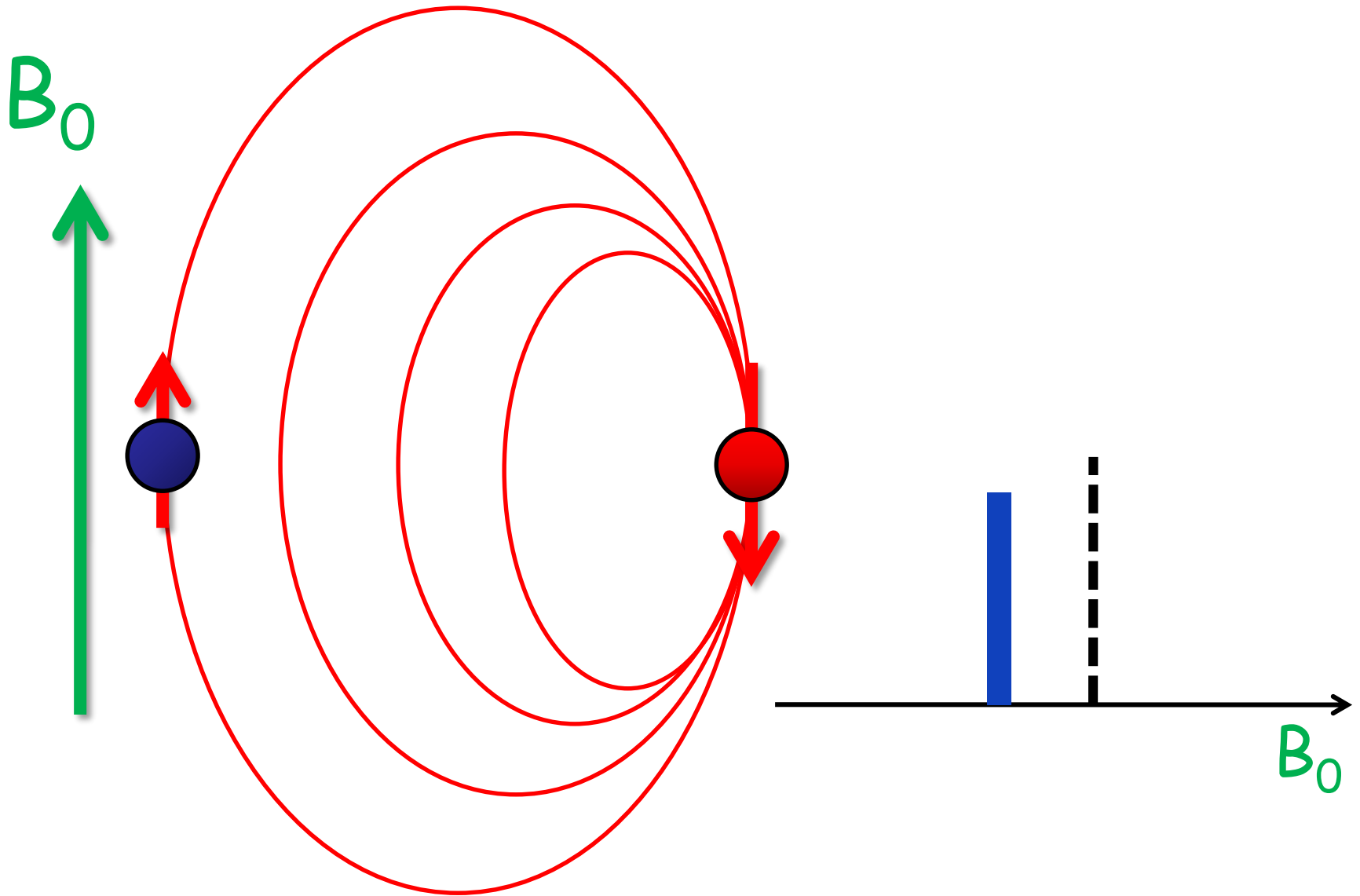
$$\omega_{\text{dip}} = \frac{\gamma_s \mu_I}{r_{SI}^3} (1 - 3 \cos^2(\theta)) \propto \frac{g_s^{\text{eff}} g_I^{\text{eff}}}{r_{SI}^3} (1 - 3 \cos^2(\theta))$$



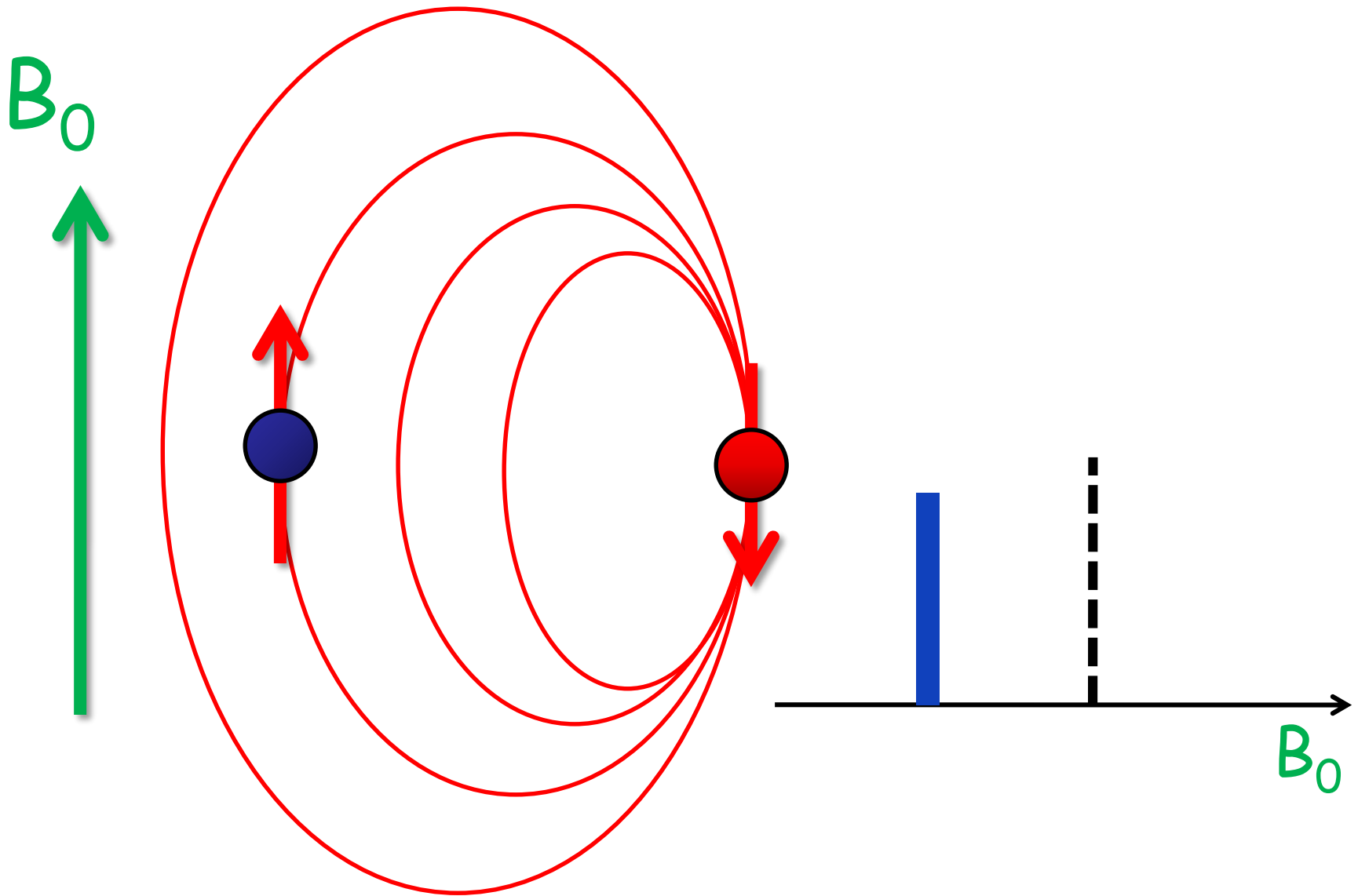
Magnetic dipole-dipole interaction between two spin labels



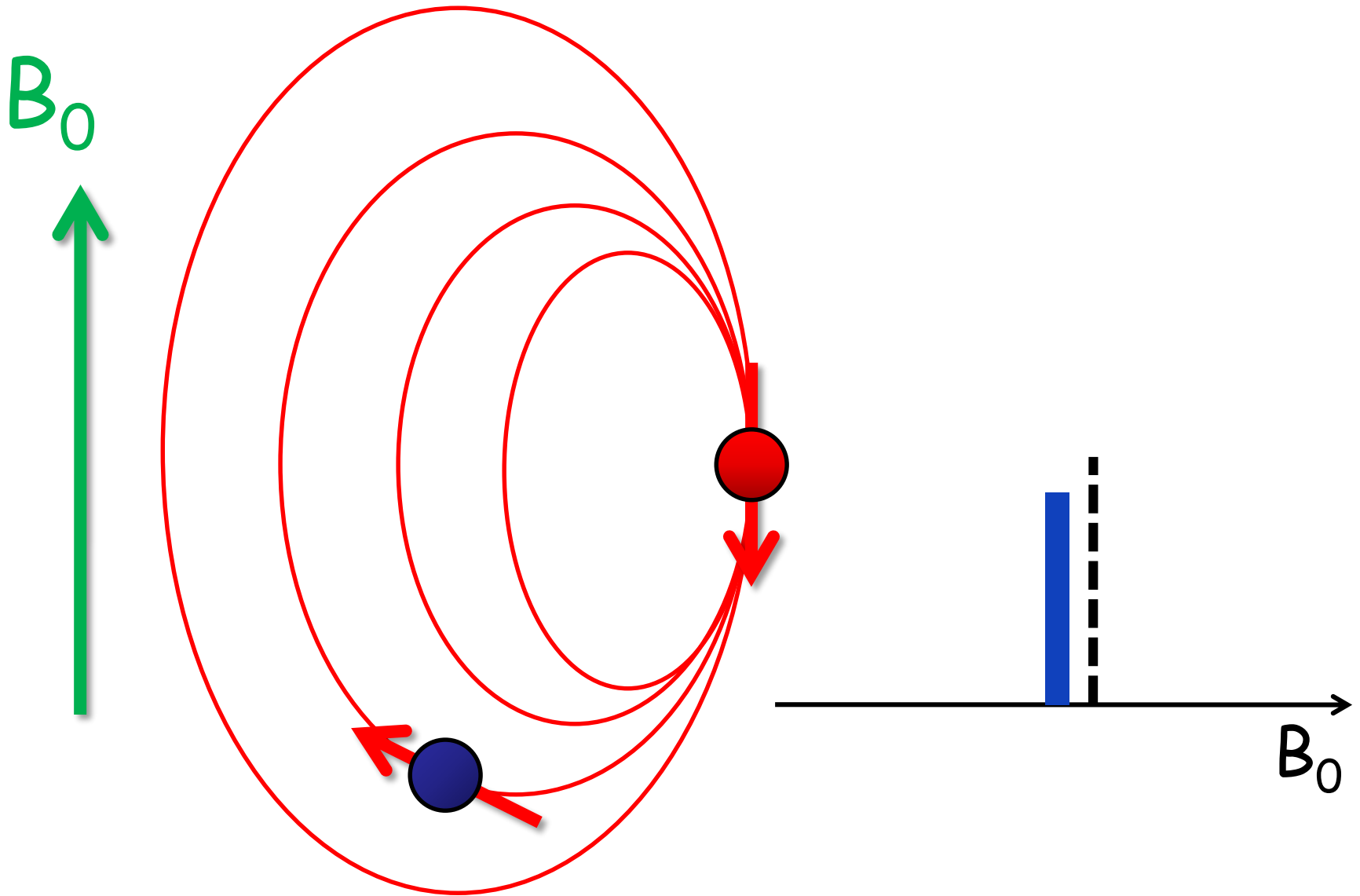
Magnetic dipole-dipole interaction between two spin labels



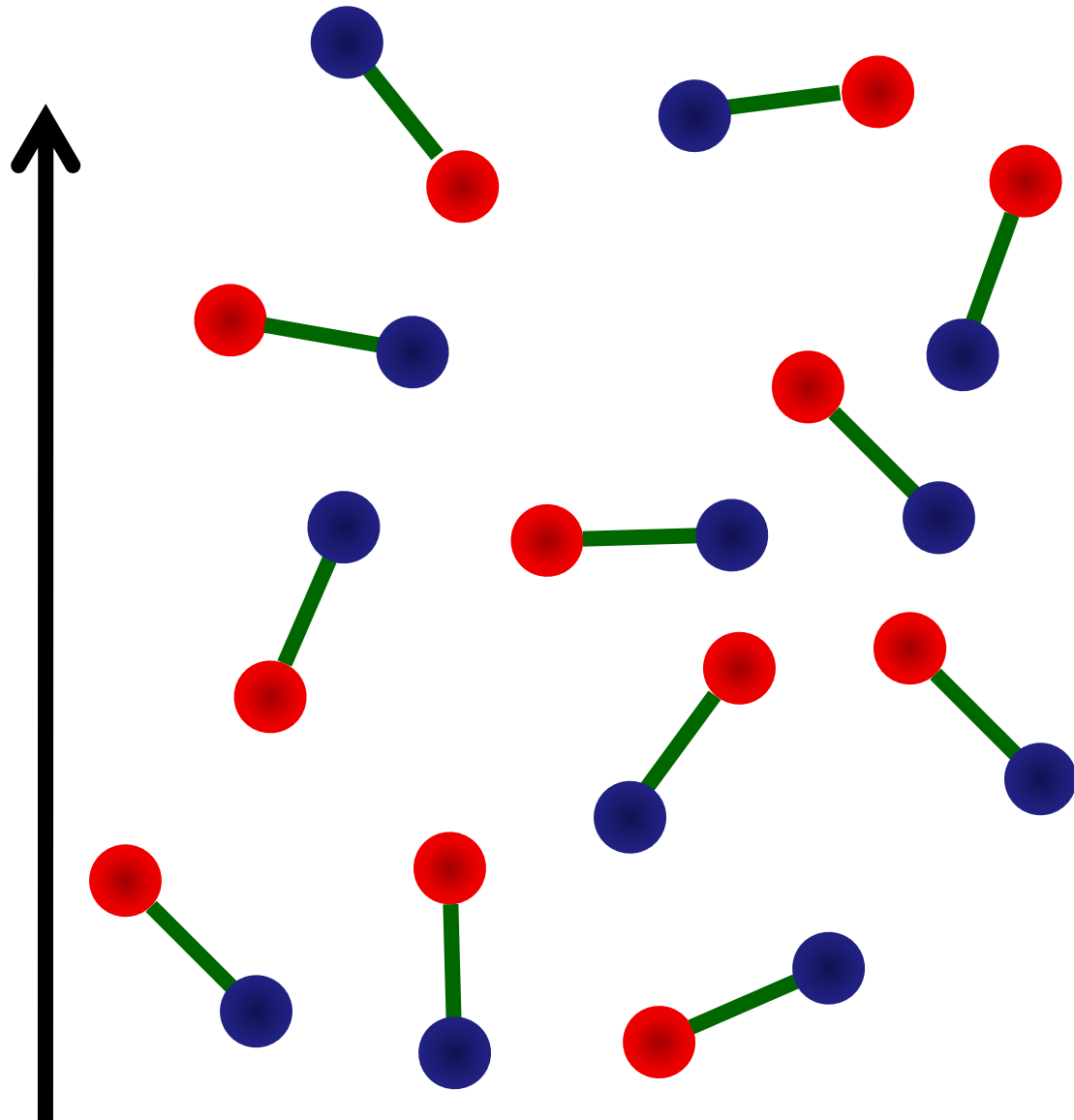
Magnetic dipole-dipole interaction between two spin labels



