Structure and Dynamics of Nucleic Acids

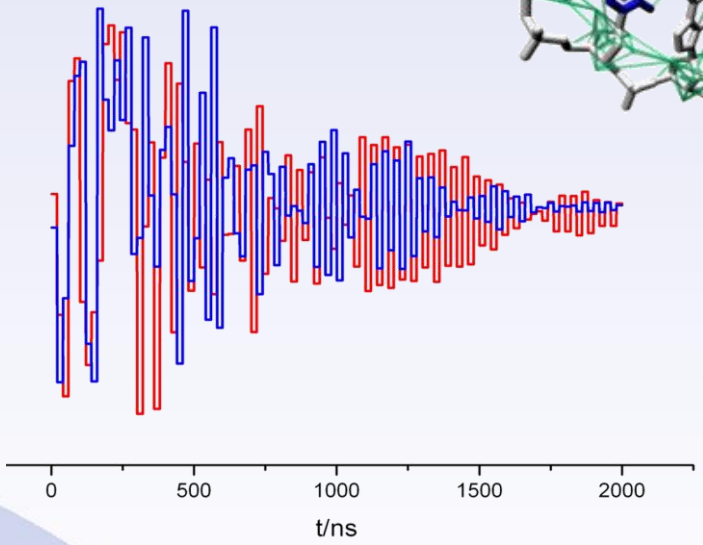
JOHANN WOLFGANG GOETHE
UNIVERSITÄT
FRANKFURT AM MAIN

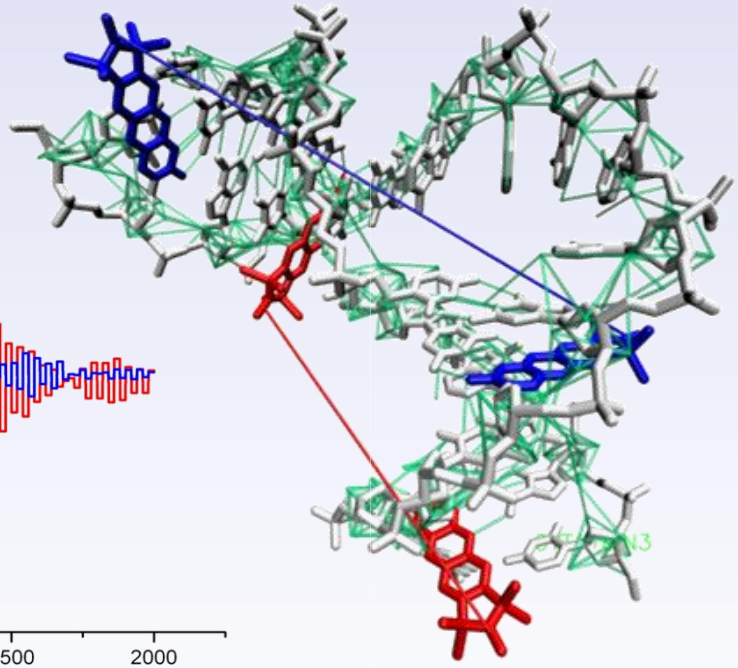
T. F. Prisner

Institute of Physical & Theoretical Chemistry

Center of Biological Magnetic Resonance

Goethe University Frankfurt





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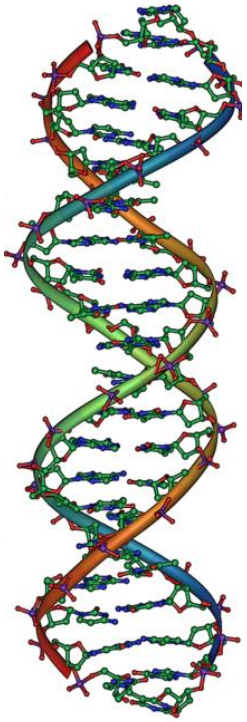
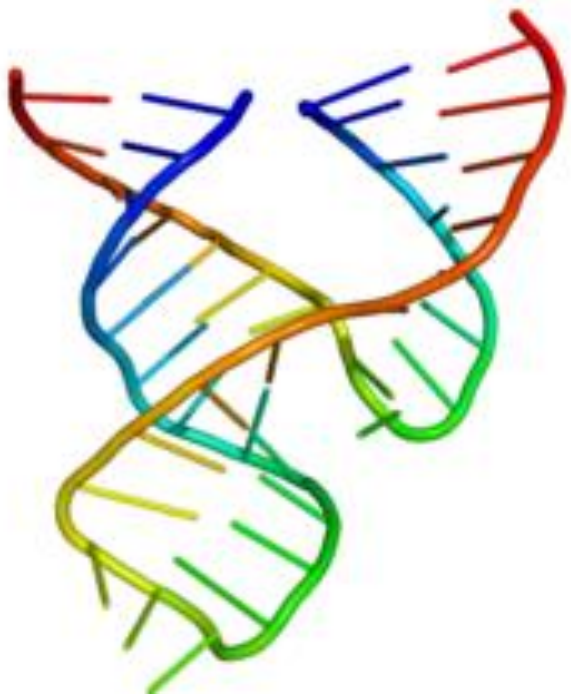
Structure and Flexibility of Nucleic Acids

Highly conserved structures
for storage and translation
of the genetic code

Highly flexible structures for
regulatory and catalytic
functions of nucleic acids



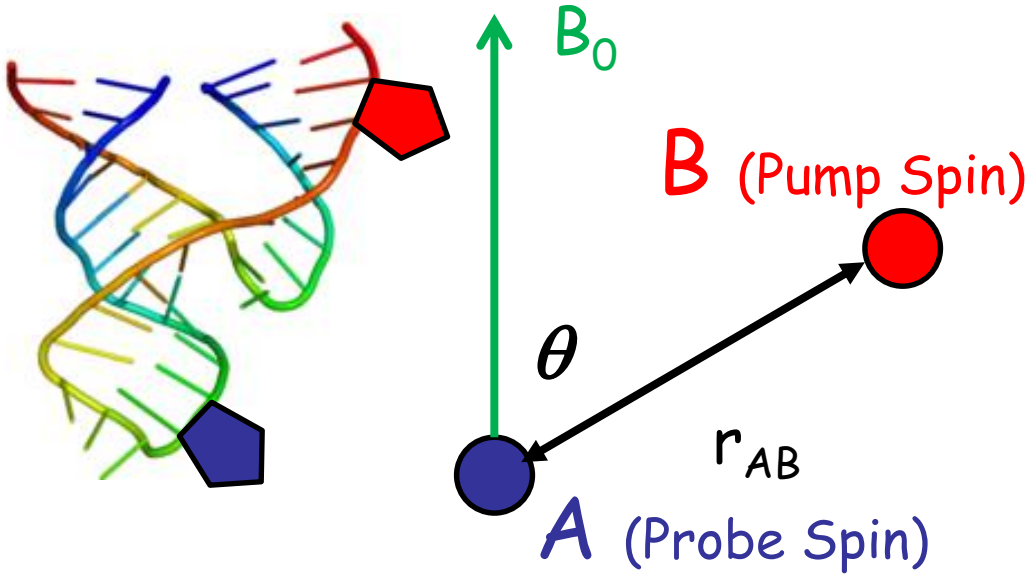


X-ray

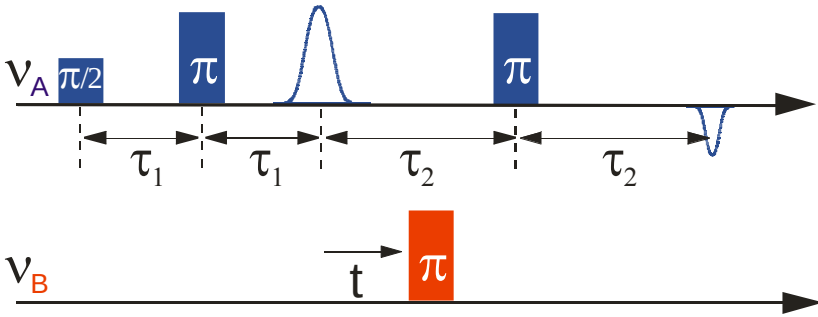
NMR, CD, IR
FRET, MD

EPR

Pulsed Electron Electron Double Resonance



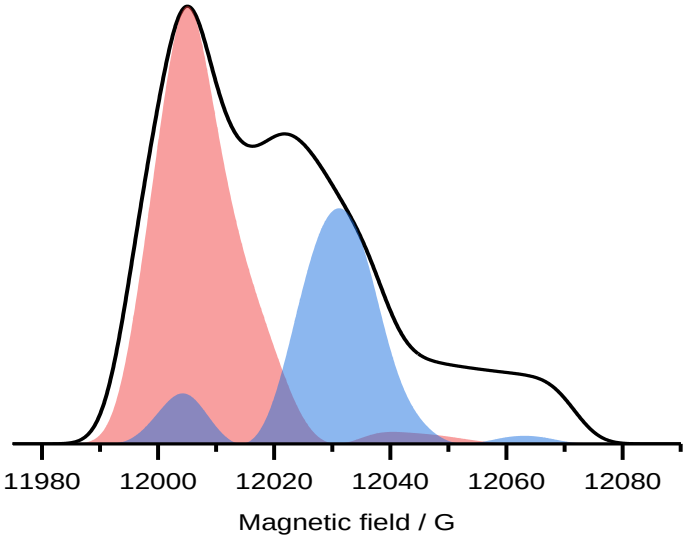
A. D. Milov, K. M. Salikov, M. D. Shirov.
Fiz. Tverd. Tela, **23**, 975 (1981)



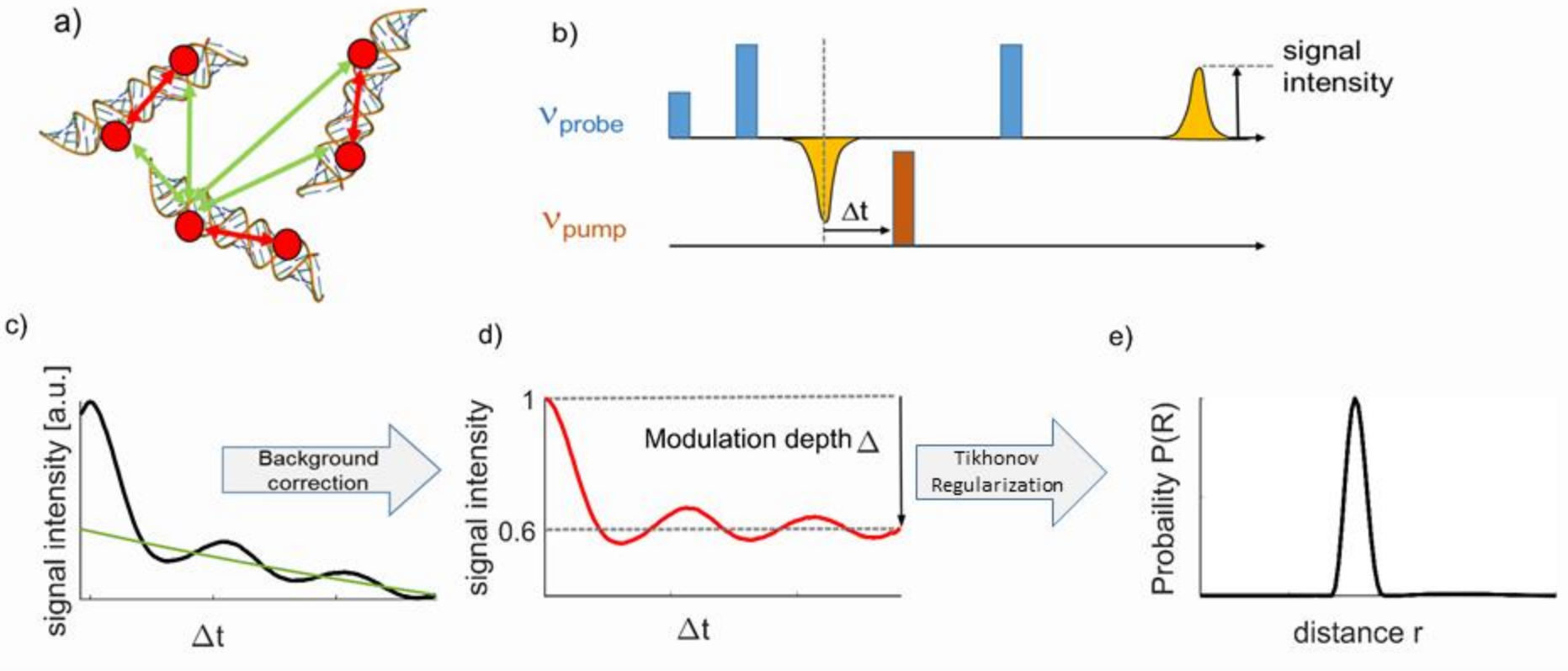
G. Jeschke, H.W. Spiess et al.,
JMR, **142**, 331 (2000)