Magnetic dipole-dipole interaction between two spin labels

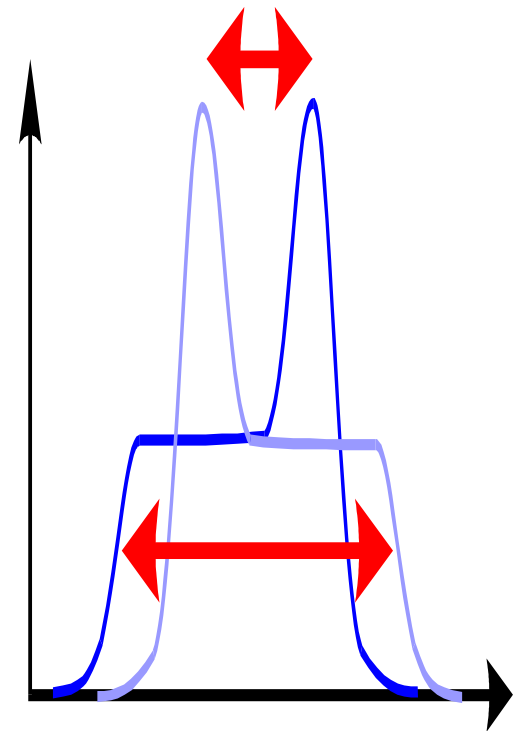


Frozen Sample with random orientation of molecules

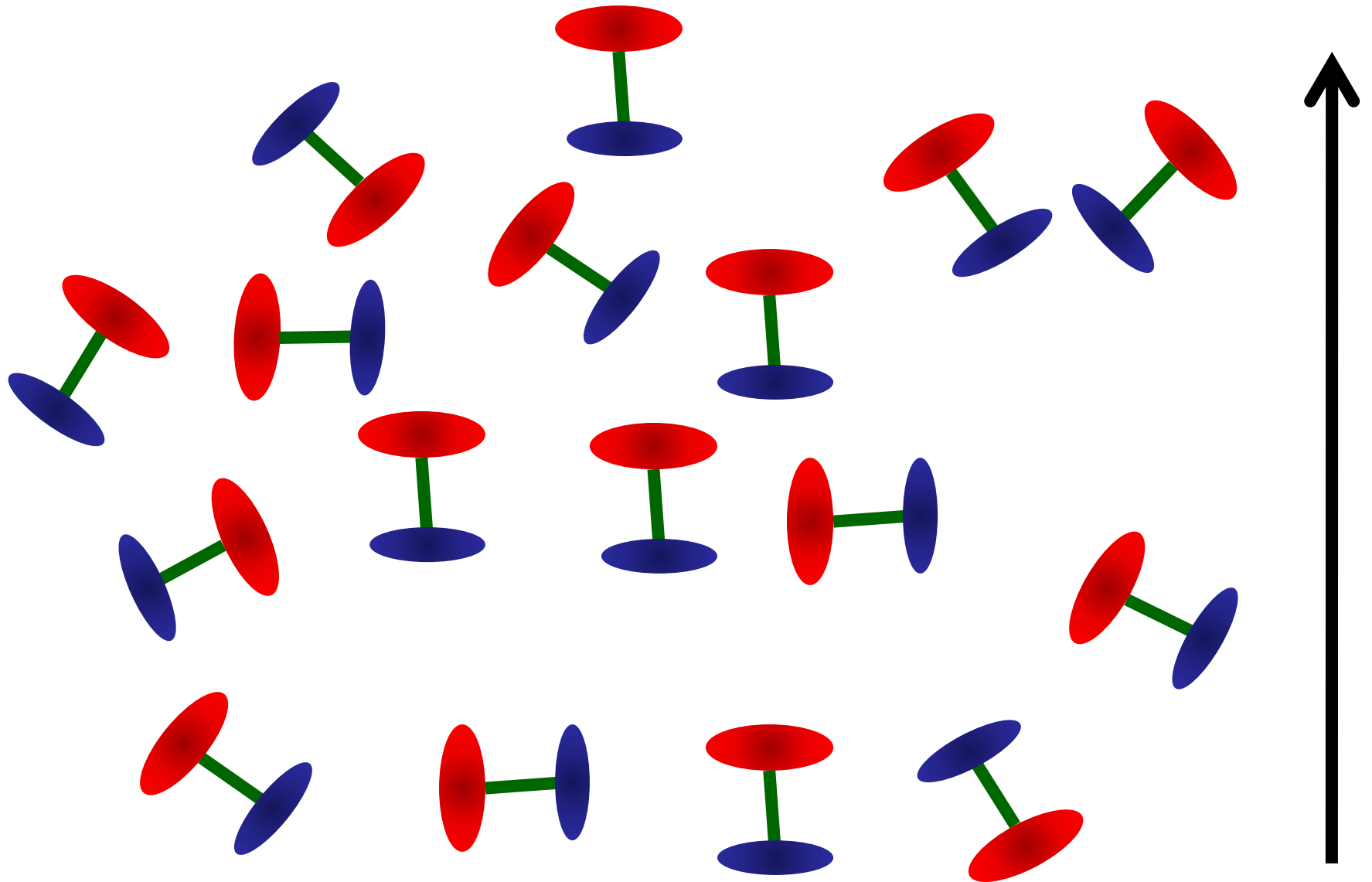


Dipolar Pake
Pattern

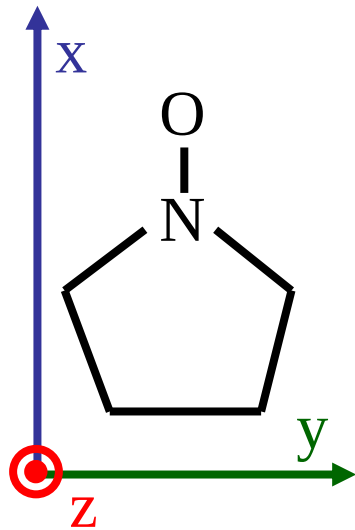
Width < MHz



Randomly oriented powder sample

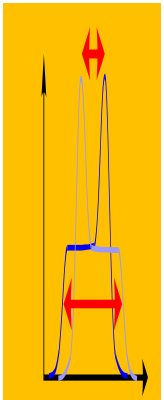


EPR linewidth of nitroxide spin labels



*Hyperfine
dominated*

0.3 T



6.4 T



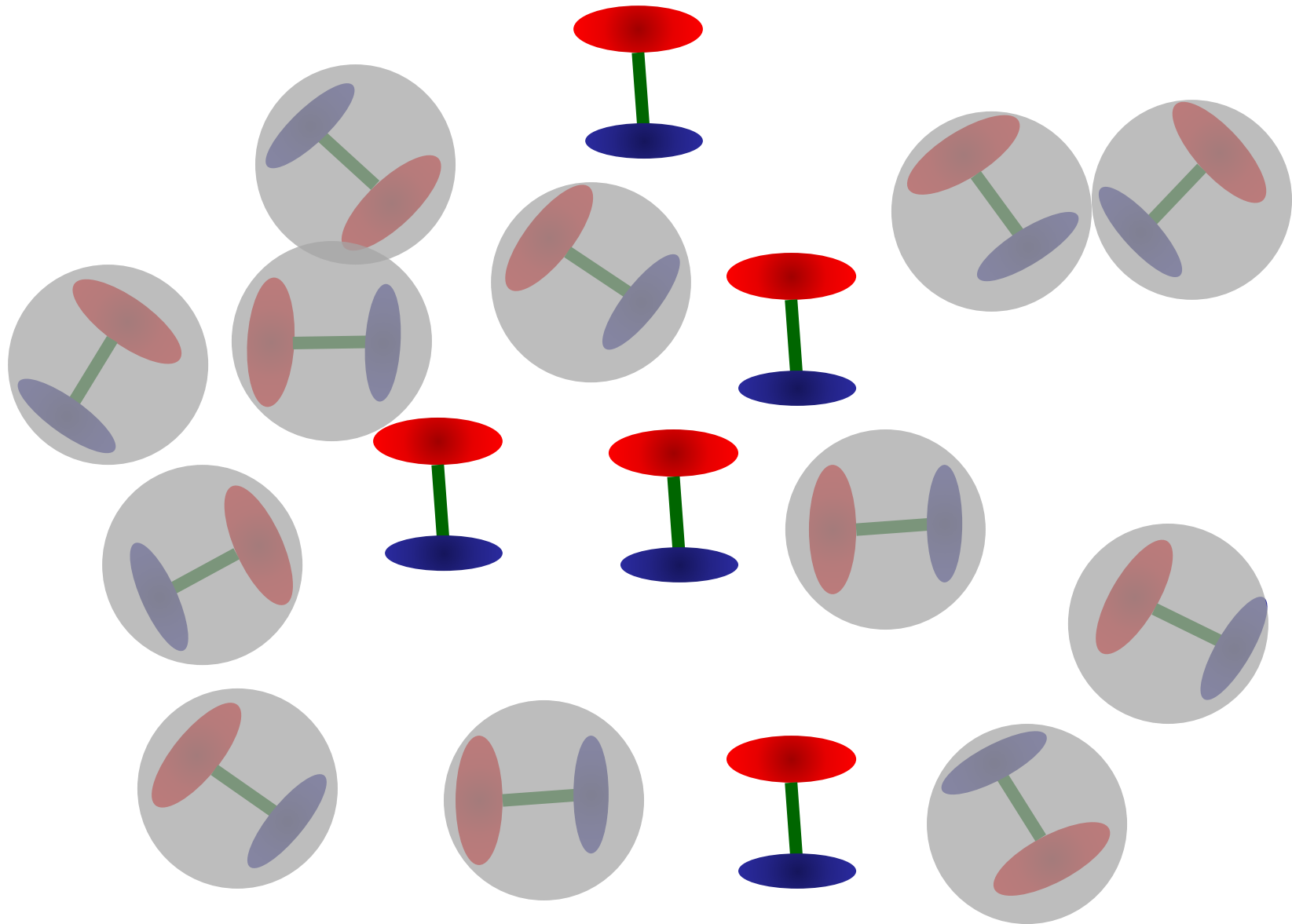