$$\omega_{ee} = \frac{2\pi \cdot C}{r^3}(1 - 3\cos^2\theta)$$

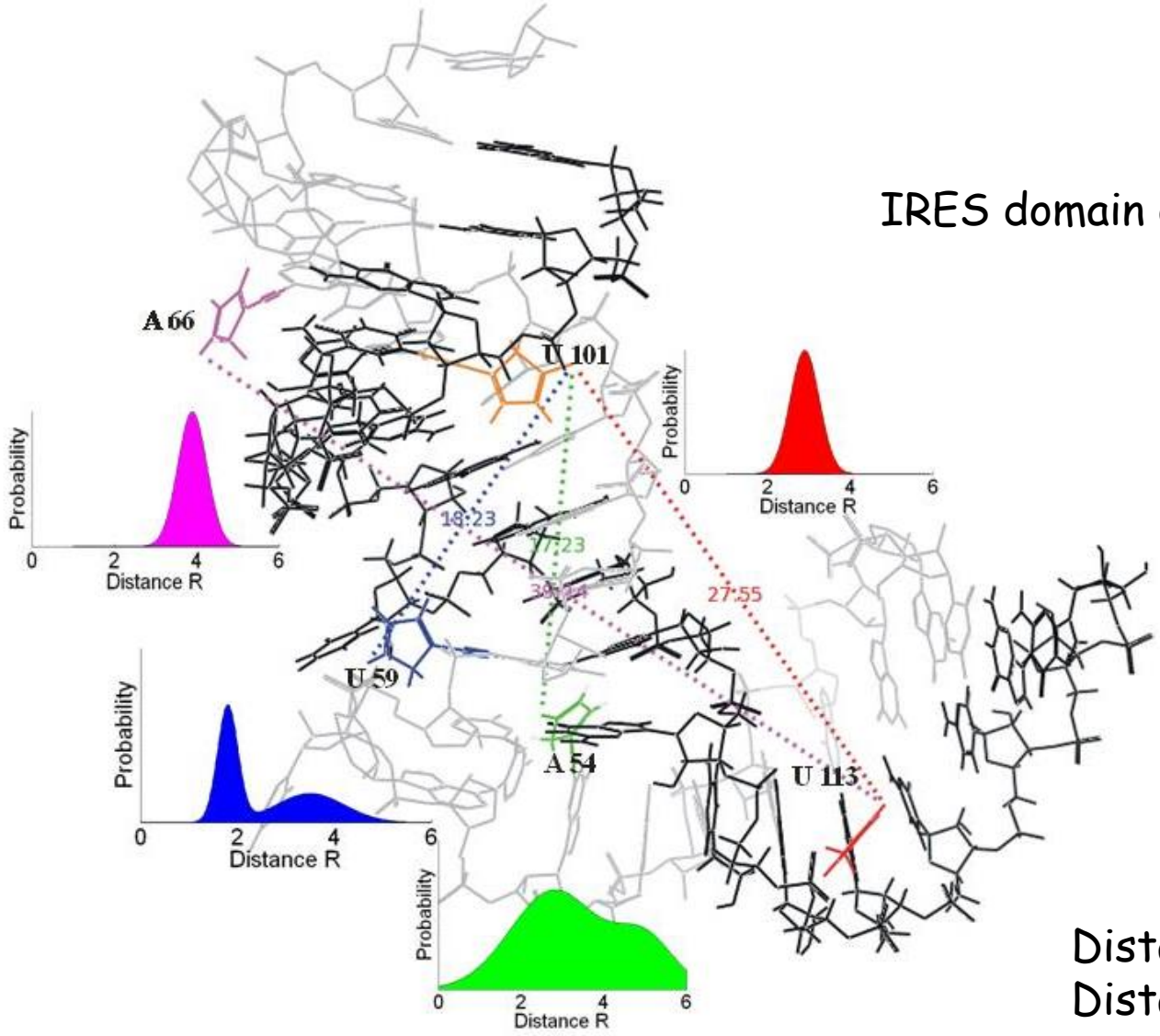
$$C = 52.04\ \text{MHz} \cdot \text{nm}^3$$



Analysis of PELDOR/DEER time traces



Distances and Distance Distributions

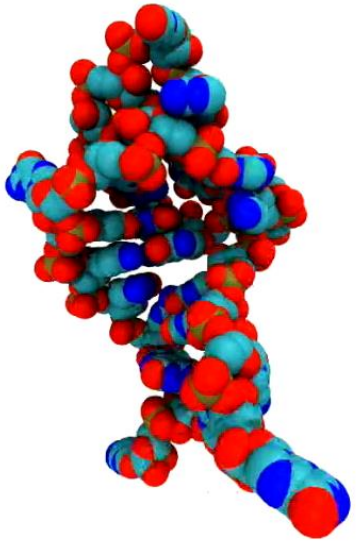


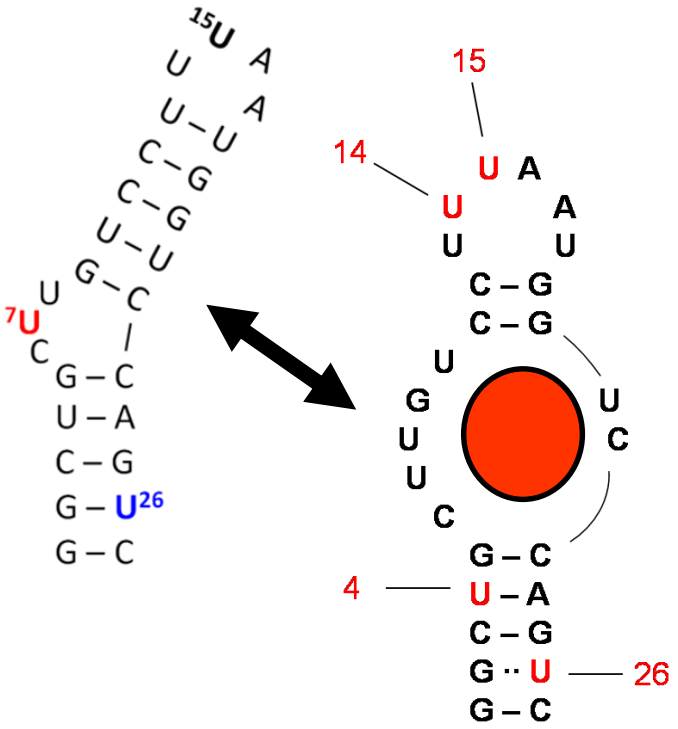
IRES domain of Hep Virus

Distances &
Distance Distributions

Conformational dynamics of RNA

Neomycin Binding Aptamer

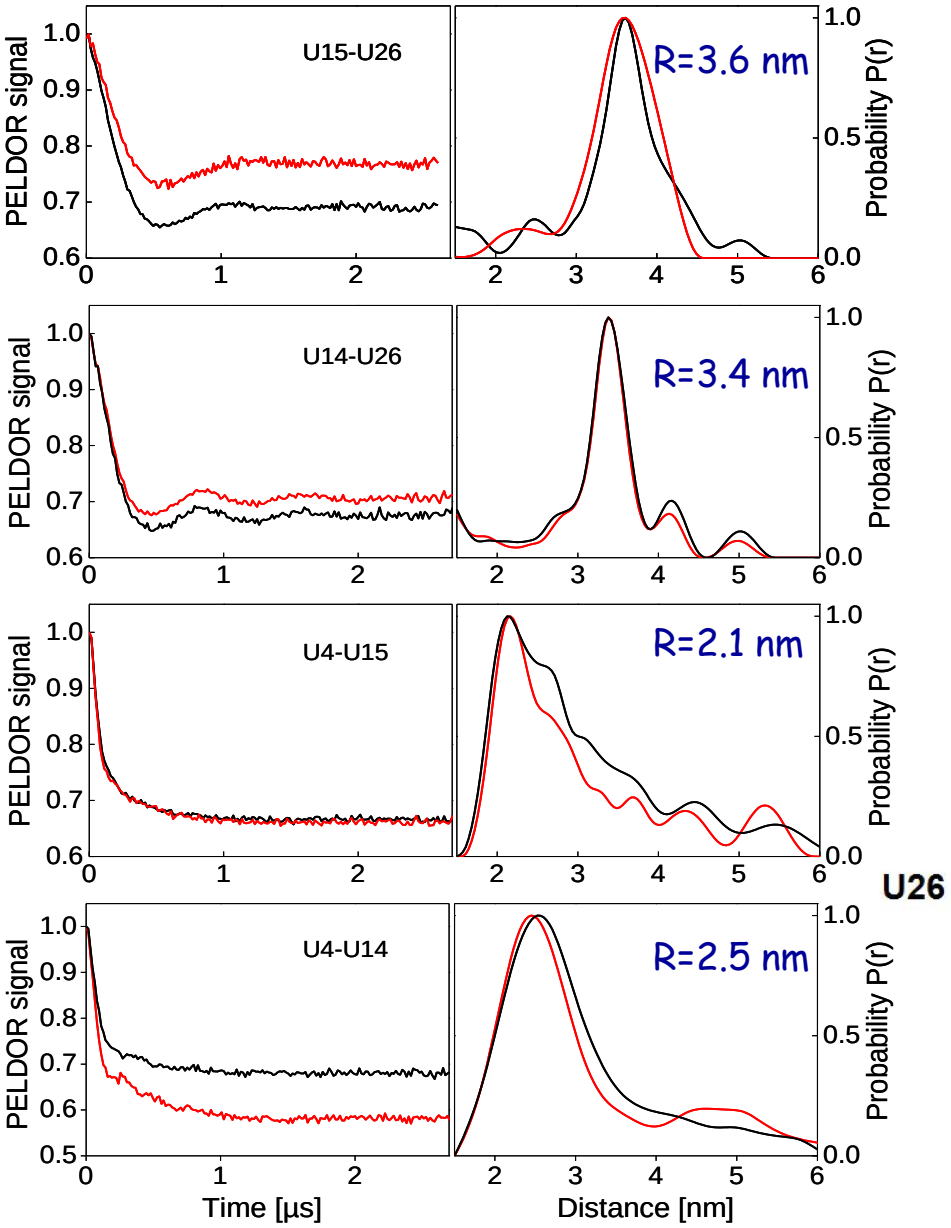


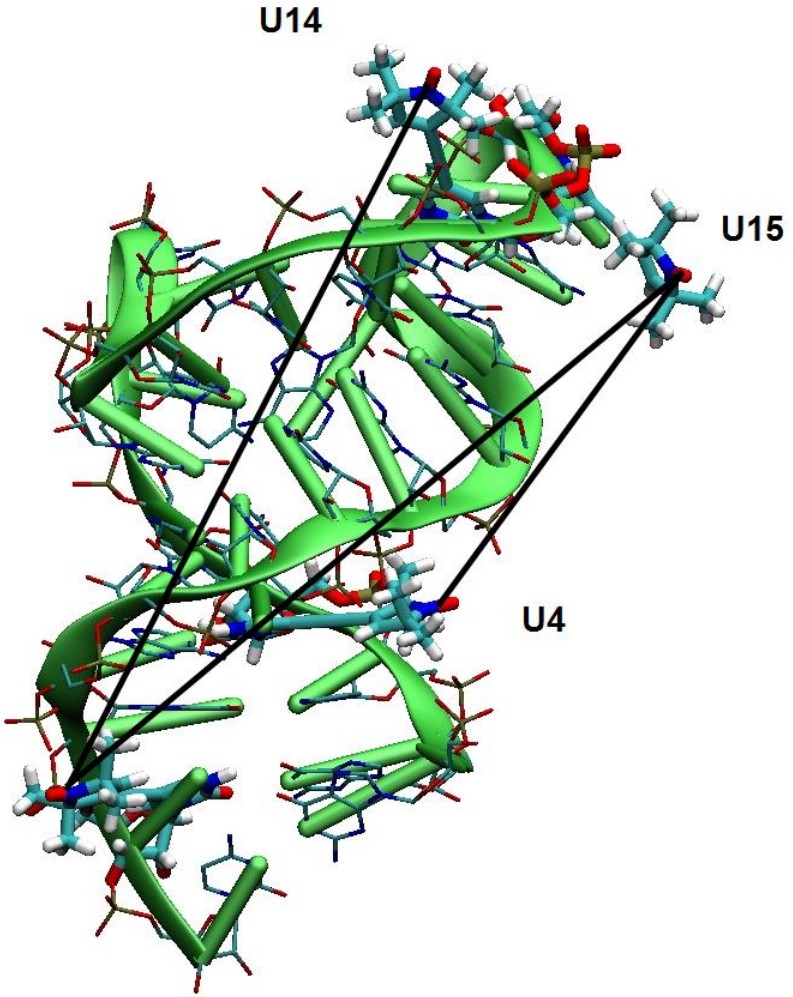


L. Stelzl, G. Hummer et al.
J. Chem. Theory Comput. **2017**, *13*, 3927, 6328

Nature Protocoll (2007)
NAR (2007)

Conformational dynamics of RNA



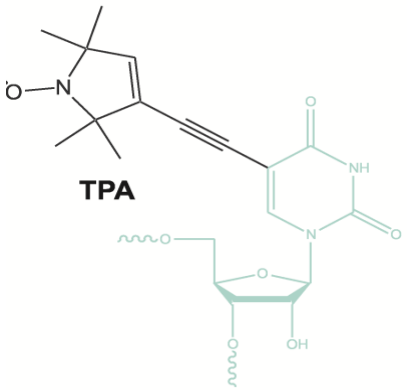


Wöhnert, J. et al Angewandte 2011

JACS (2011)

Spin labels for Nucleic Acids

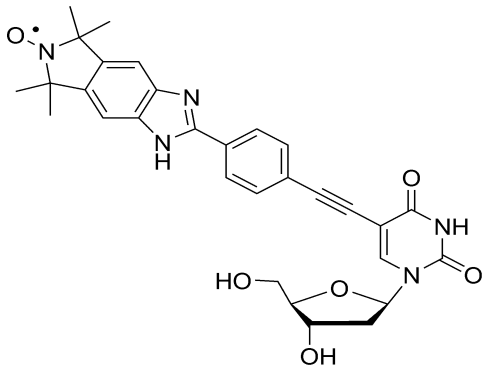
Joachim Engels (Uni Frankfurt)

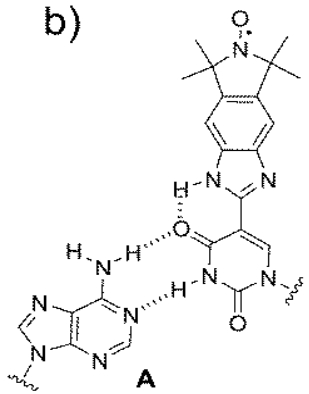


Rotate around triple bond

Nature Protocoll (2007)
NAR (2007)

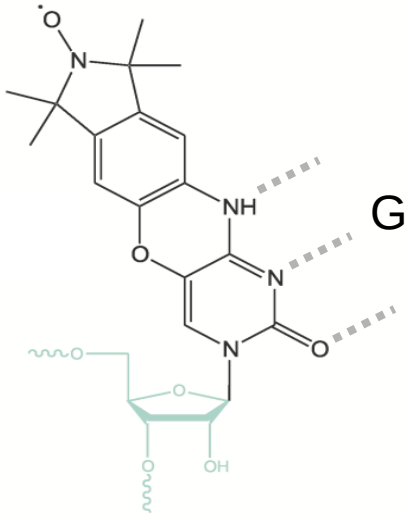
Snorri Sigurdsson (University of Iceland)





Rigid in double stranded structures

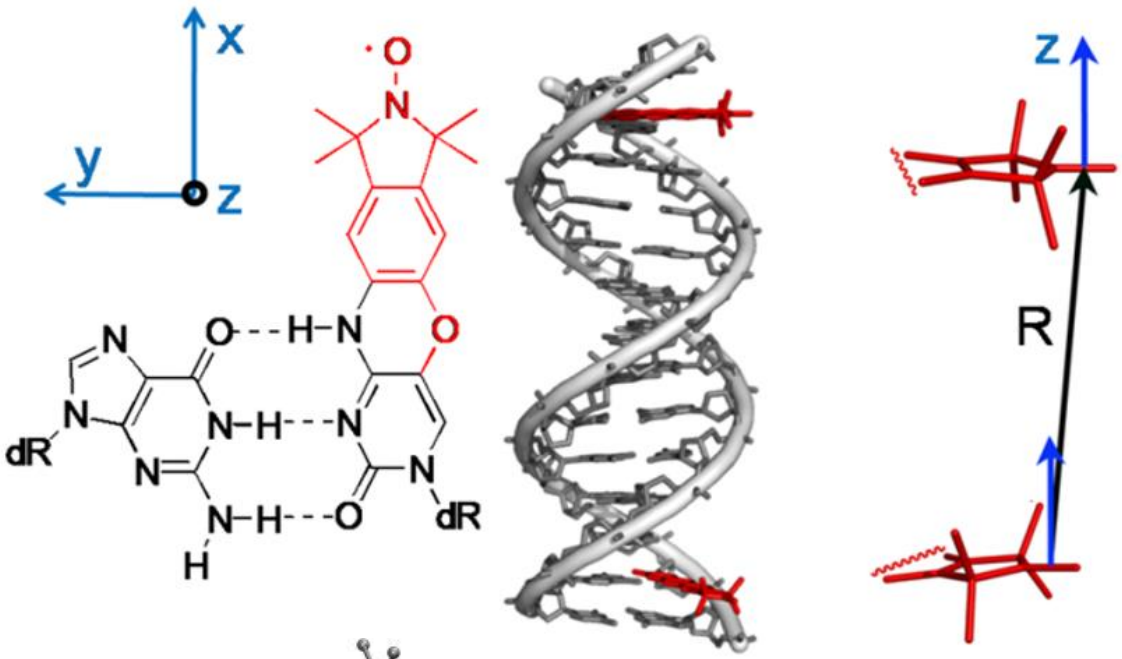
Chem. Eur. J. (2014)



Angewandte (2009)
JACS (2011)

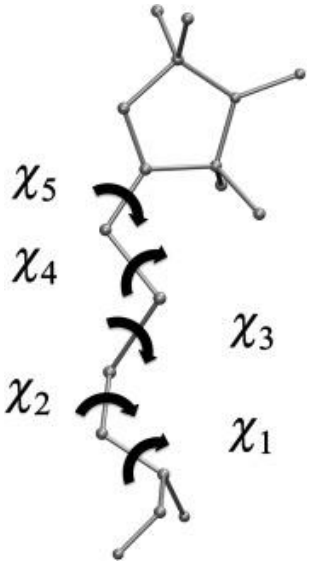
- All nucleotides in DNA and RNA can be spin-labeled
- Different degrees of rotational flexibility of spin label
- Different orientation with respect to nucleotide
- Synthesis with modified nucleobase

A rigid spin-label analog of cytidine



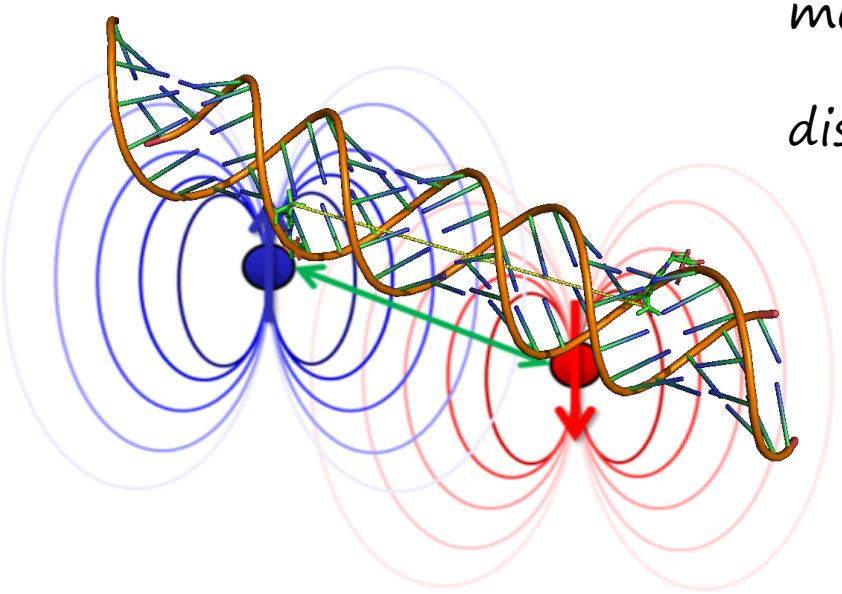
Distance between two unpaired electron spins can be directly converted in structural information of nucleic acid molecule

Snorri Sigurdsson
(University of Iceland)



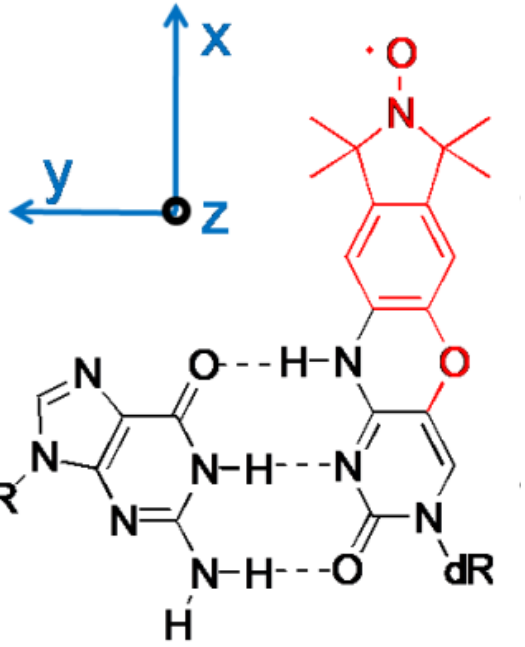
Much better defined distance compared to the classical used MTSL spin label for proteins

Distance measurements of spin-labeled NA



Magnetic dipole-dipole interaction measured by pulsed EPR

distances of ~ 1-10 nm

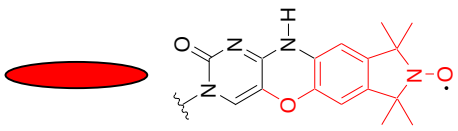


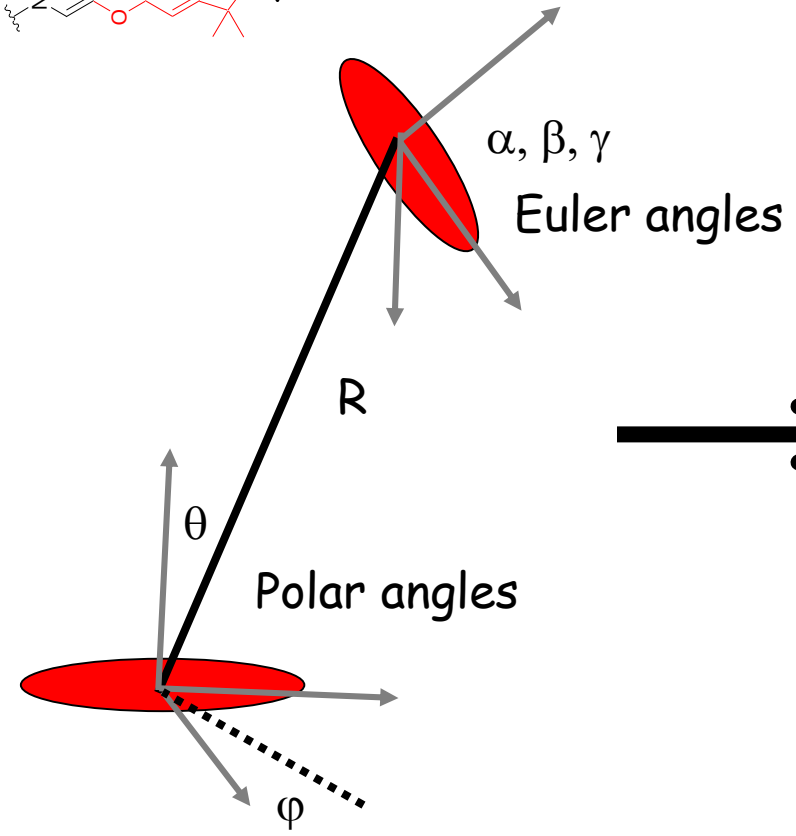
Cytidine analog spin label **rigidly** incorporated into ds DNA or ds RNA