*G-Tensor
dominated*



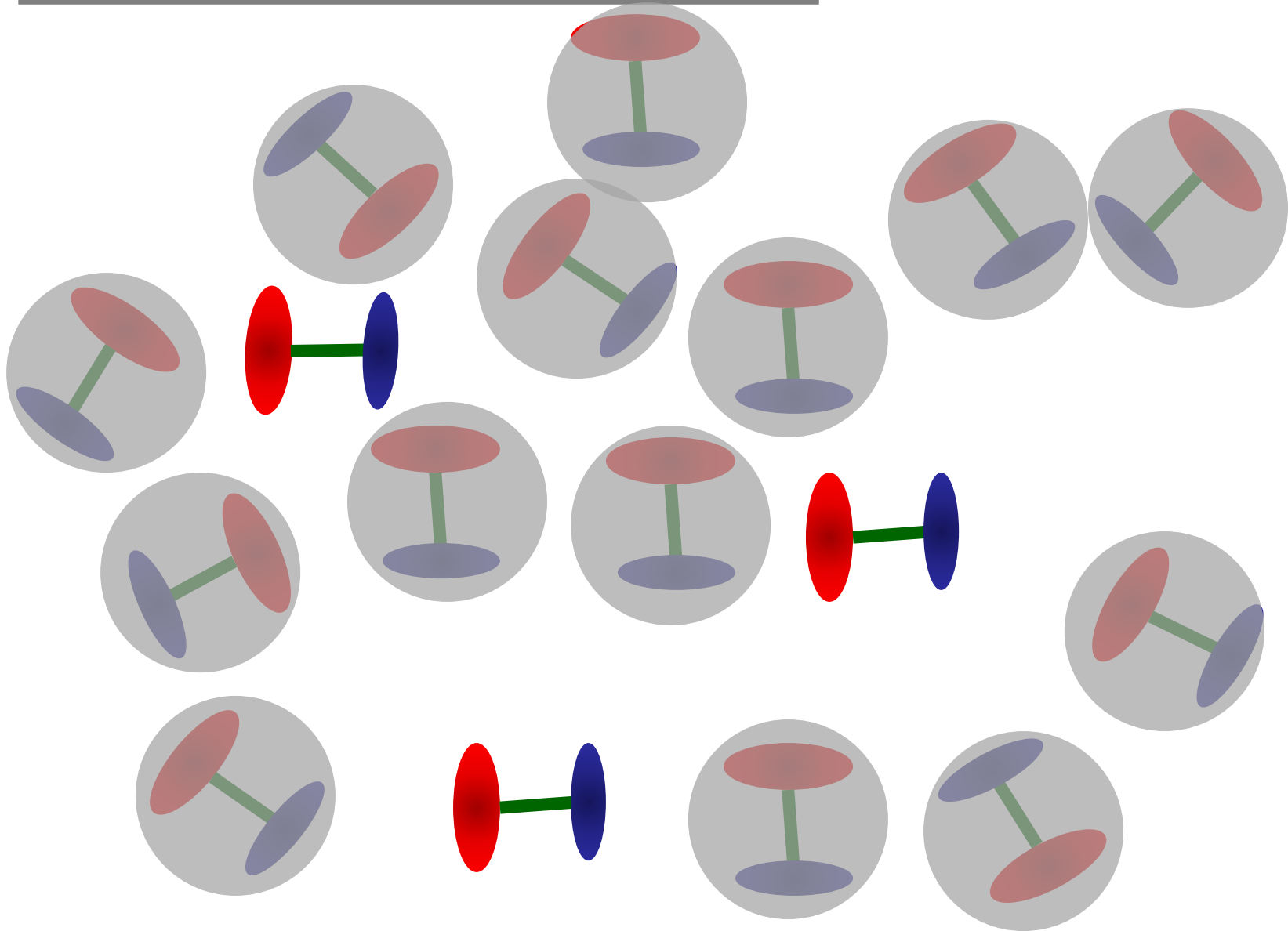
B_0

B_0

Select only small g value

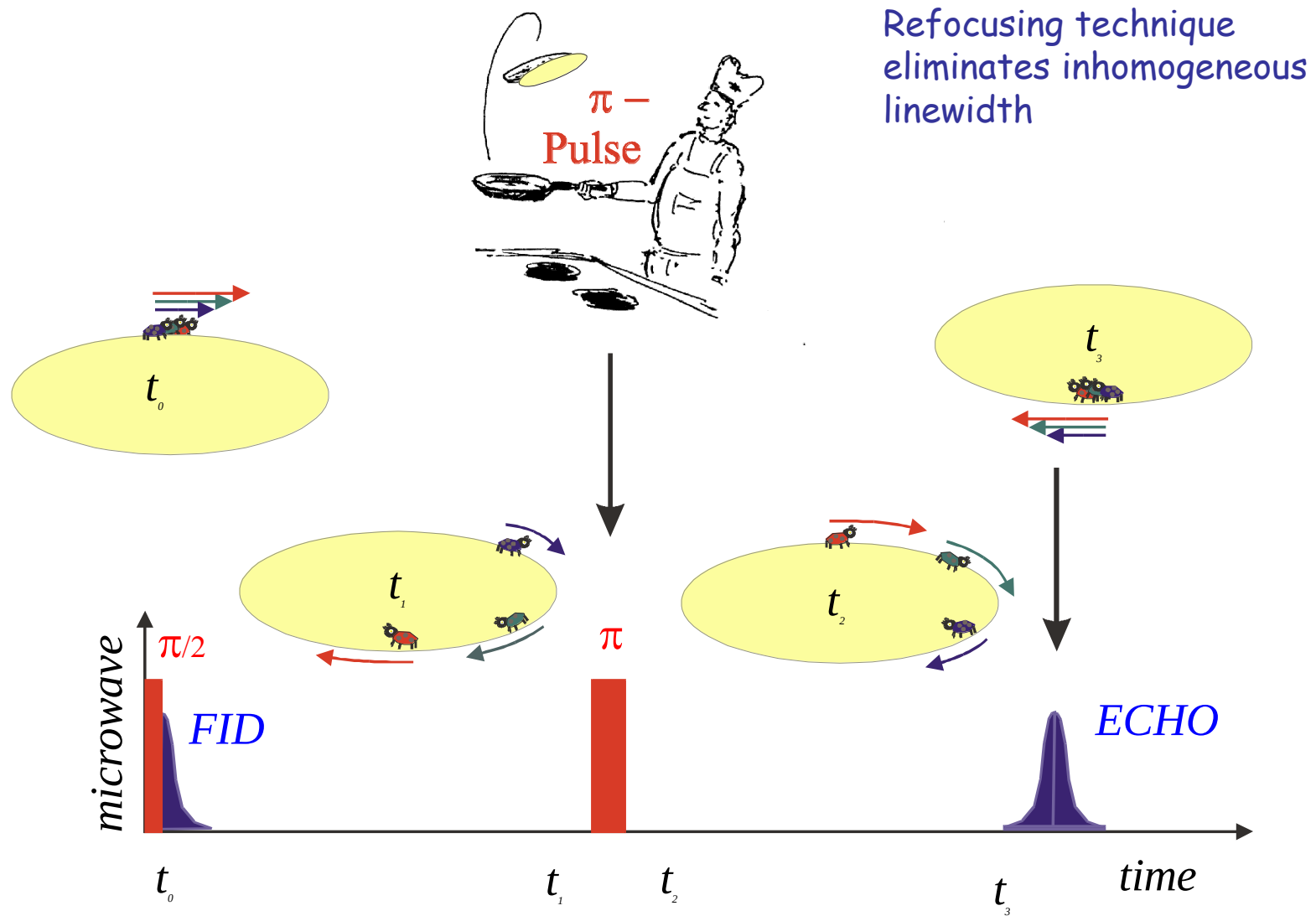


Select only large g-value



Pulsed EPR

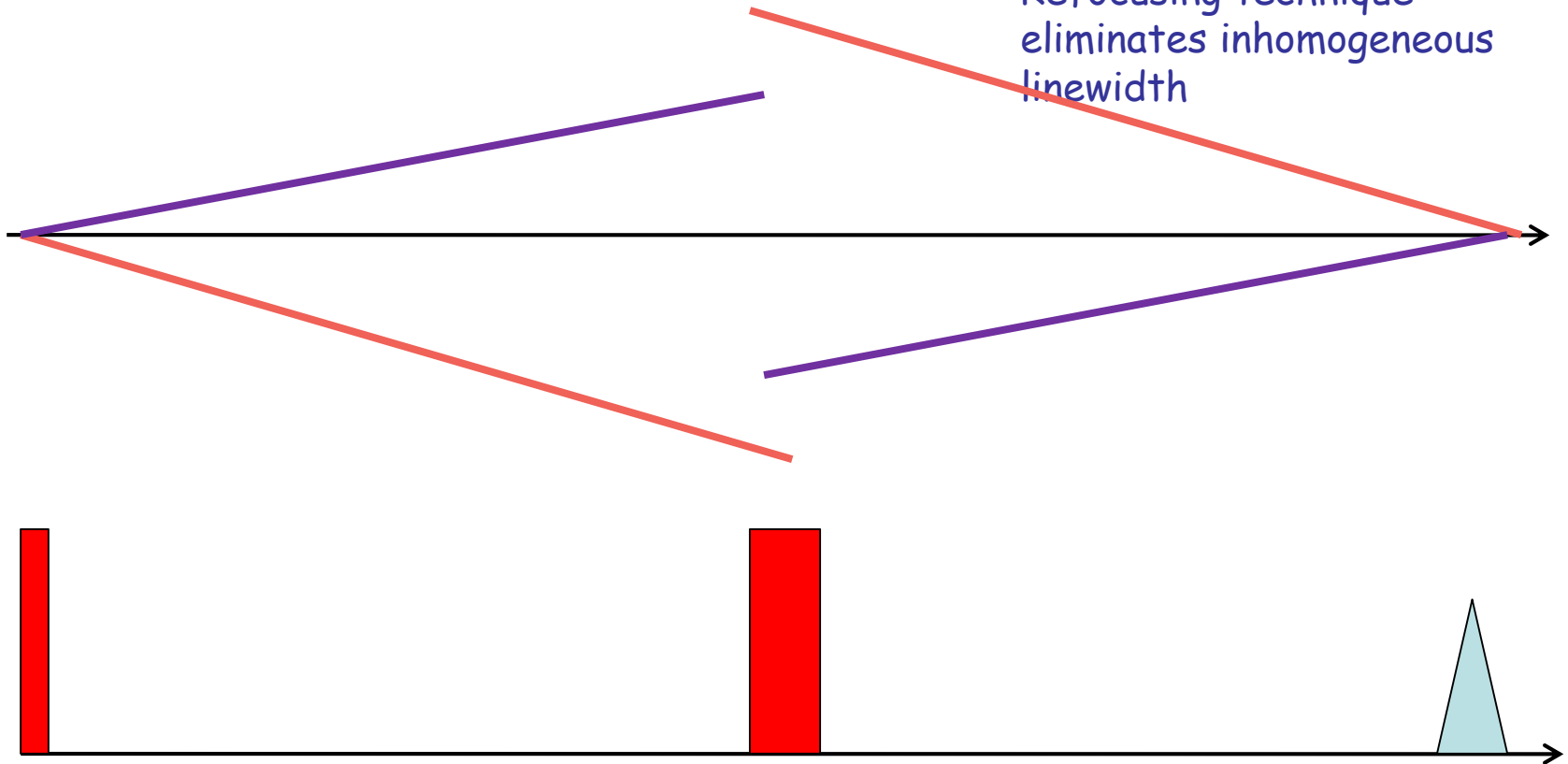
Hahn Echo sequence



Pulsed EPR

Hahn Echo sequence

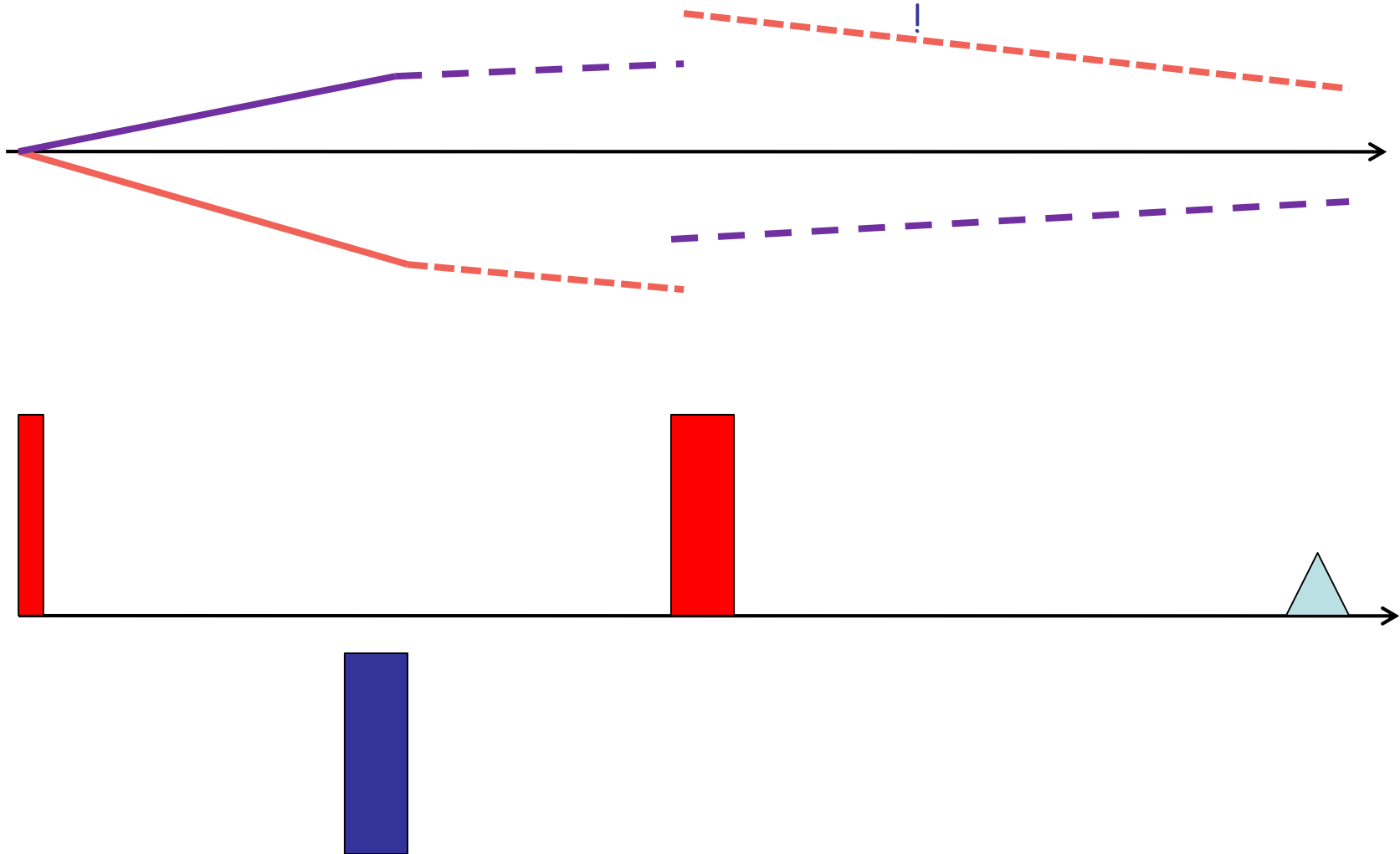
Refocusing technique
eliminates inhomogeneous
linewidth



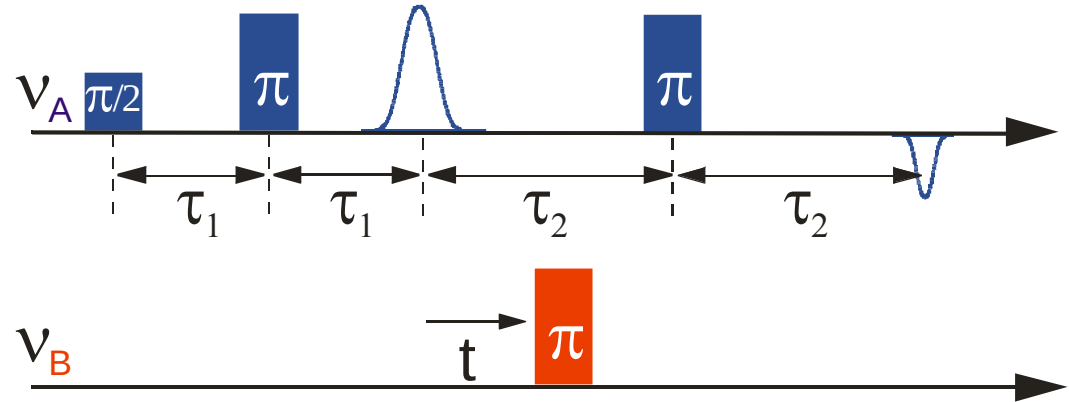
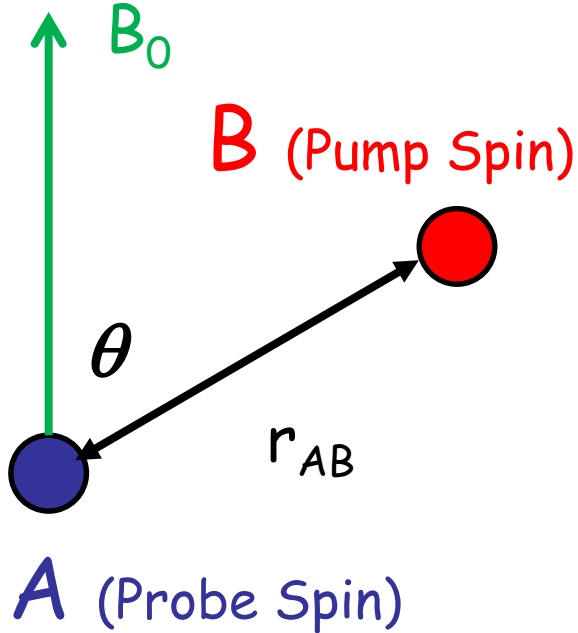
Pulsed EPR