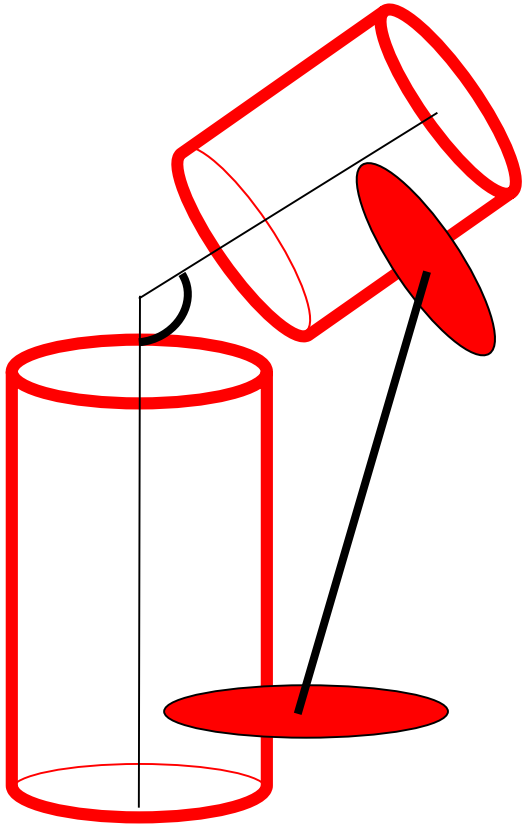
Collaboration with Snorri Sigurdsson (Uni. of Iceland)

Angewandte (2009)

Investigation of conformational flexibility of dsDNA

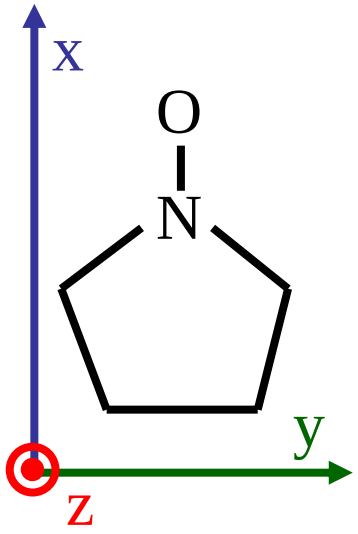






Determination of distance R and geometry of spin labels

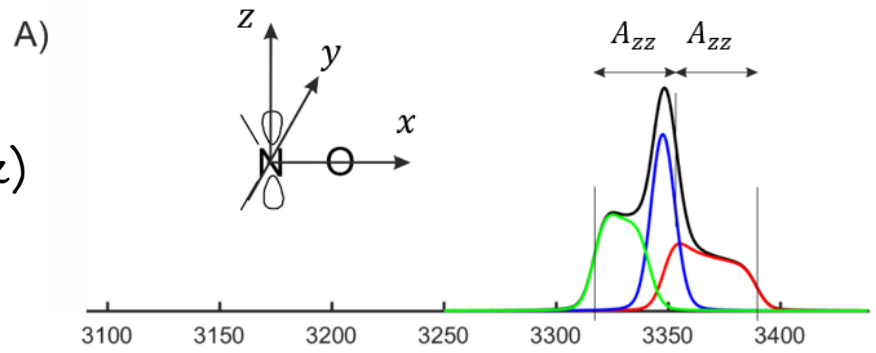
Relates directly to structure of helical parts in RNA / DNA



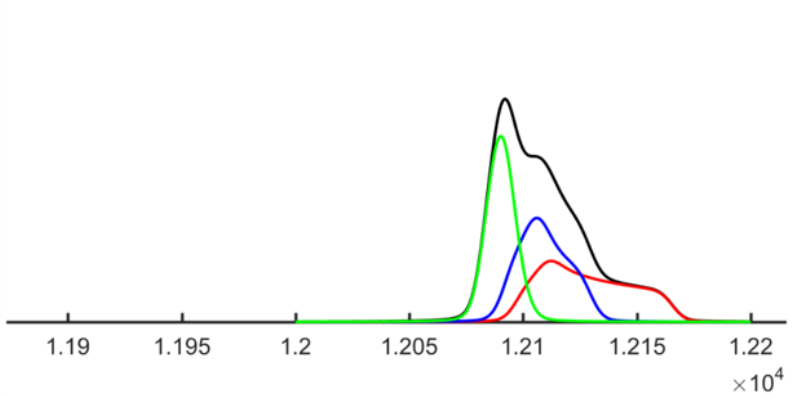
^{14}N (I=1)
3 hyperfine lines (red, blue, green)

Anisotropic g-tensor from spin-orbit coupling (depending strongly on field)

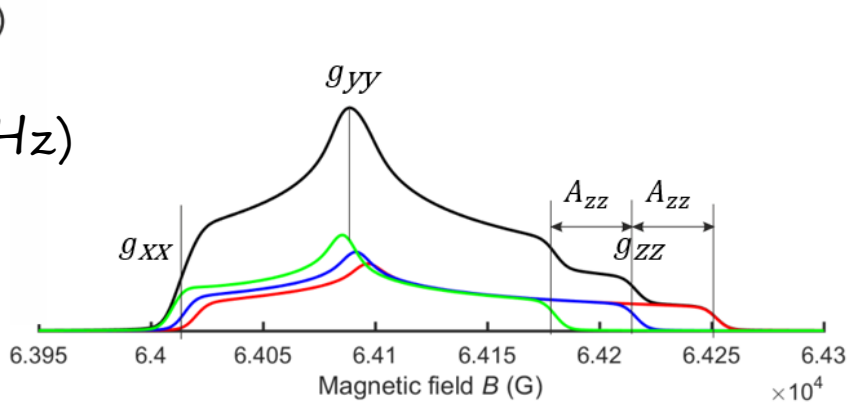
X-band (9 GHz)
0.3 T

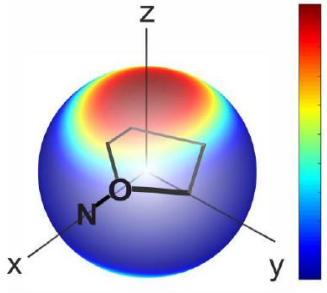


Q-band (35 GHz)
1.2 T

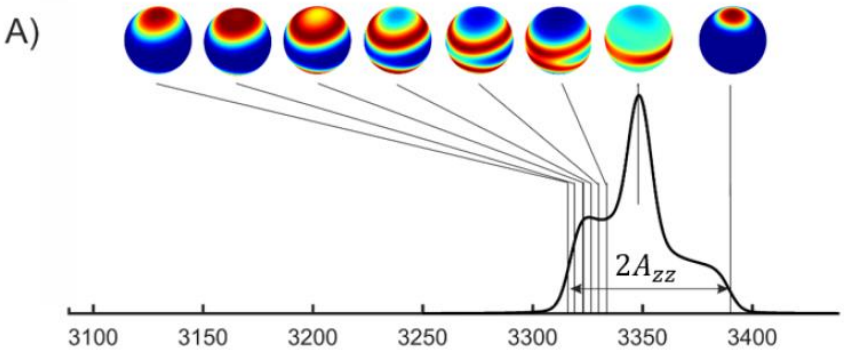


G-band (180) GHz)
6.4 T



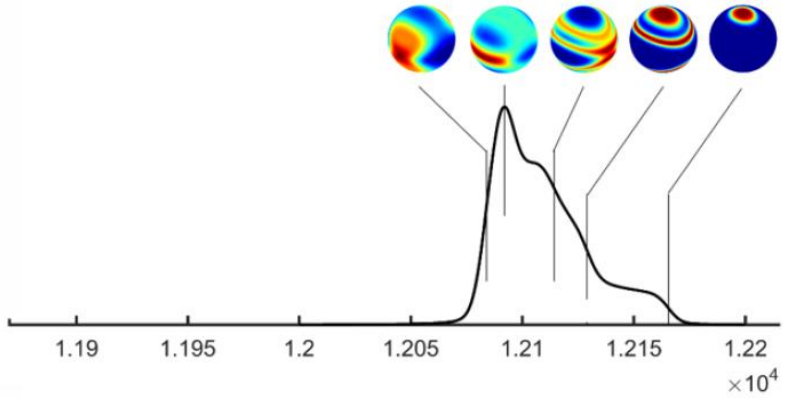


X-band (9 GHz)

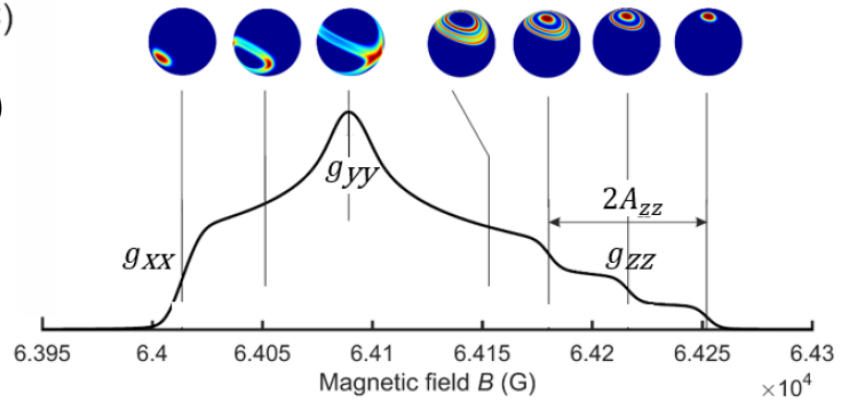


Selection of specific molecular orientations with respect to the magnetic field by frequency of MW pulses

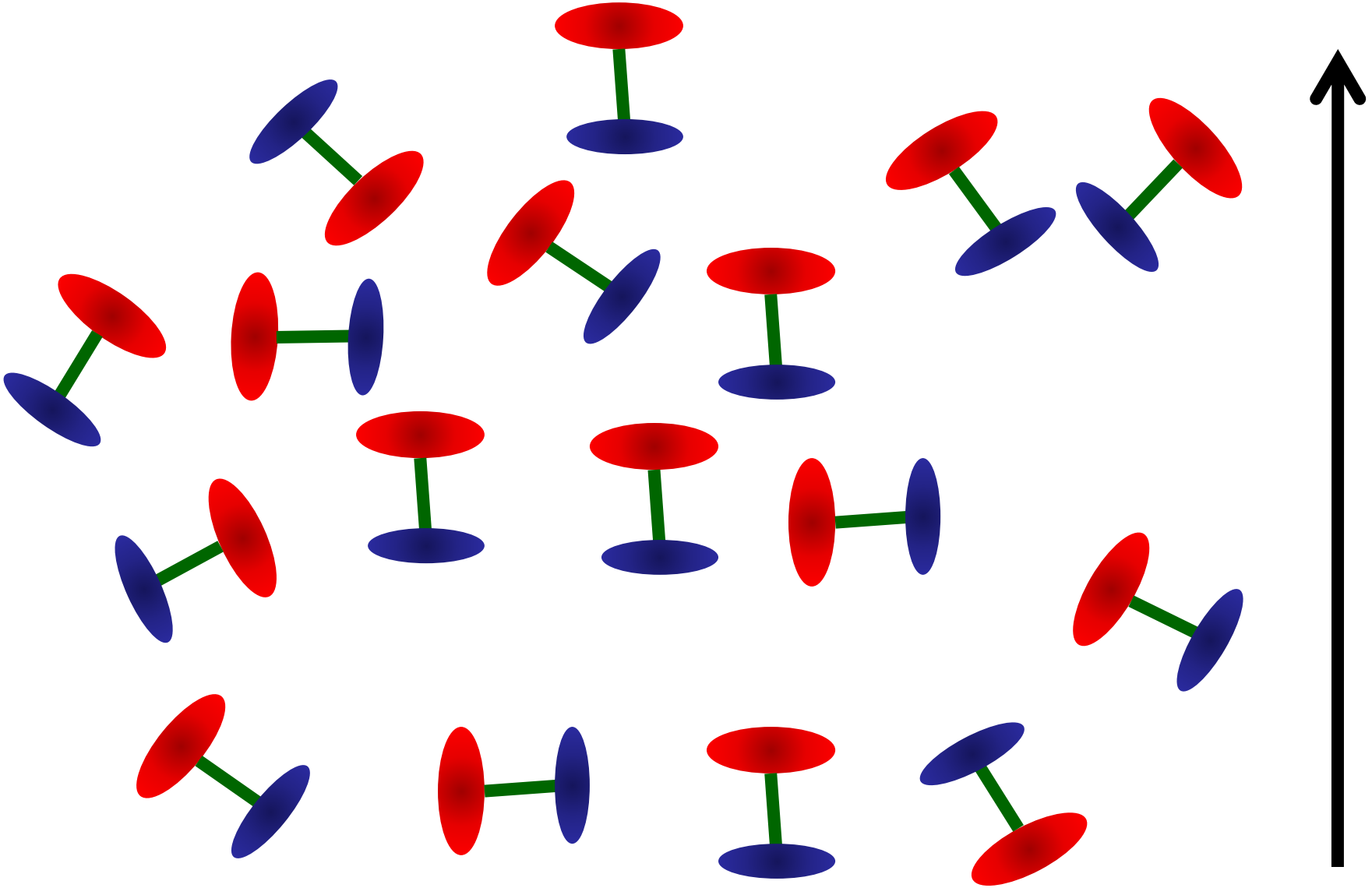
Q-band (35 GHz)