Hahn Echo sequence

Pulse on coupled spin brakes
the symmetry - less refocusing

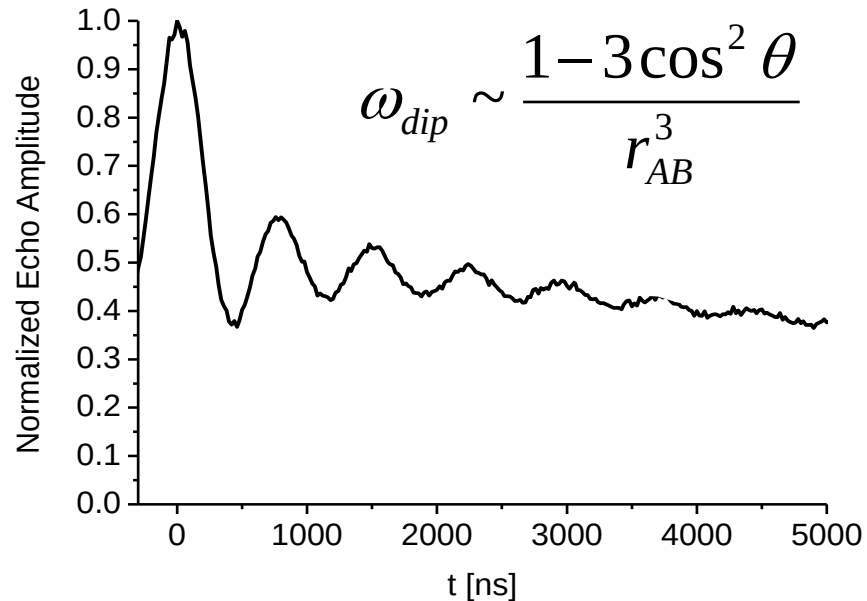


PELDOR Time Trace oscillates with dipolar frequency

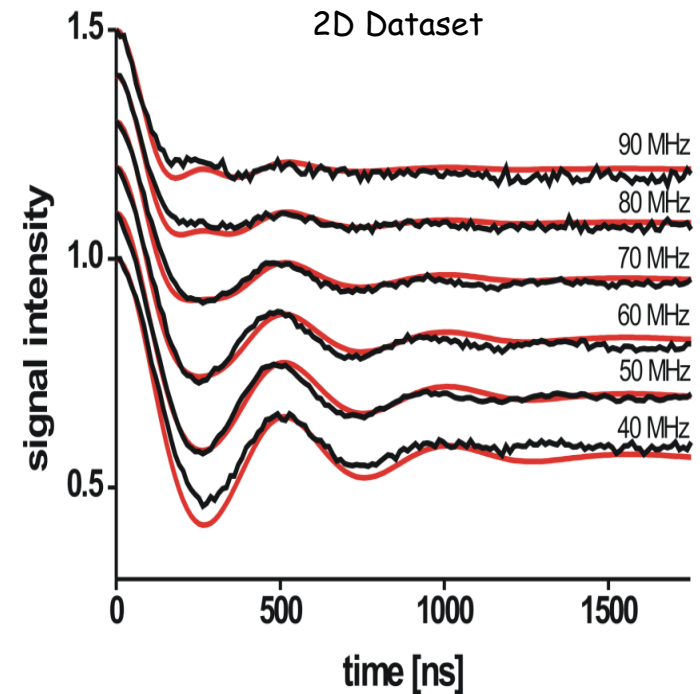
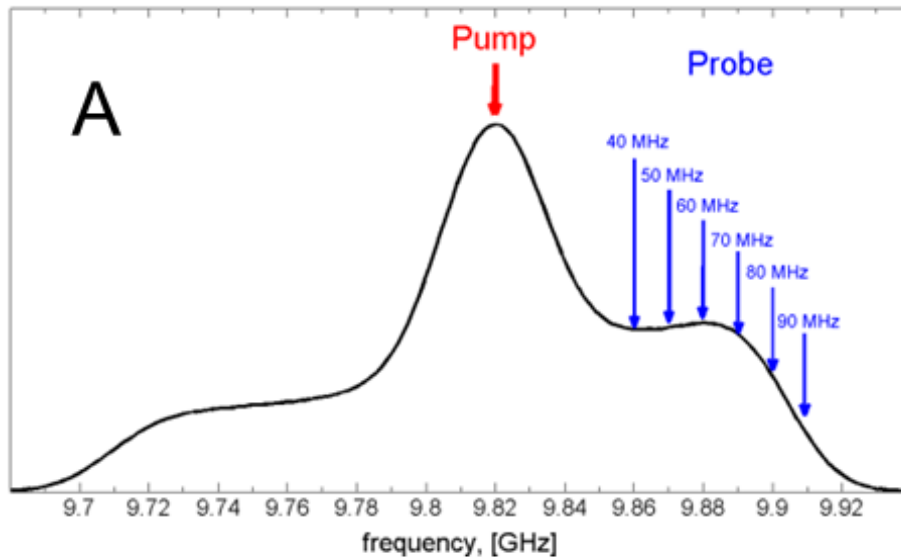
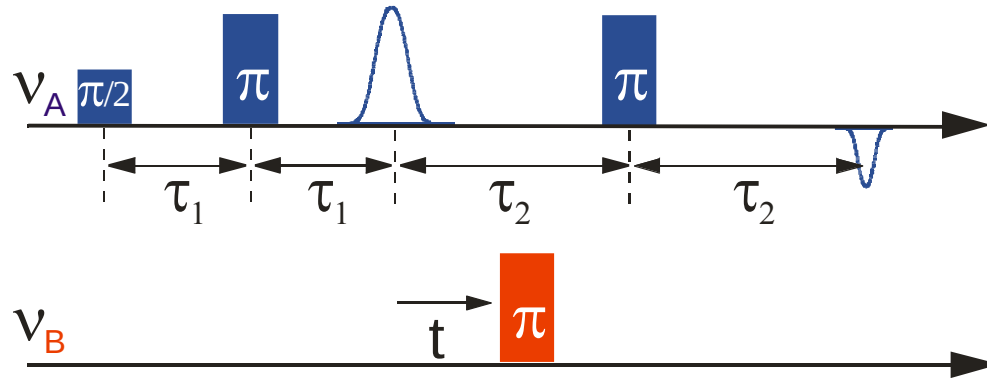


$$S(t) \sim \int \cos(\omega_{dip} t) d \cos \theta$$

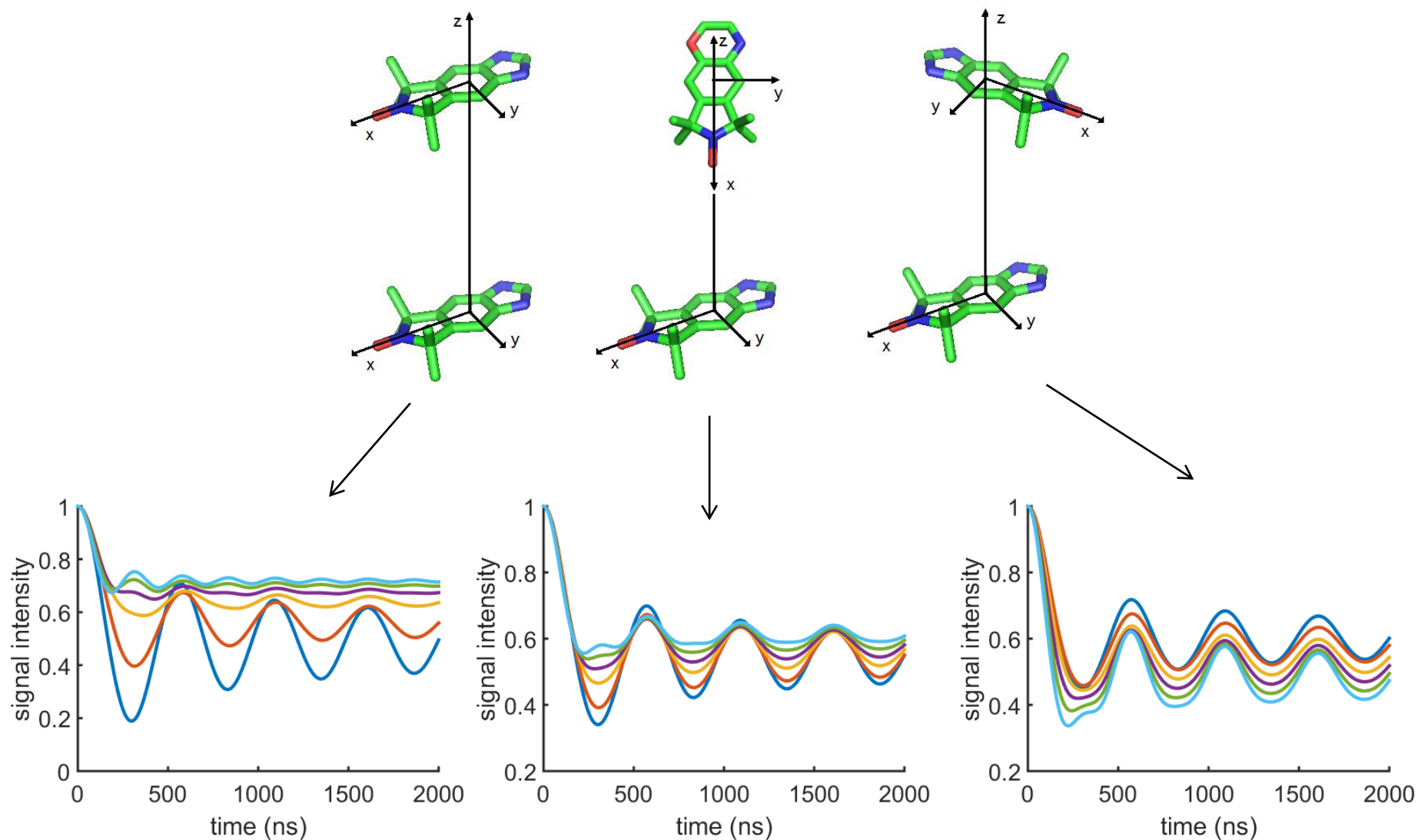
$$\omega_{dip} \sim \frac{1 - 3 \cos^2 \theta}{r_{AB}^3}$$



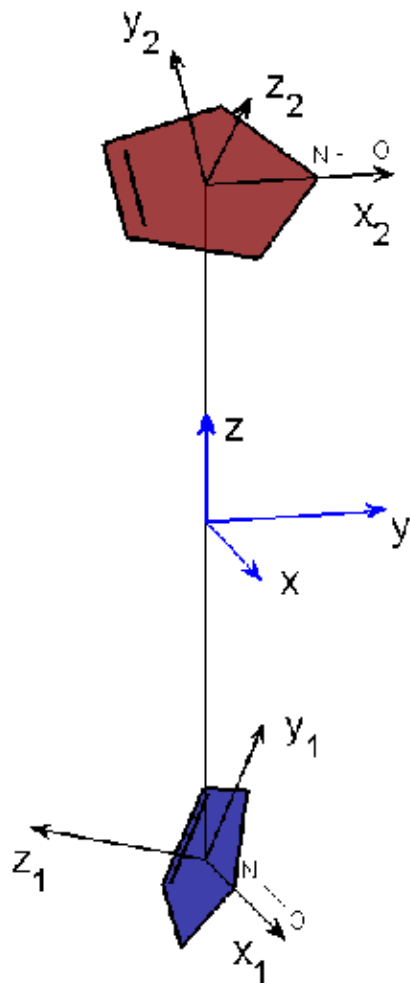
2 Dimensional Experimental Procedure



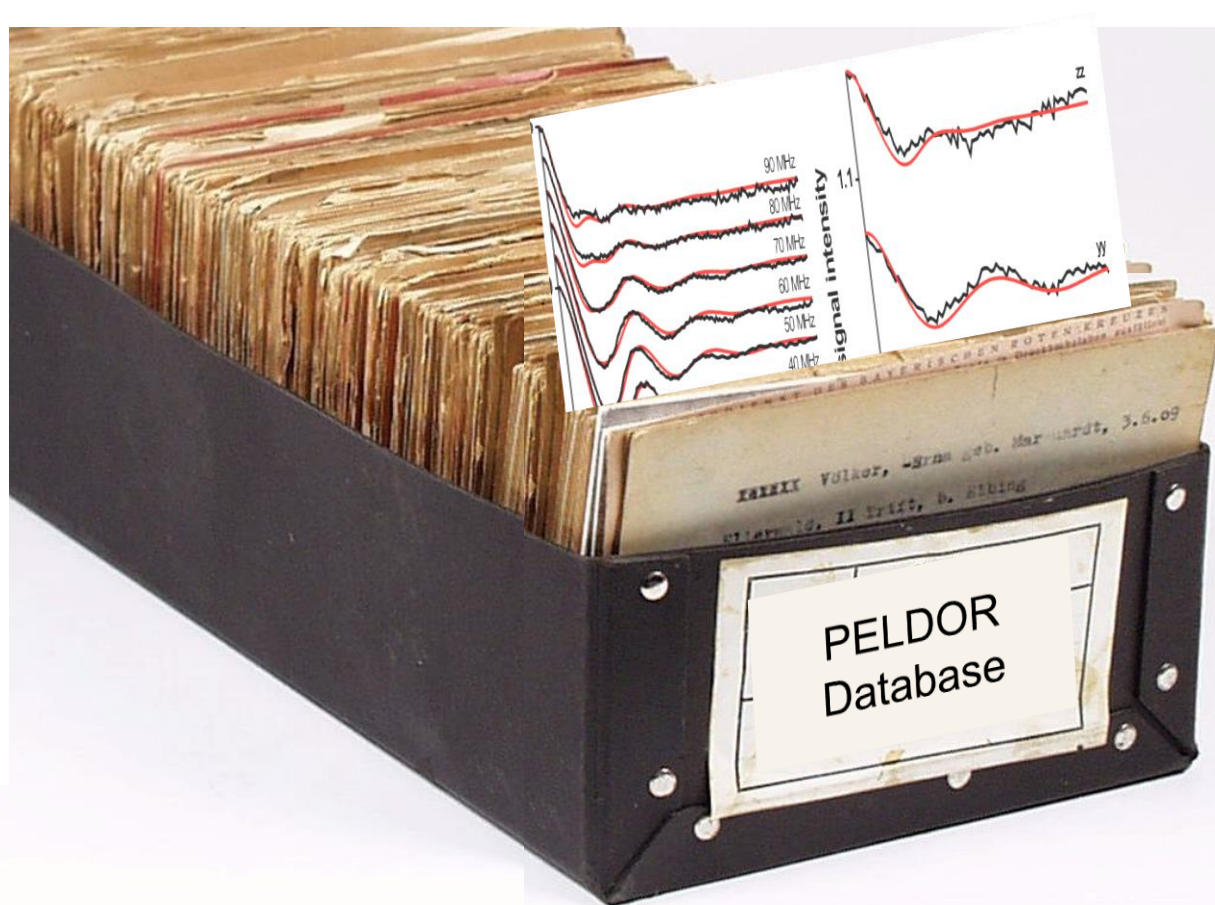
Unique pattern for a given distance / geometry



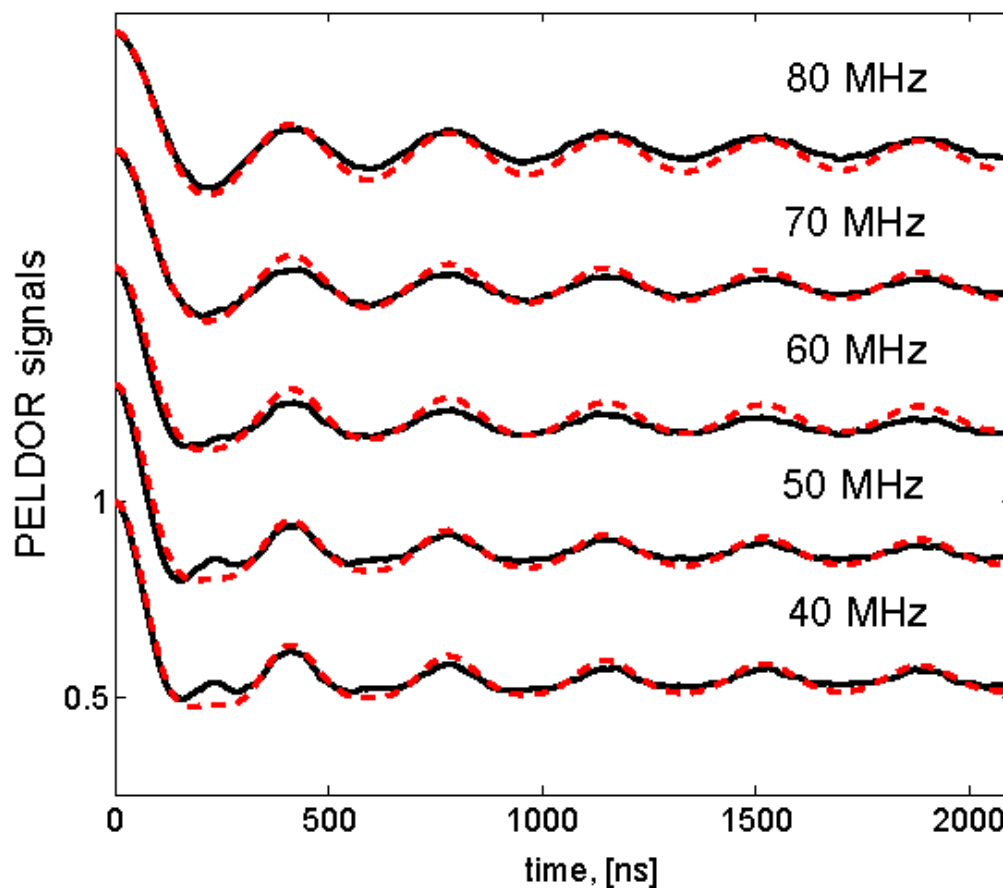
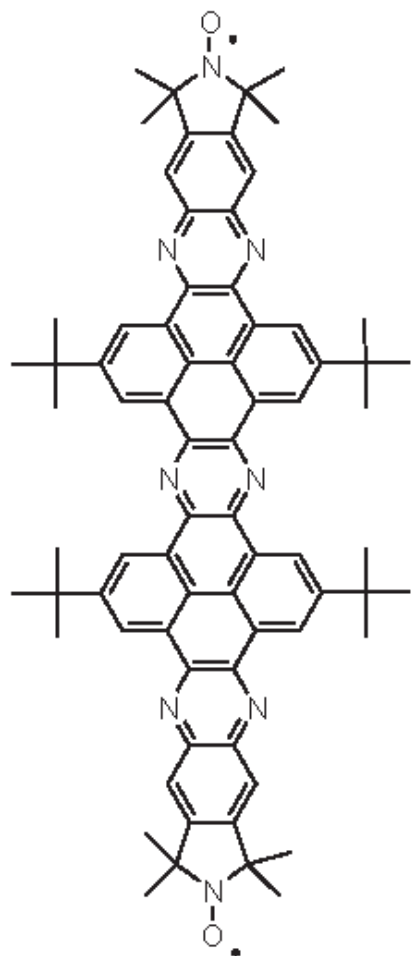
PELDOR Database



Simulated Multi-Frequency / Multi-Field Datasets
for each orientation and distance (conformer)



Test of method with rigid planar biradical

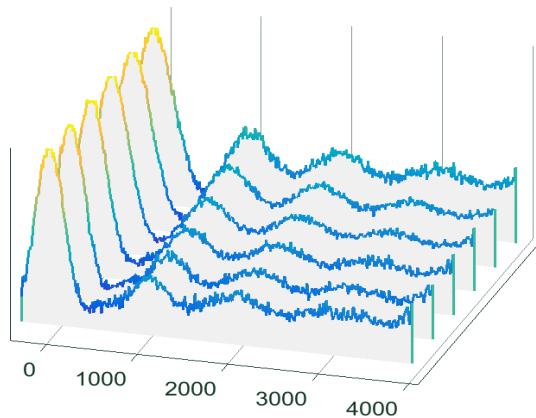
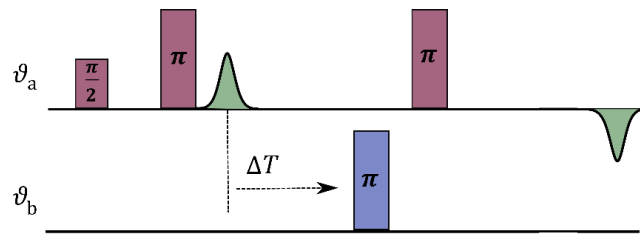


Unique solution found with single conformer

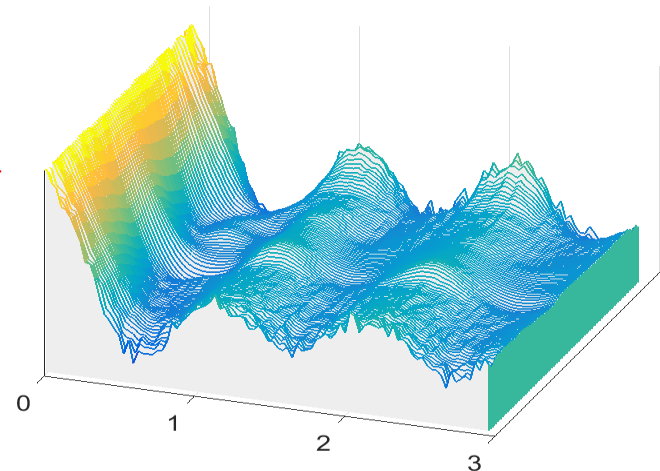
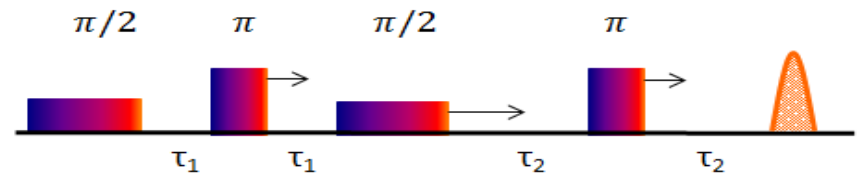
Distance ☒ & Orientation ☒

2D SIFTER experiment using broadband pulses

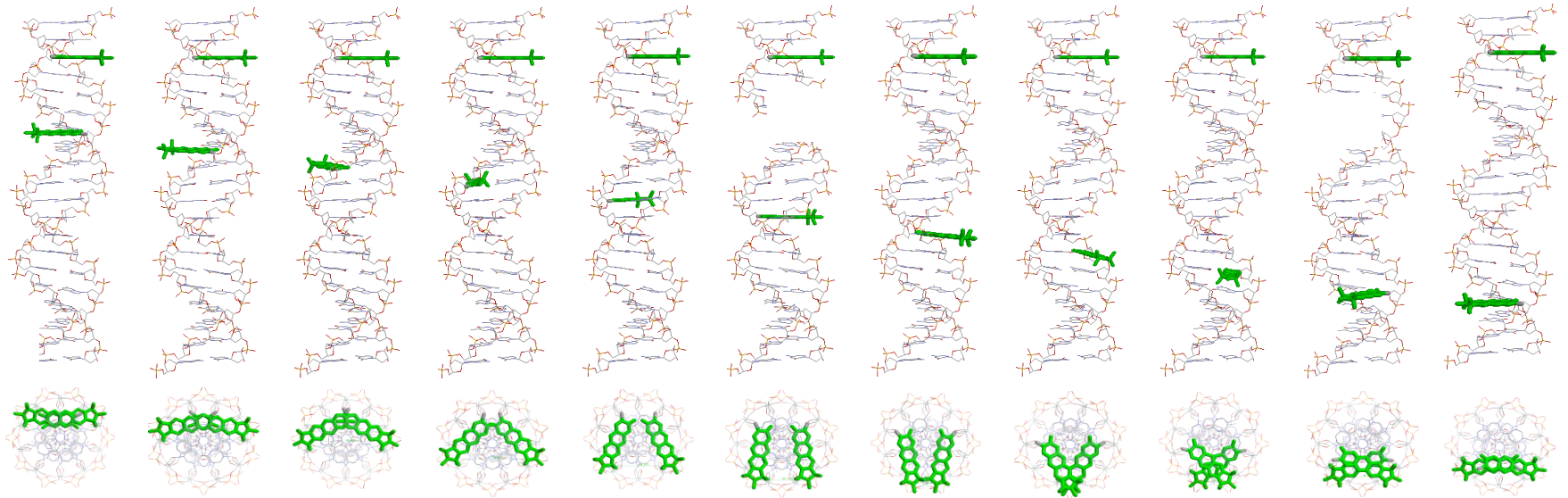
4 pulse PELDOR/DEER
with several probe
frequencies



Broadband SIFTER with FT
of Echo signal

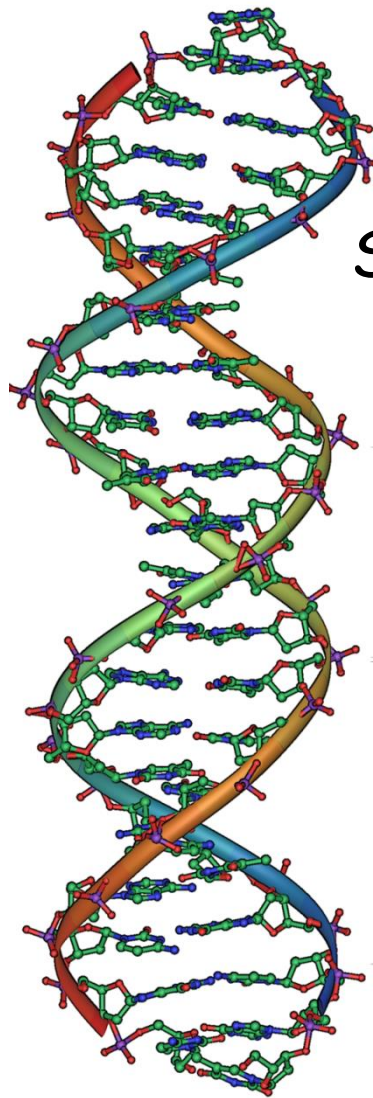


Investigation of dynamics of dsDNA



11 samples with variable distance between both spinlabels
have been synthesized and measured