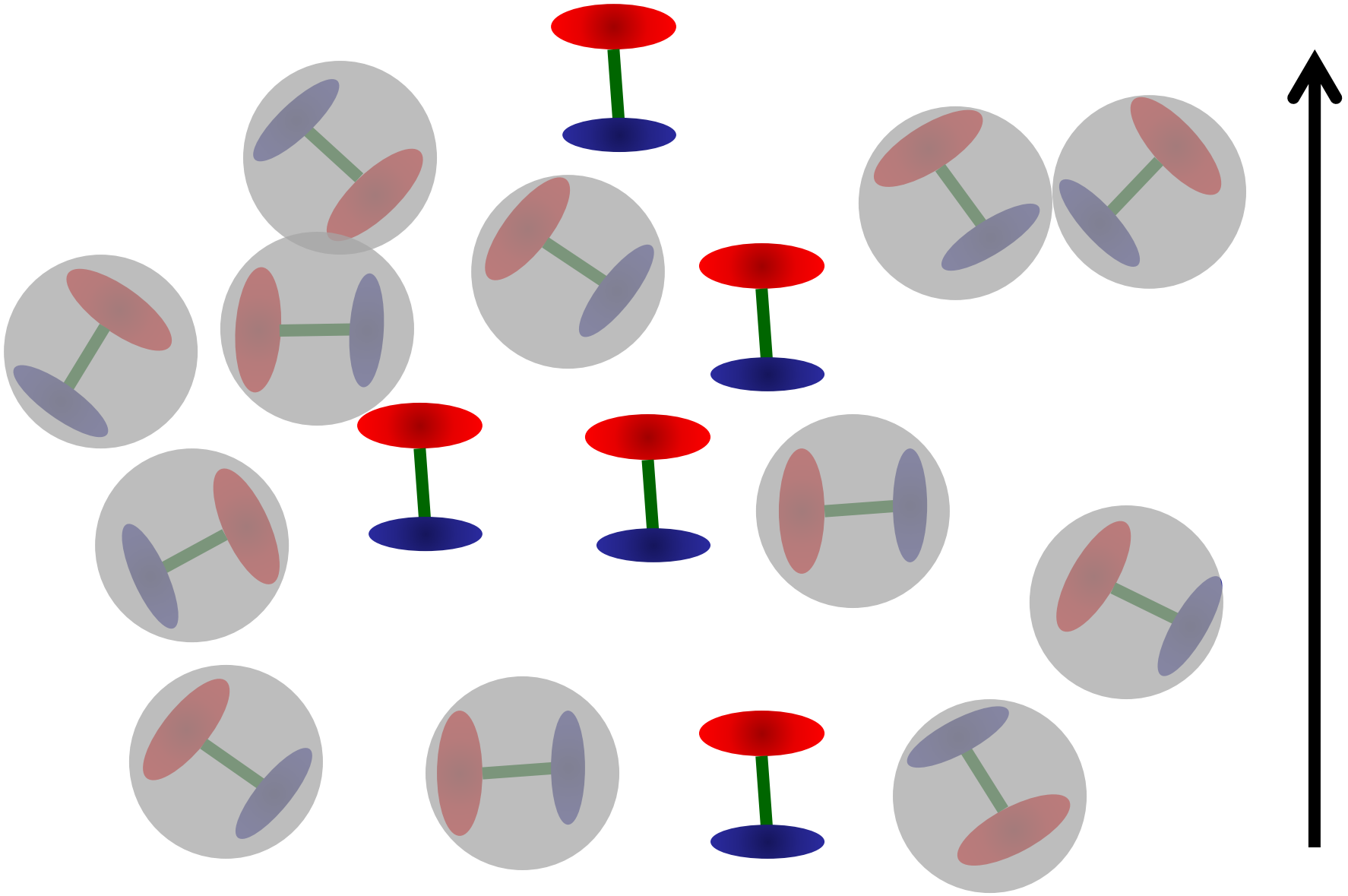
G-band (180) GHz)



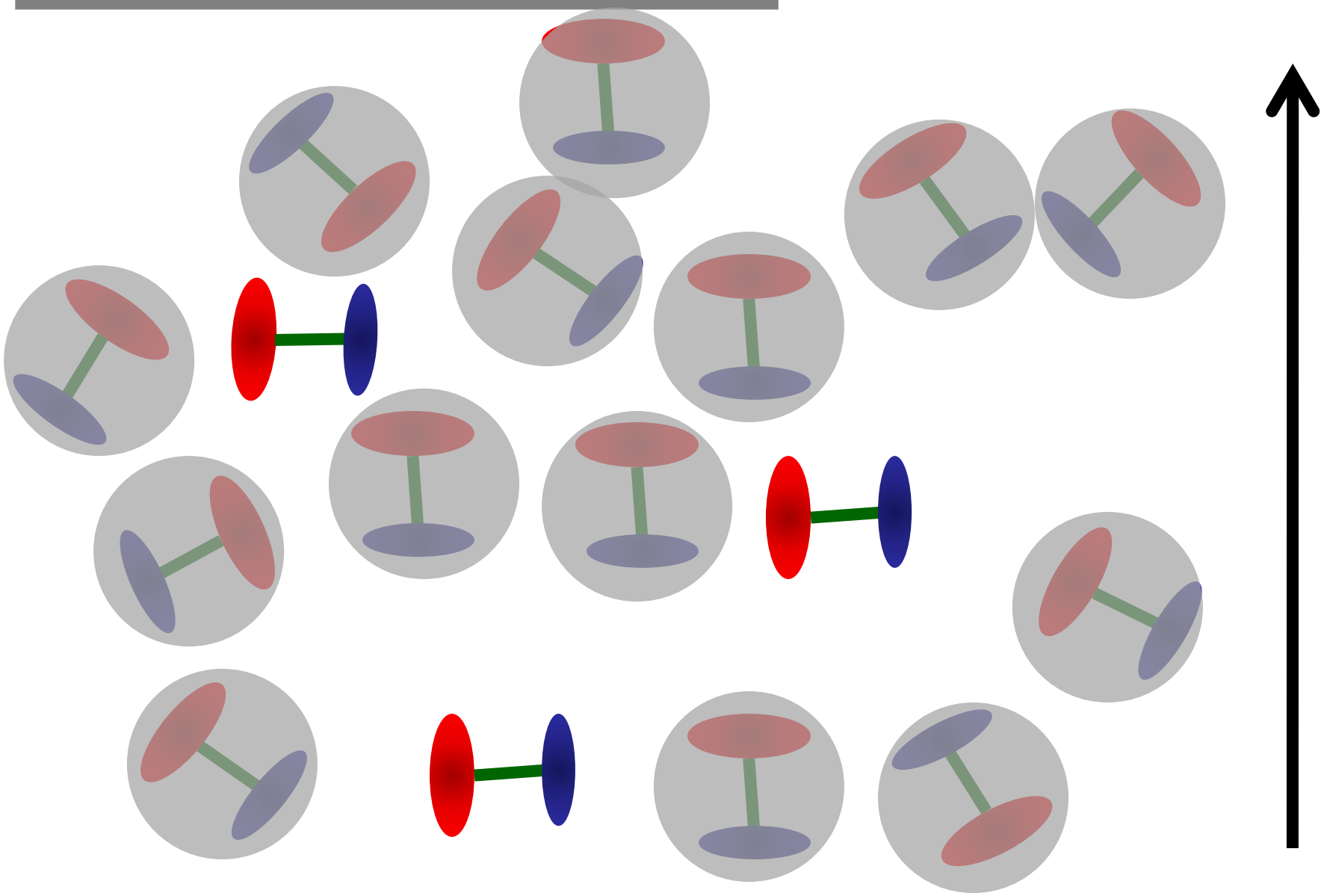
Randomly oriented powder sample



Select only small g value

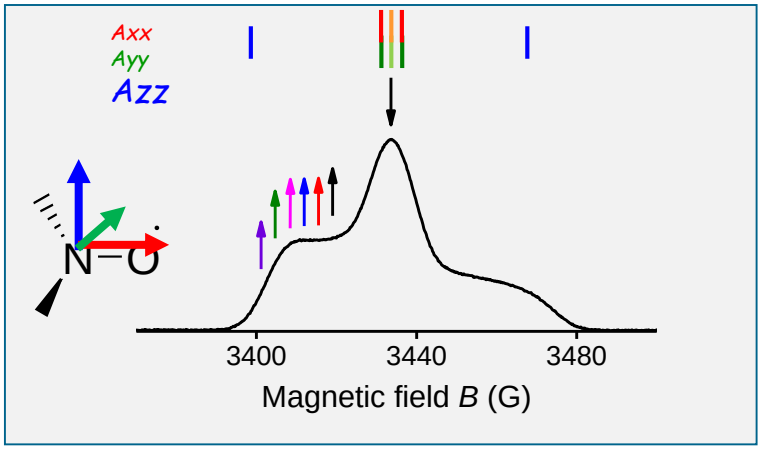


Select only large g-value

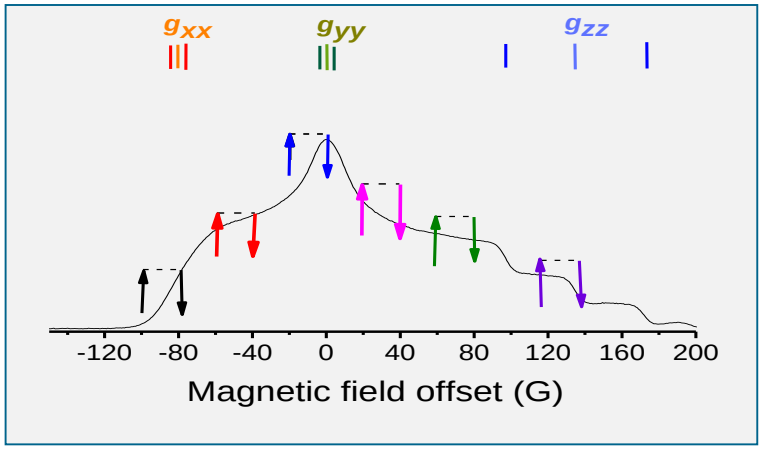


Multifrequency & Multifield PELDOR

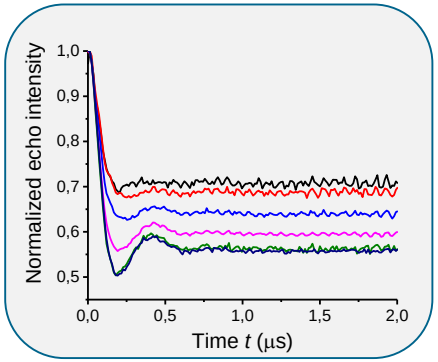
X-band (0.3 T / 9 GHz)



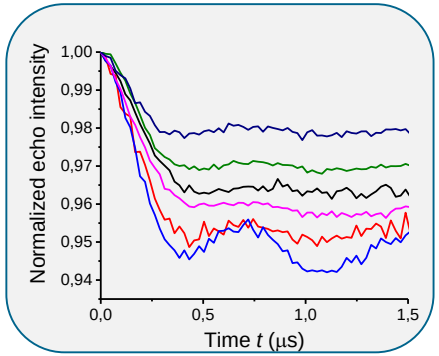
G-band (6.4 T / 180 GHz)