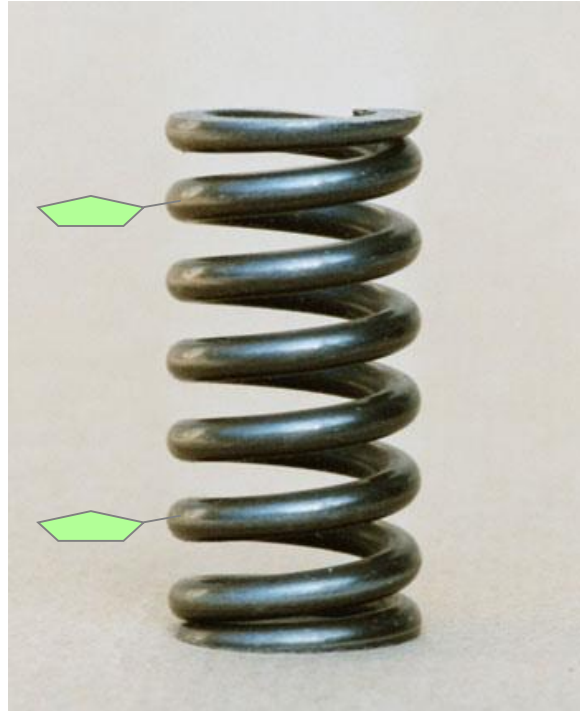
Simple modes of dynamics of dsDNA



Stretching



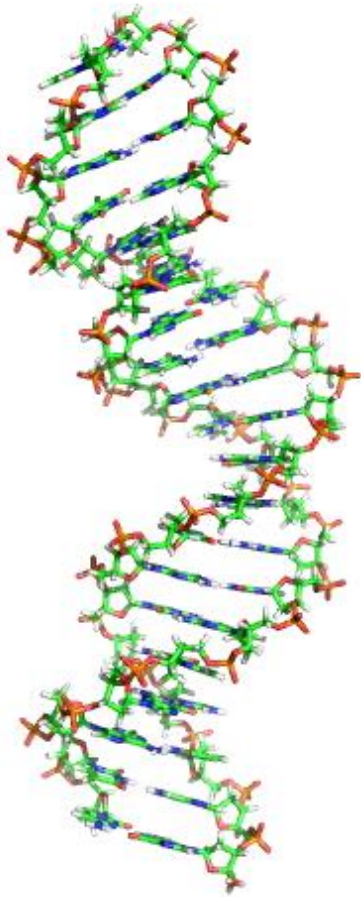
$$\Delta x^2 \sim L$$



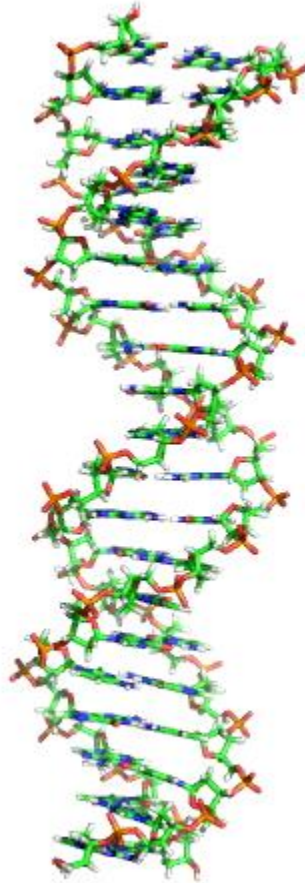
Bending

Twisting

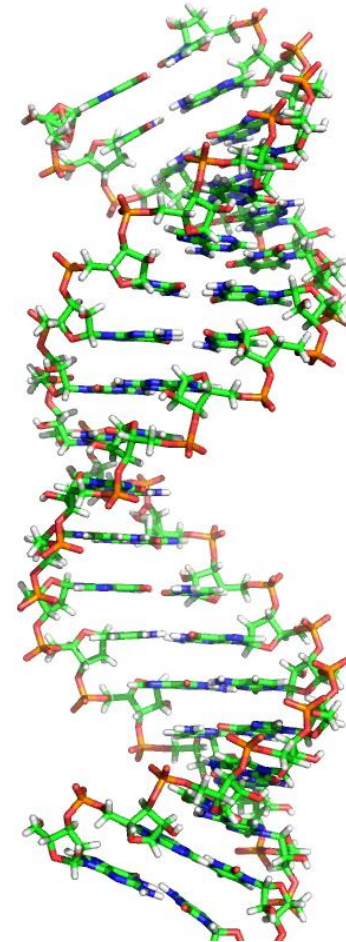
Models for conformational dynamics of dsDNA



Bending
($\Delta\alpha$)



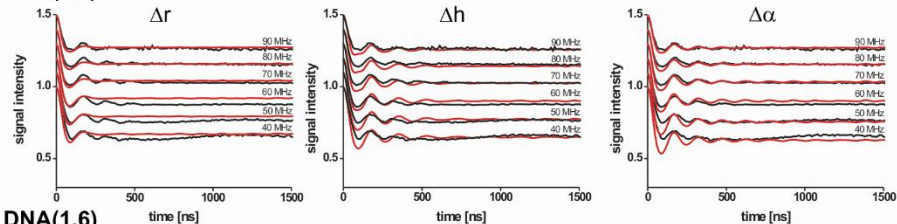
Twisting
 Δr



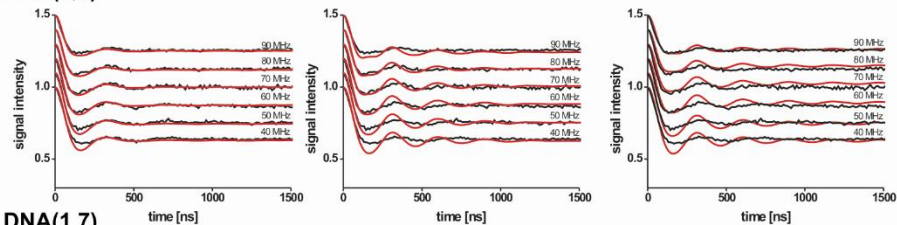
Stretching
 Δh

2D-PELDOR datasets and best model fits at 0.3 T

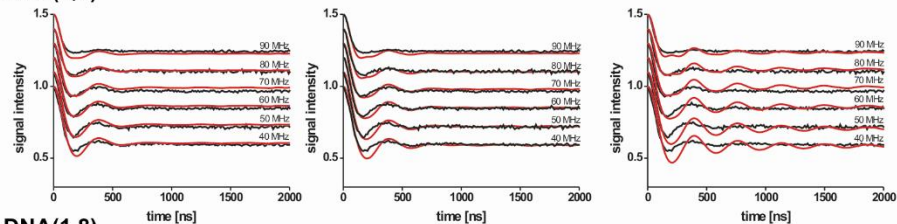
DNA(1,5)



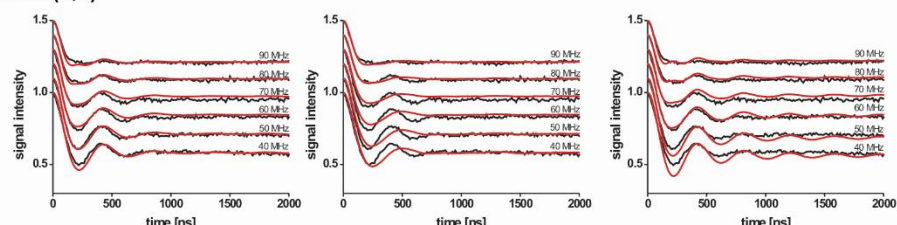
DNA(1,6)



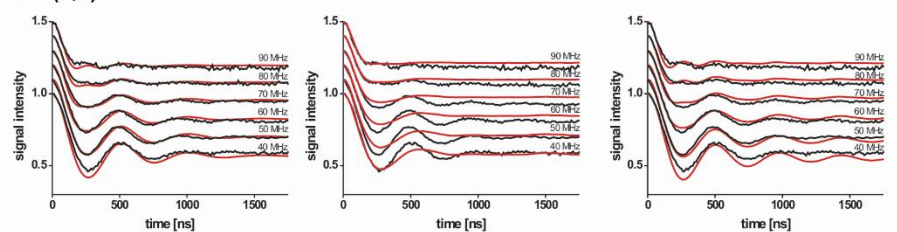
DNA(1,7)



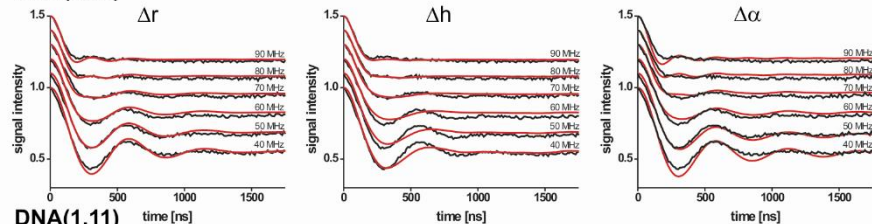
DNA(1,8)



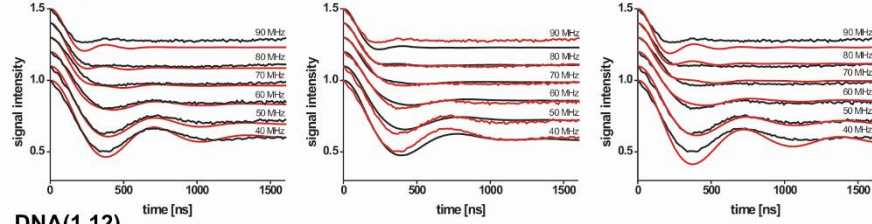
DNA(1,9)



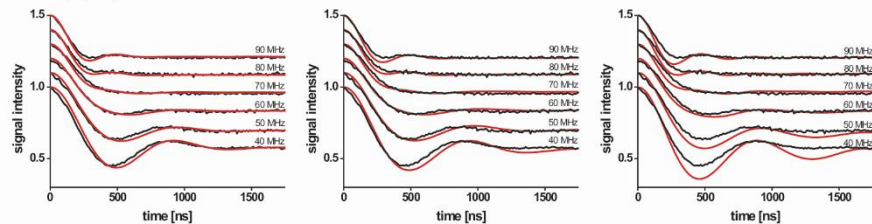
DNA(1,10)



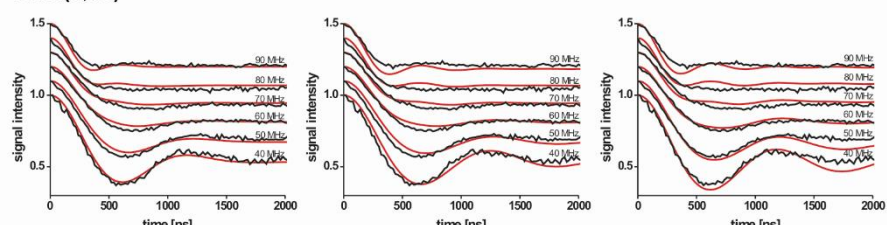
DNA(1,11)



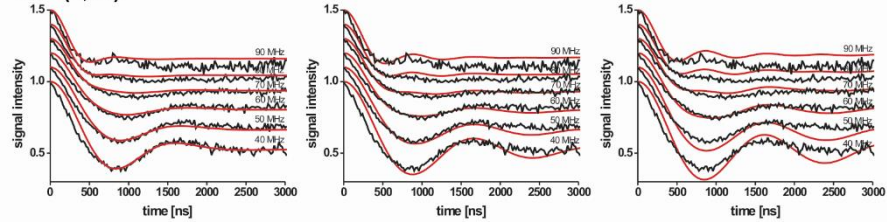
DNA(1,12)



DNA(1,13)



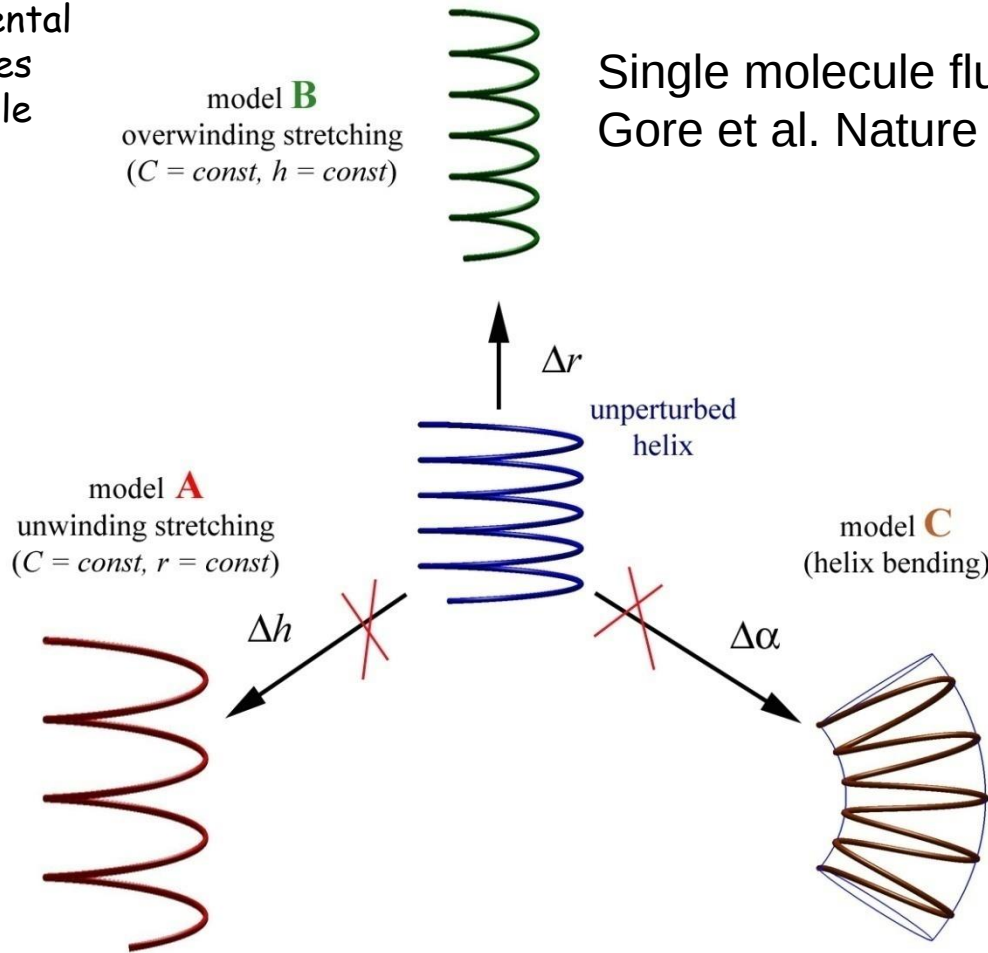
DNA(1,14)



Models for conformational dynamics of dsDNA

The radius changing mode fits best our experimental data but with a 10 times softer stretching module

JACS 2011

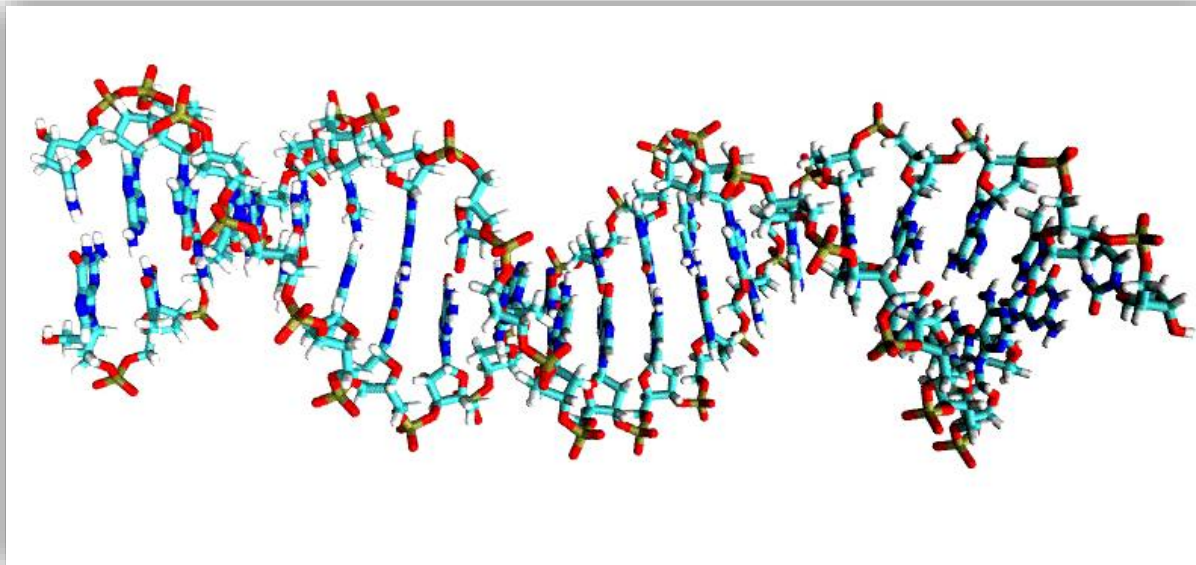


Single molecule fluoreszenz
Gore et al. Nature (2006)

SAXSI
Mathew-Fenn et al. Science (2008)

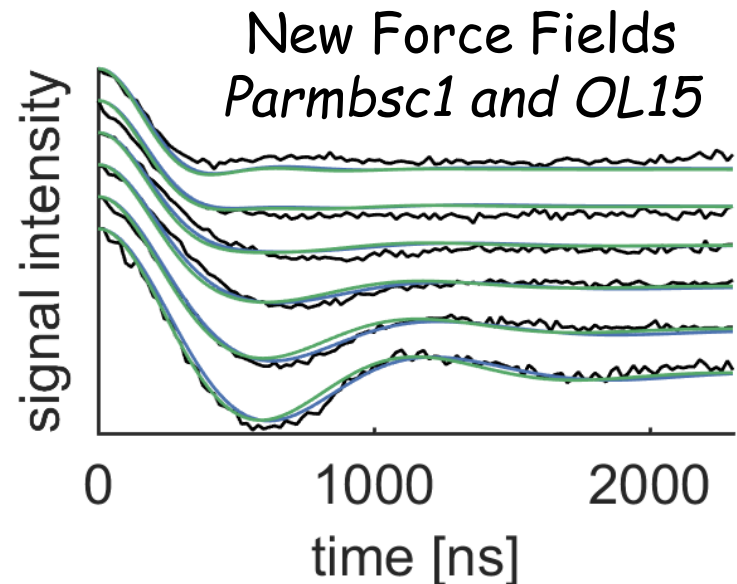
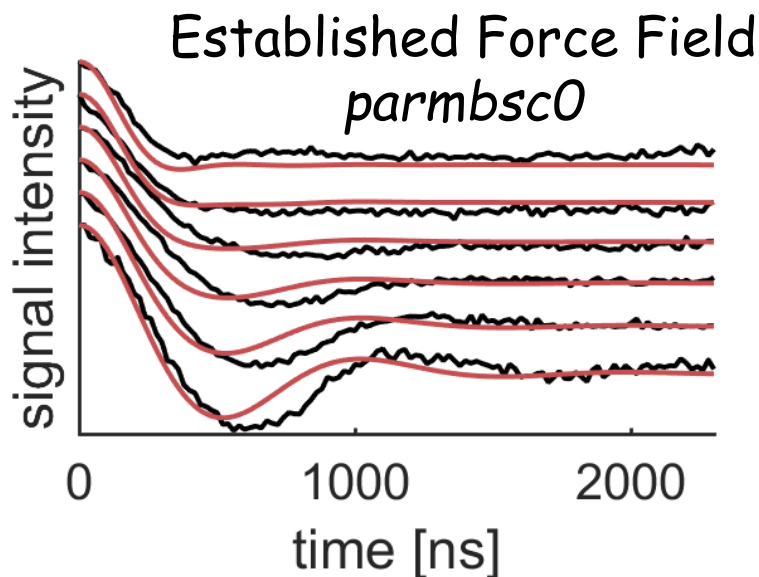
Modeling
Becker et al. Science (2009)

Comparison with MD simulations of dsDNA



Collaboration with
G. Hummer
(MPI Biophysics,
Frankfurt)

JACS 2017



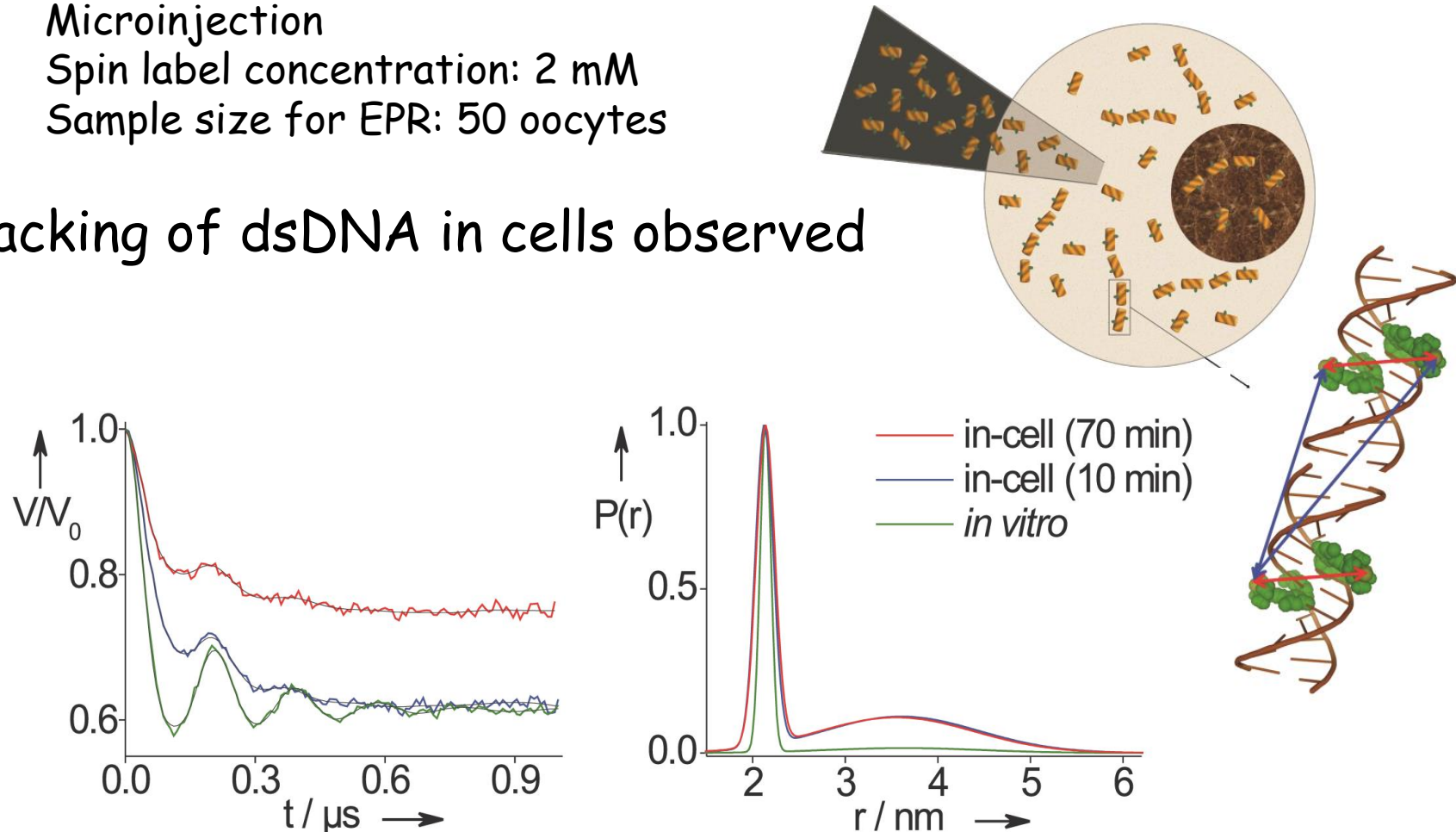
In-cell application is possible

Microinjection

Spin label concentration: 2 mM

Sample size for EPR: 50 oocytes

Stacking of dsDNA in cells observed

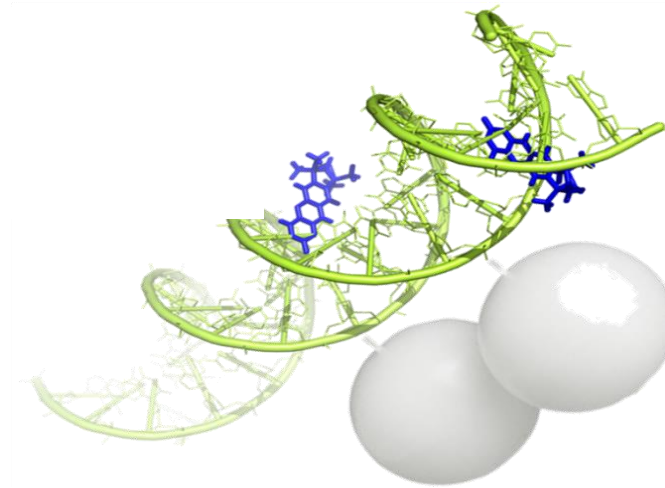
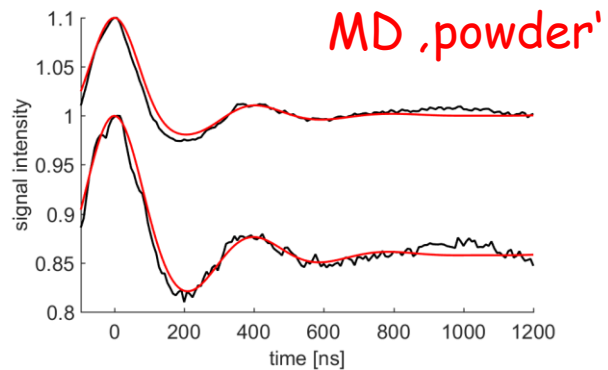


Collaboration with Volker Dötsch (Uni Frankfurt)

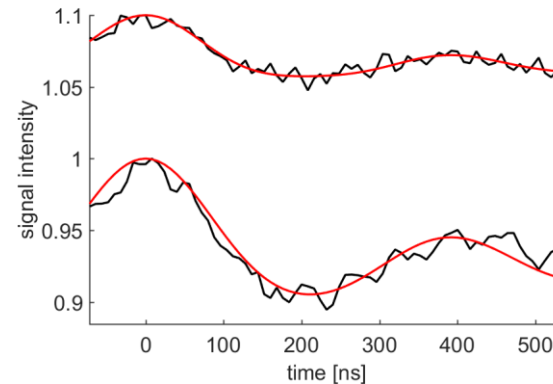
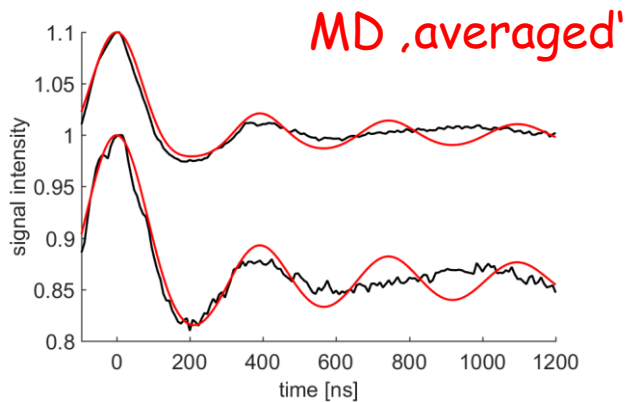
Angewandte 2011

PELDOR Experiments at Room Temperature

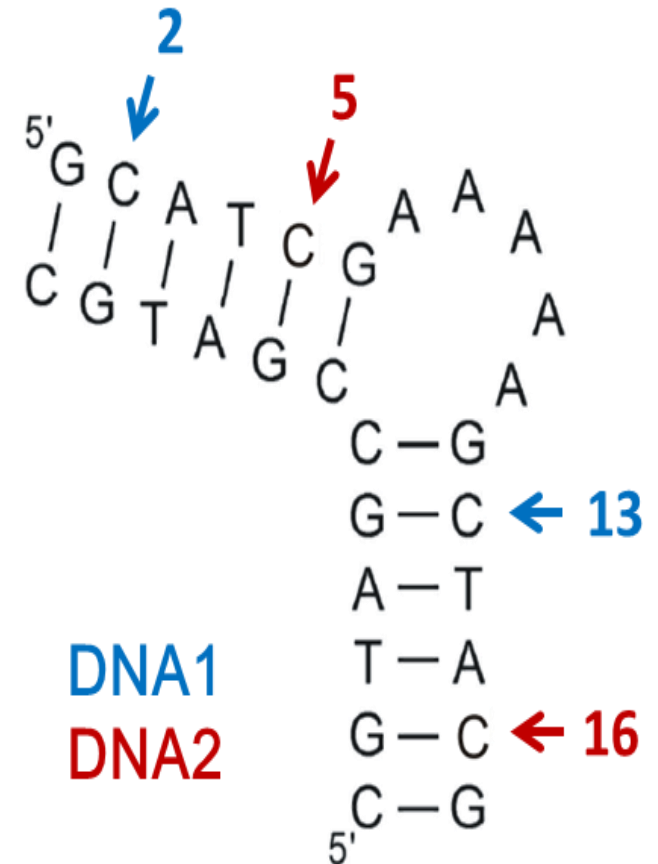
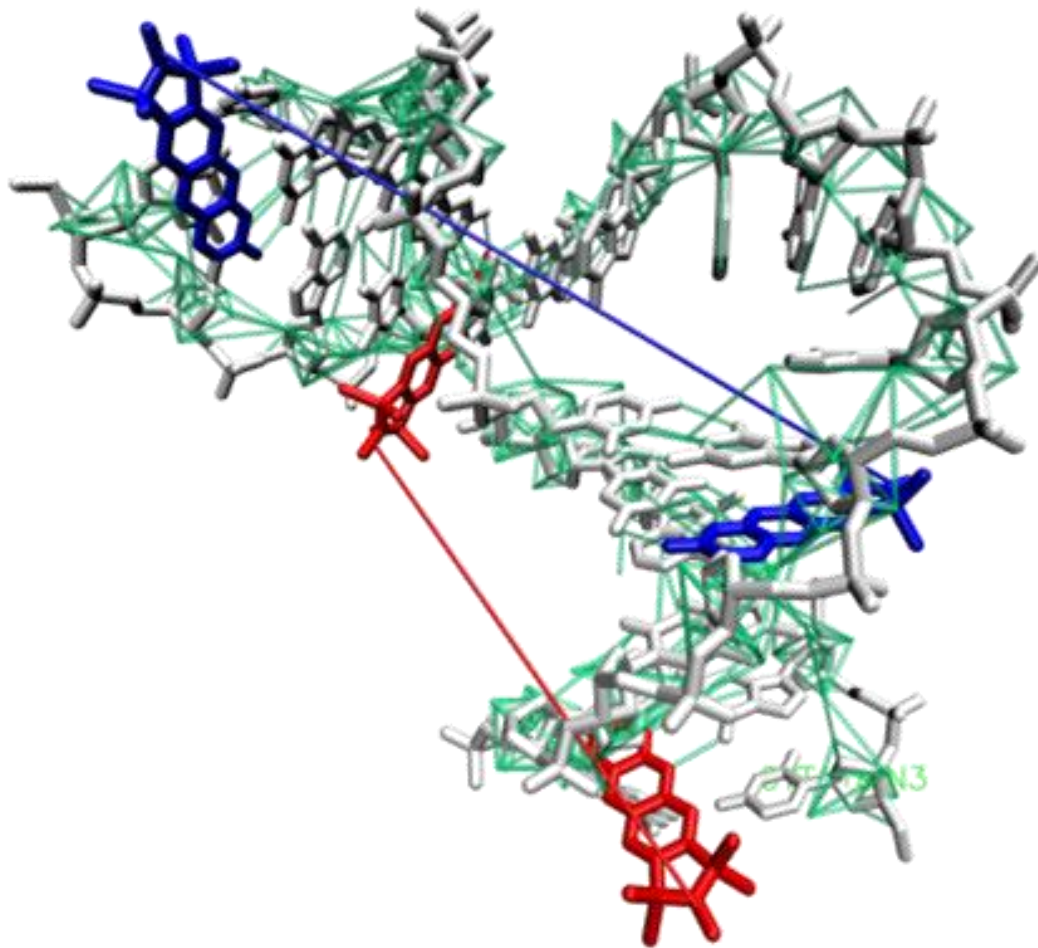
40 K experiment