Anisotropic ^{14}N resolution



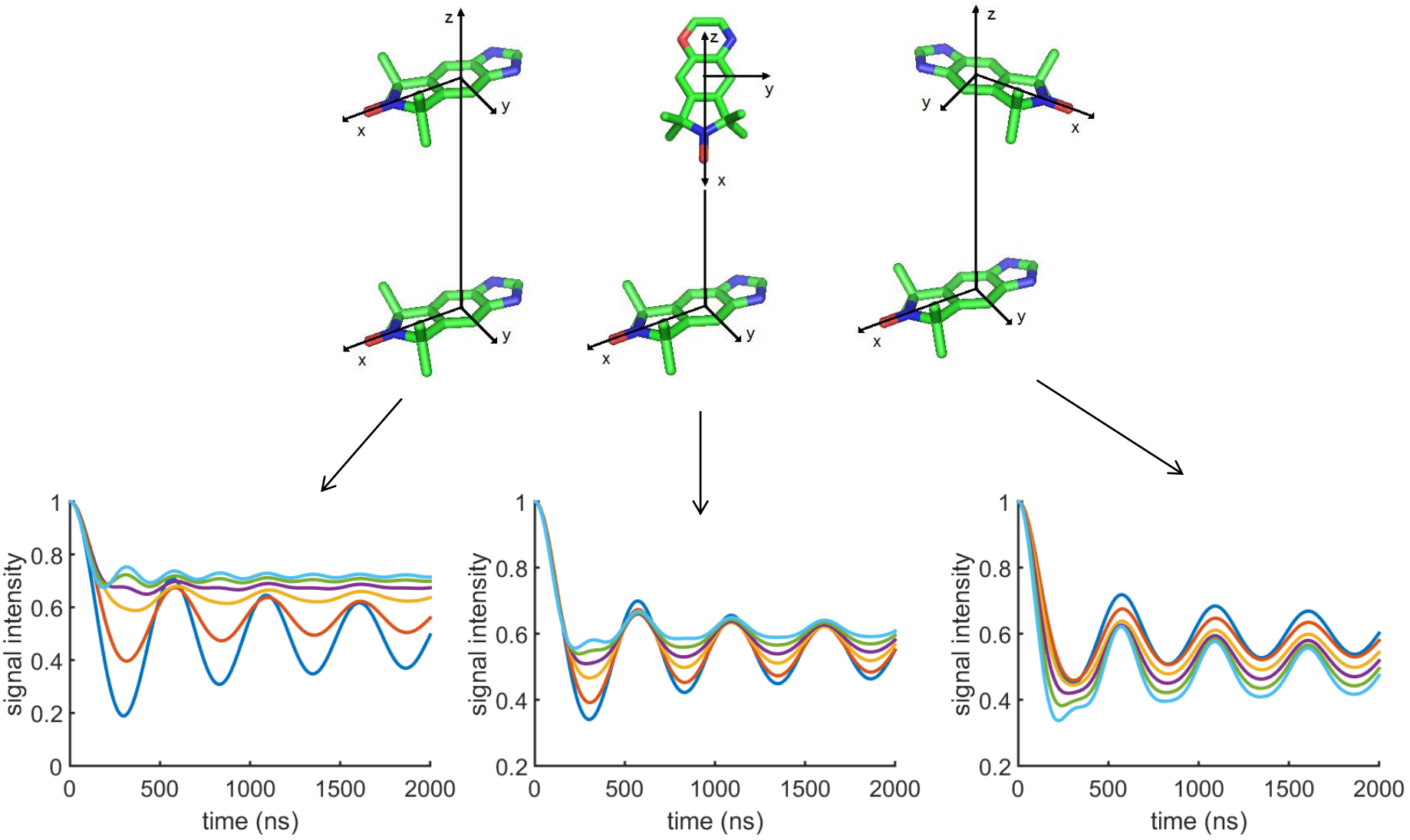
Anisotropic g tensor resolution



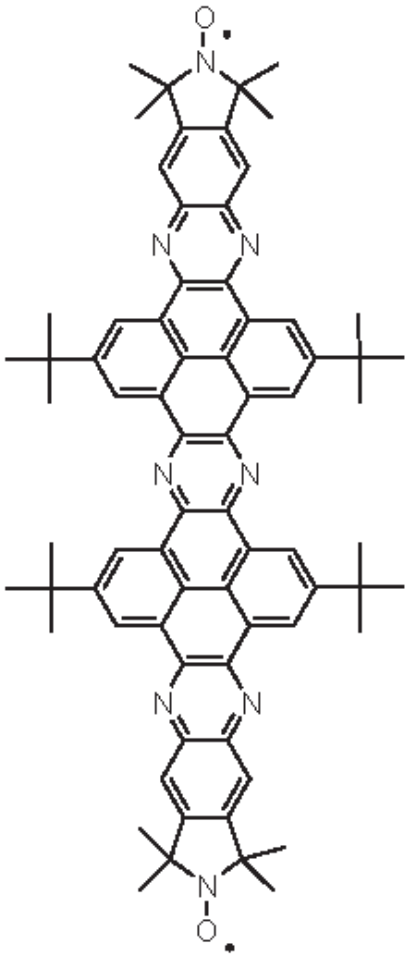
Multi-frequency / Multi-Field PELDOR Dataset

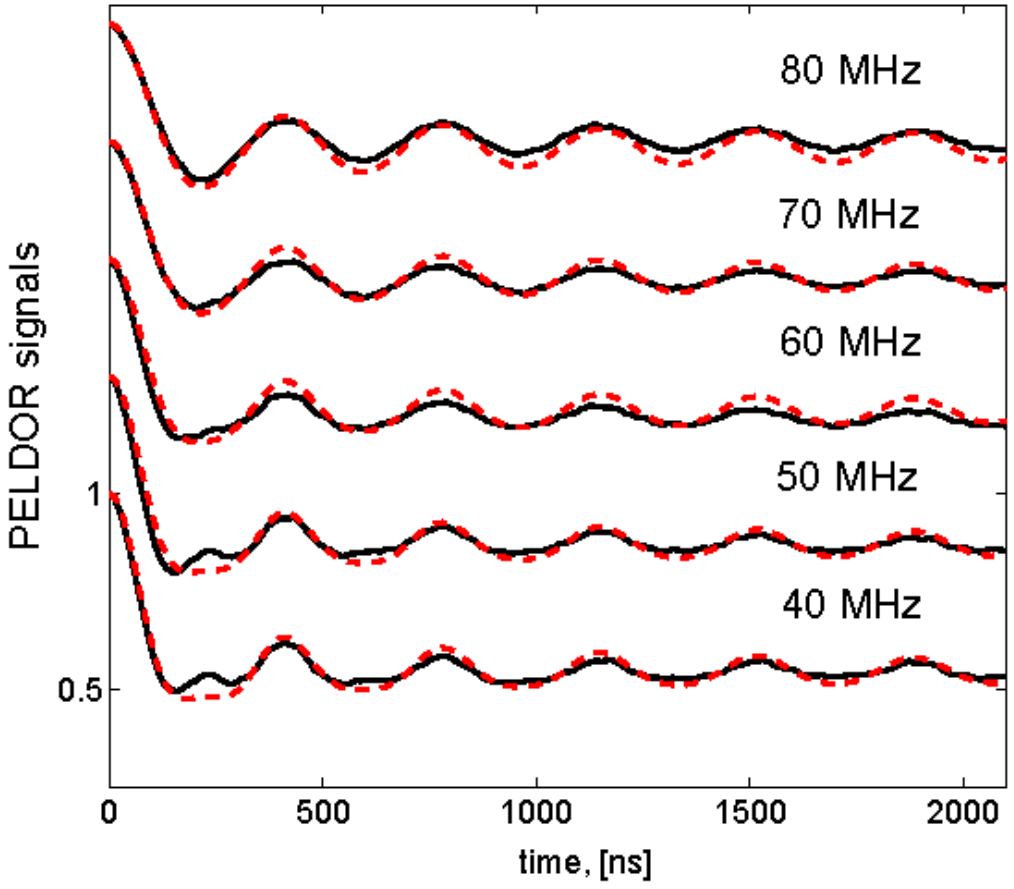
JMR (2015)

Unique pattern for a given distance / geometry



Test with rigid planar biradical

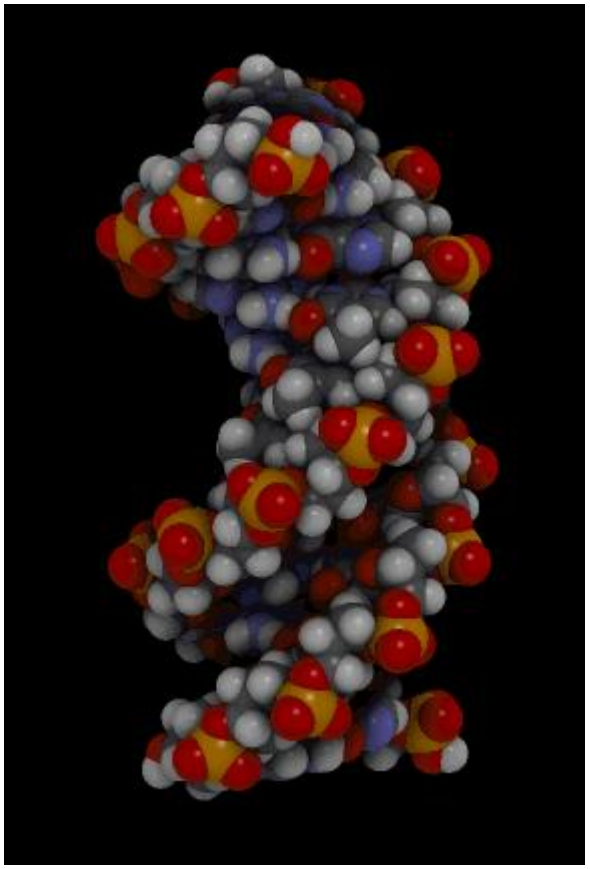




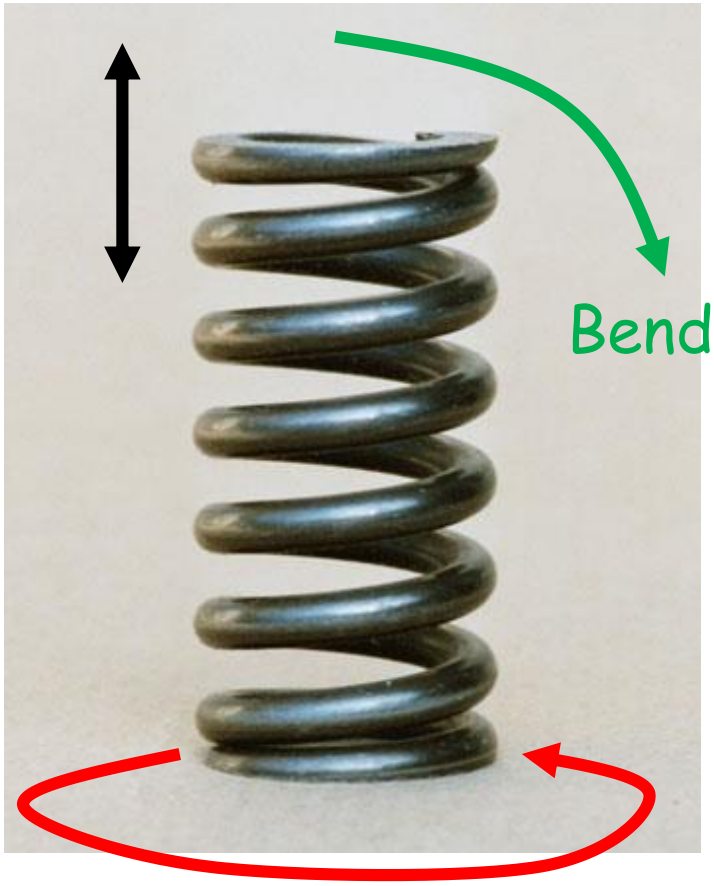
Unique solutions can be obtained for rigid molecules with a single conformation, if experiments performed at several magnetic field strengths.