RT PELDOR data

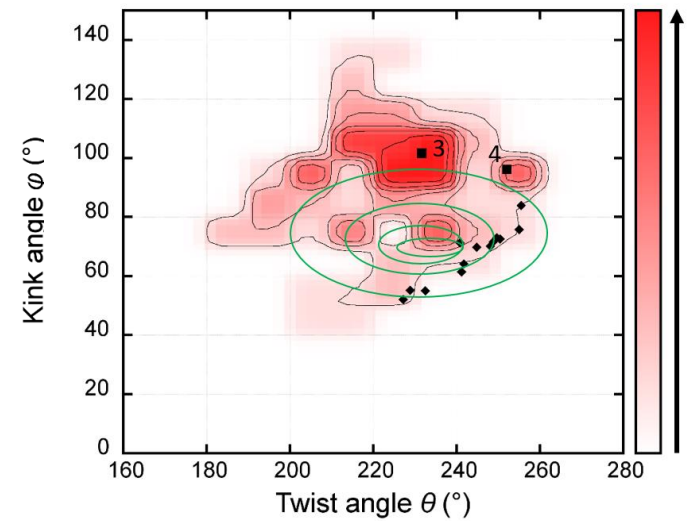
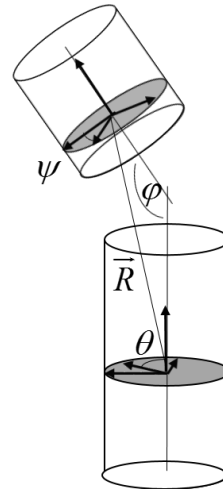
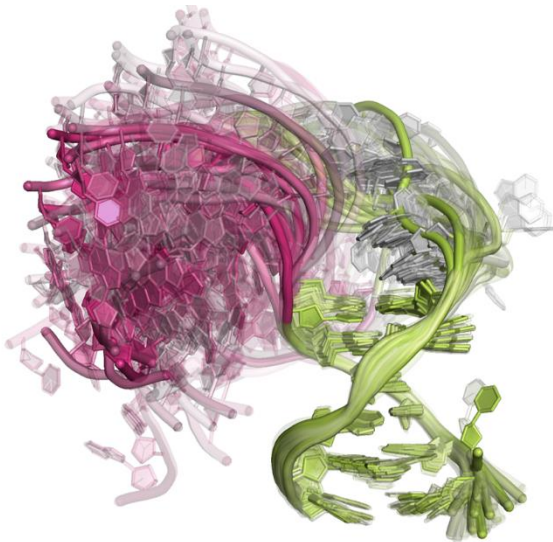
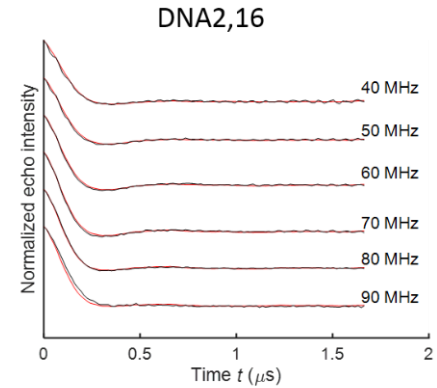
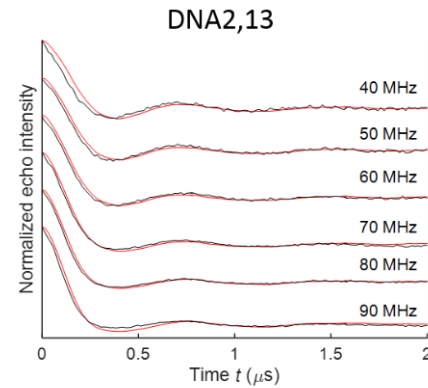
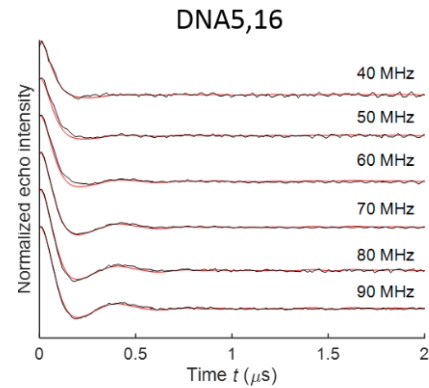
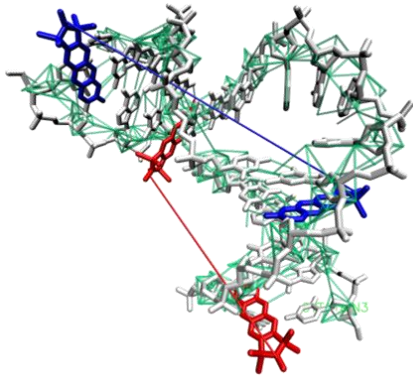


Investigation of DNA with A-bulge



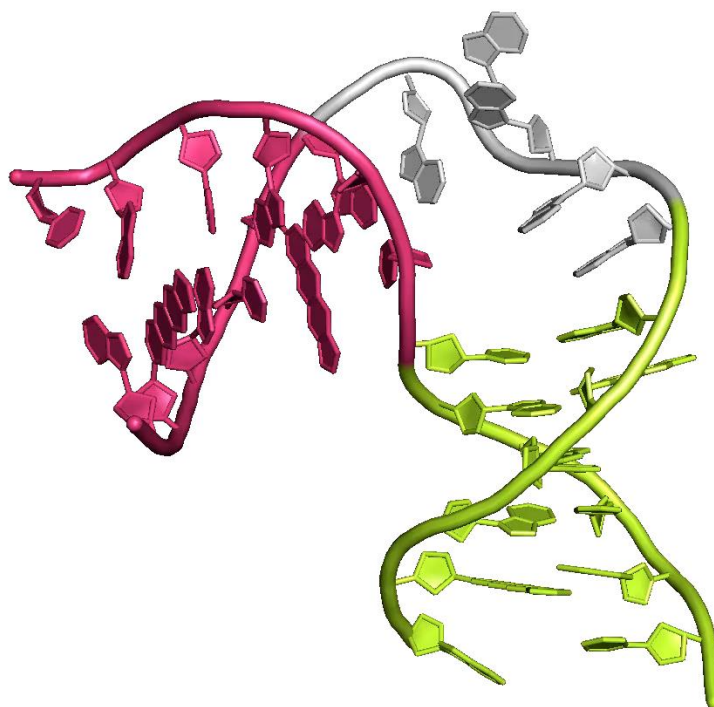
NMR structure from NOE's
Dornberger et al. Biochem. 38, 12860 (1999)

Investigation of DNA with A-bulge

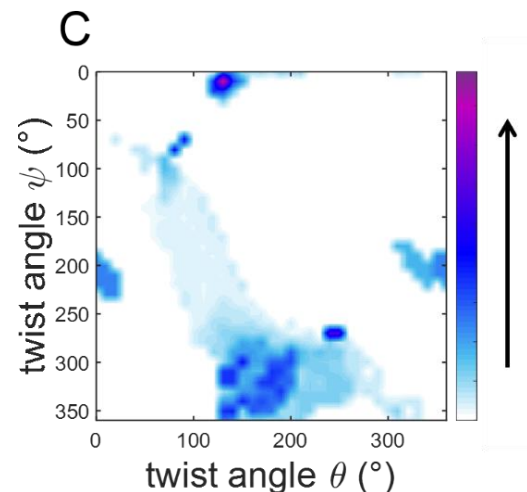
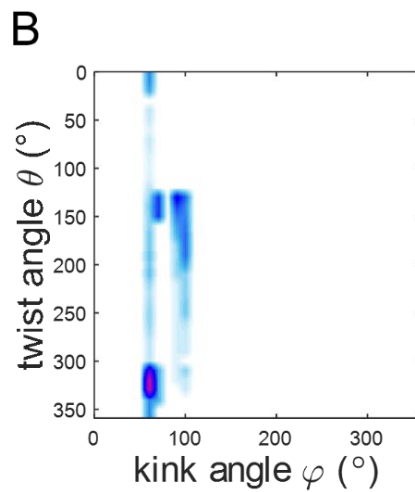
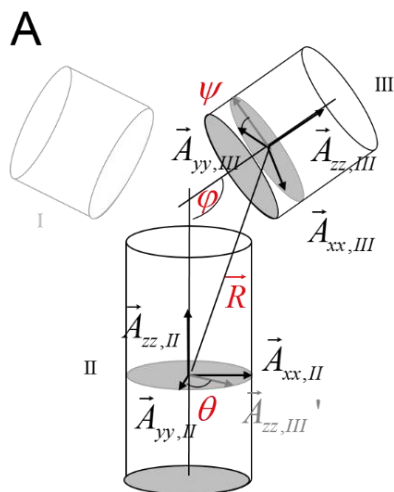
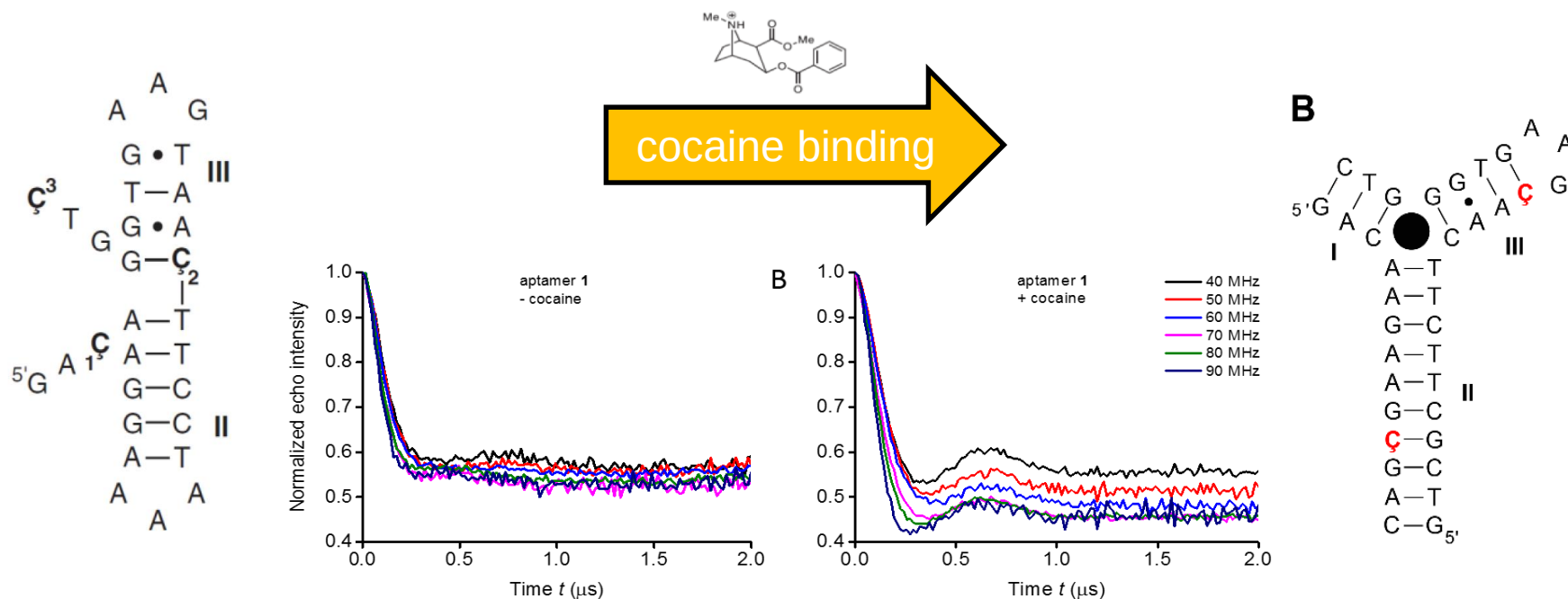


Investigation of DNA with A-bulge

Most populated structure



Investigation of cocaine binding aptamer



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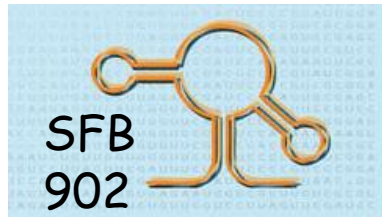
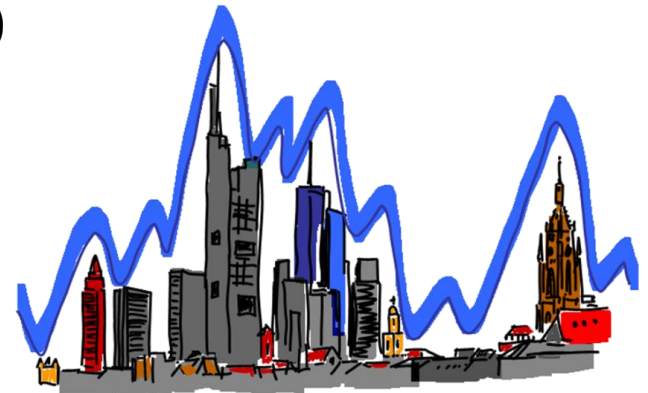
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