PCCP 2013

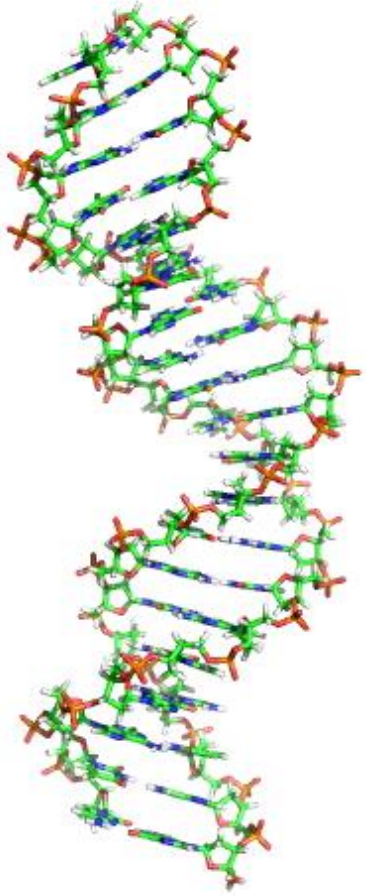
Determination of internal dsDNA dynamics



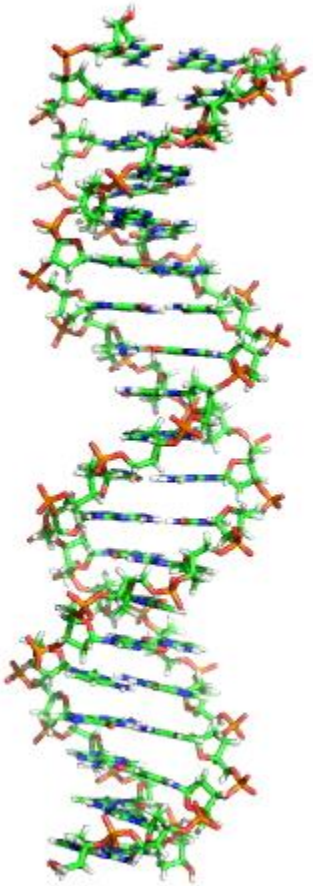
Stretching



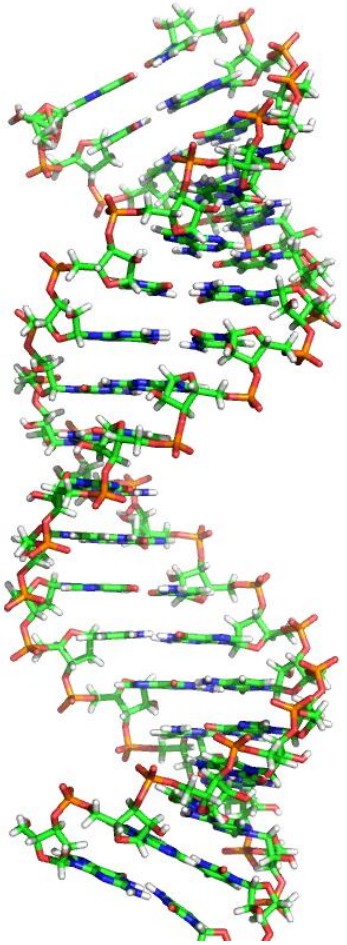
Investigation of dynamics of dsDNA



Bending
(Δa)

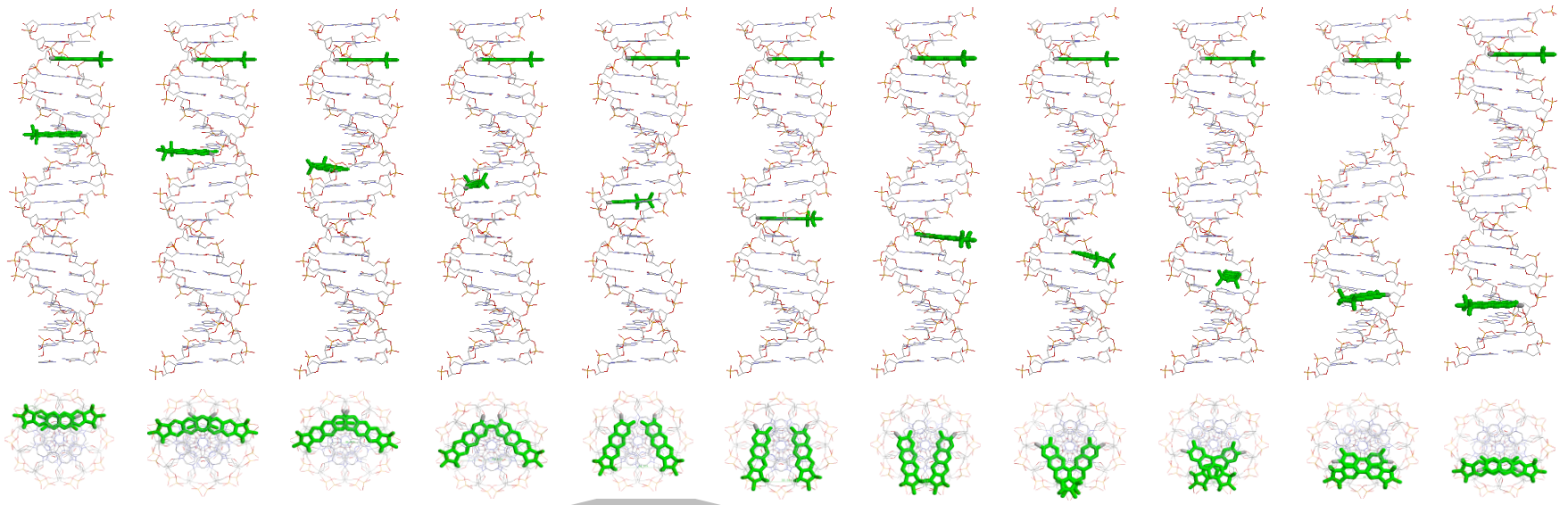


Twisting
Δr

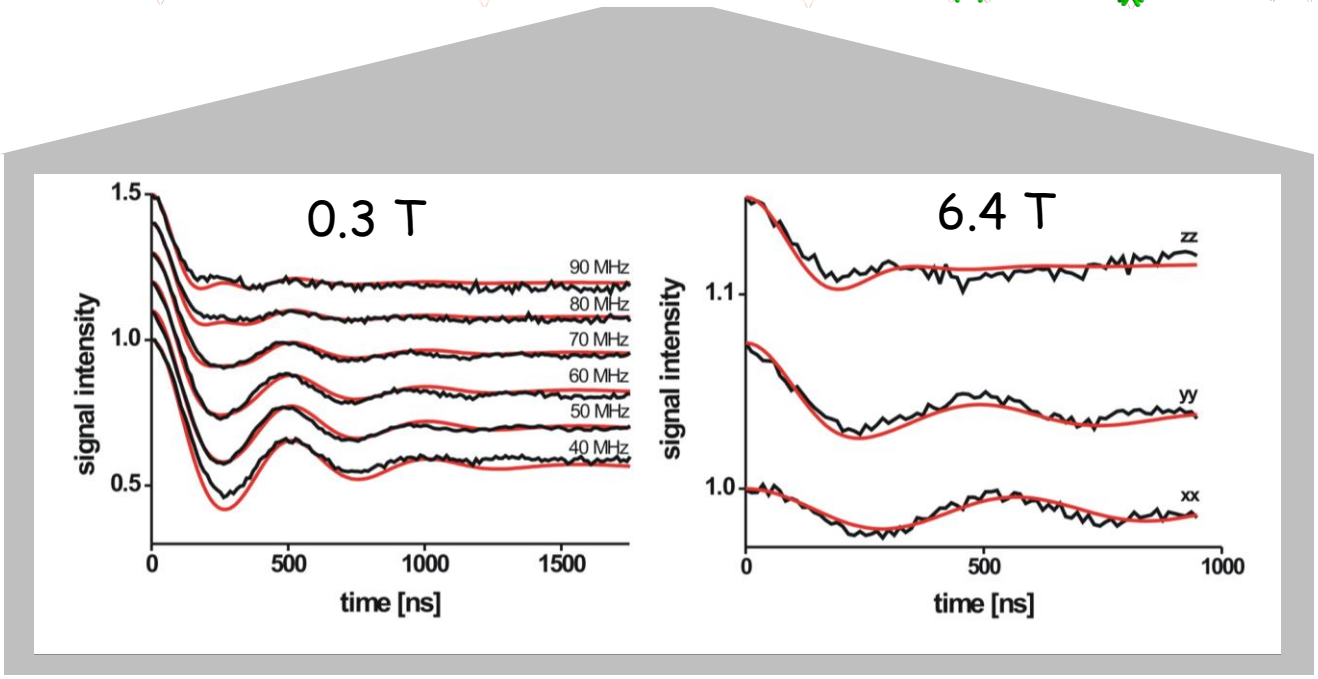


Stretching
Δh

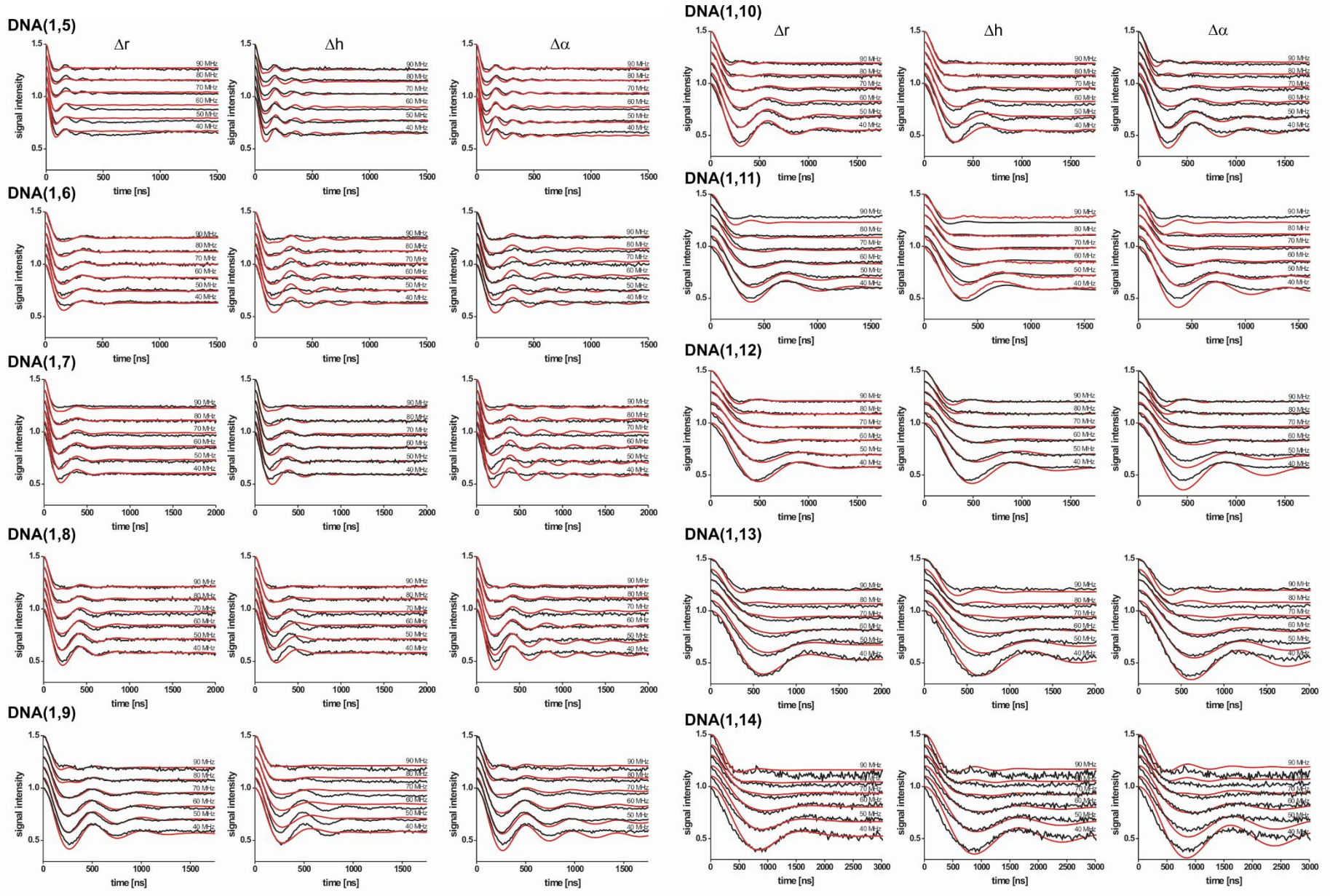
Set of samples with variable distance between spinlabels



Marko JACS (2011)



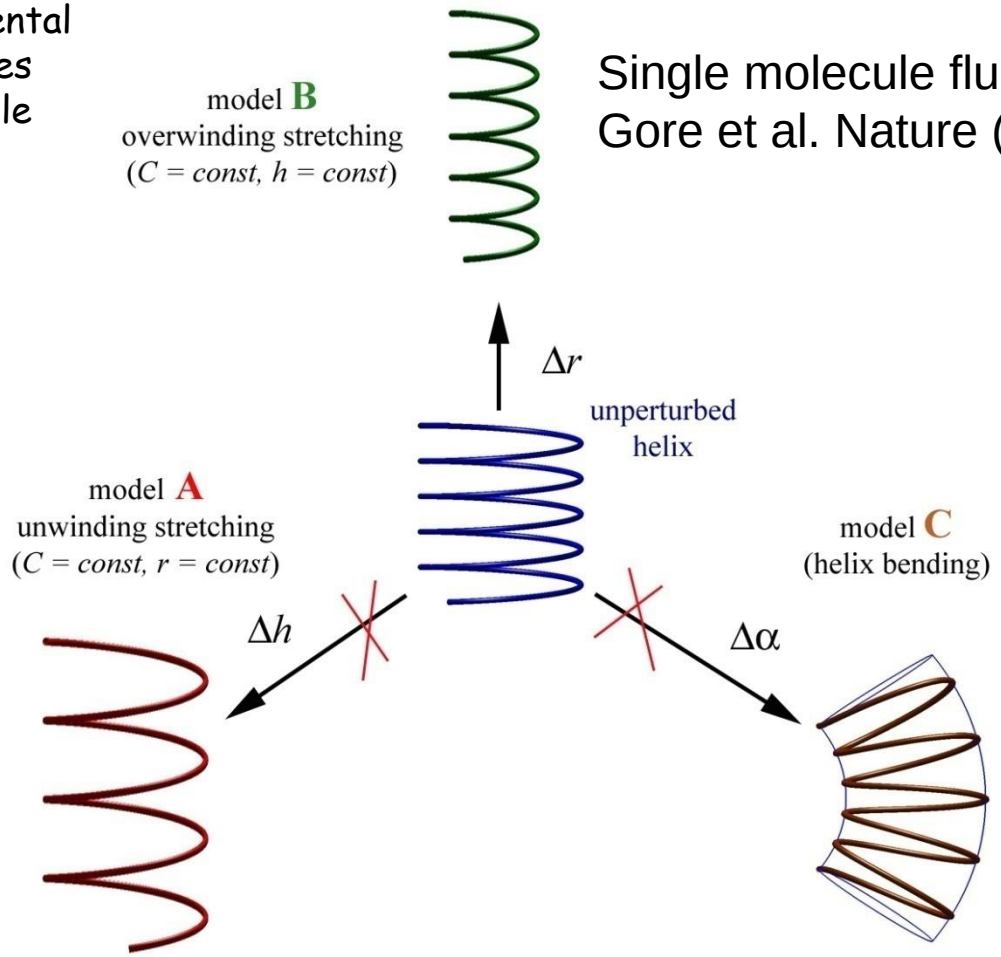
2D-PELDOR datasets and best model fits at 0.3 T



Models for conformational dynamics of dsDNA

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

JACS 2011

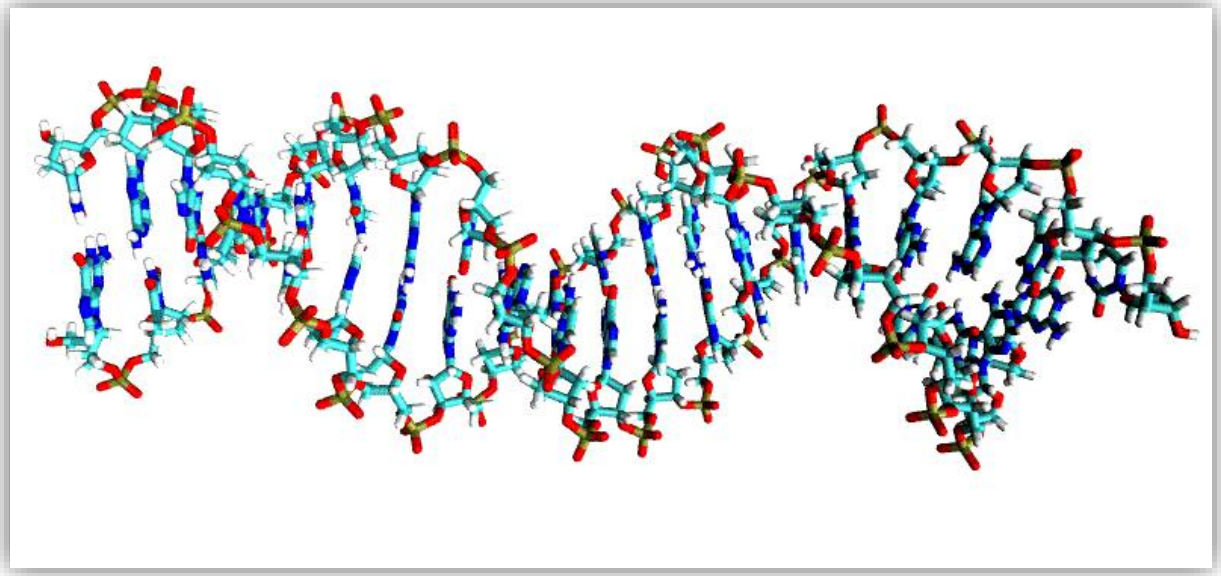


Single molecule fluoreszenz
Gore et al. Nature (2006)

SAXSI
Mathew-Fenn et al. Science (2008)

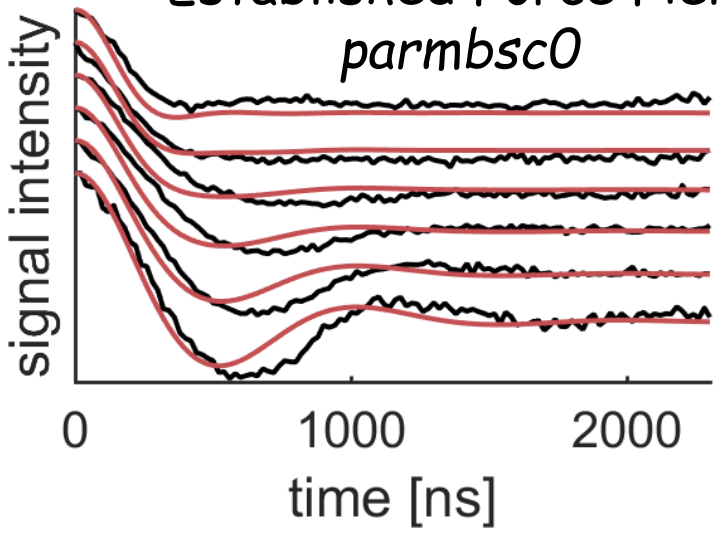
Modeling
Becker et al. Science (2009)

Comparison with MD simulations of dsDNA

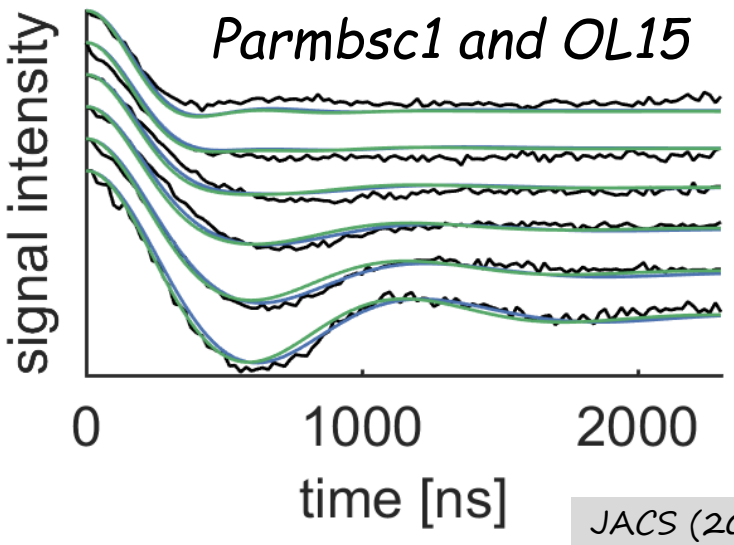


Collaboration with
G. Hummer
(MPI Biophysics,
Frankfurt)

Established Force Field
parmbsc0

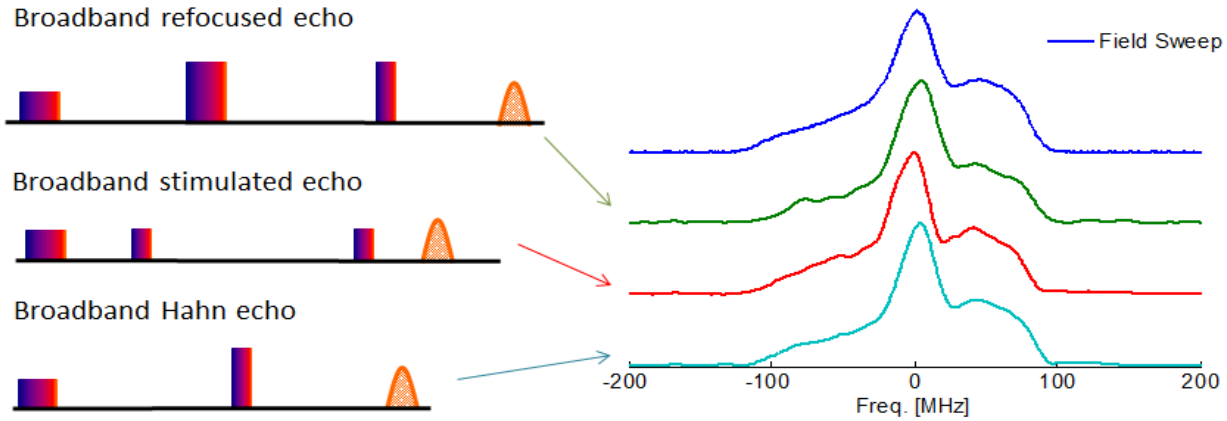


New Force Fields
Parmbsc1 and OL15

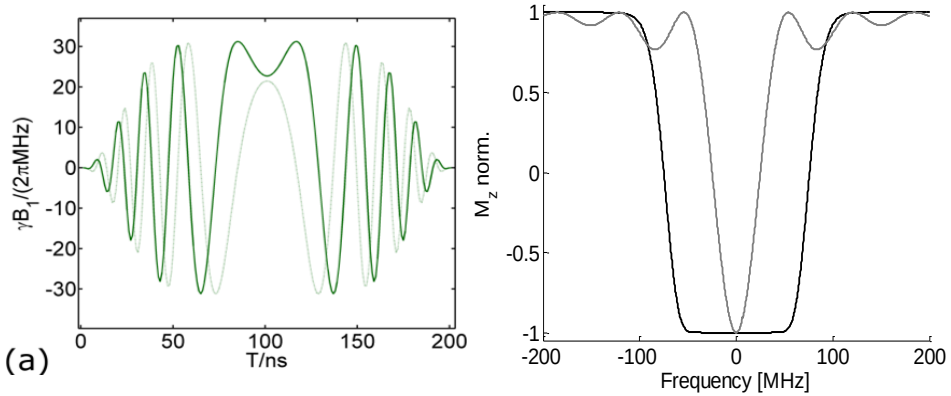


JACS (2017)

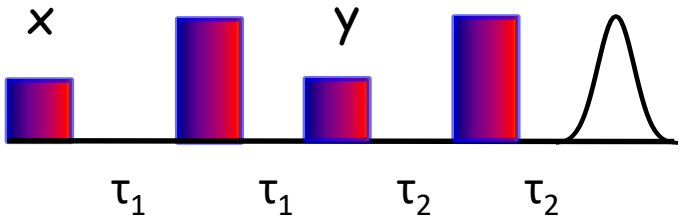
Echo sequences with broadband shaped pulses

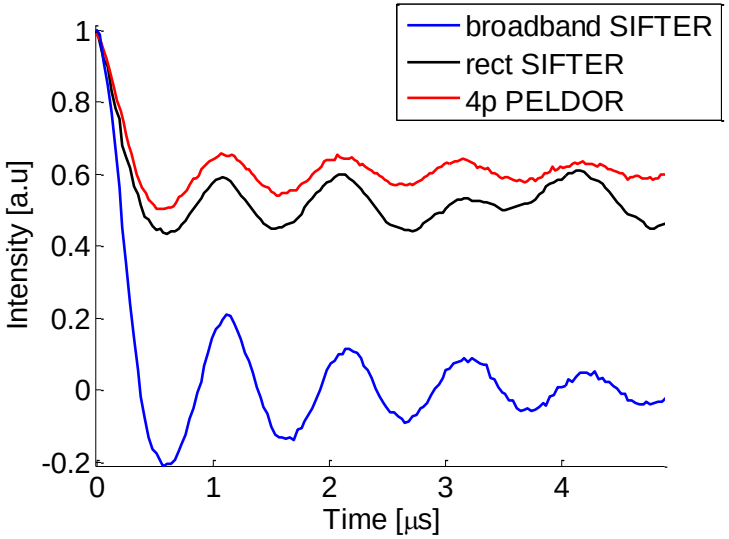


Full excitation of nitroxide spectra at X-band possible



Sech/tanh Pulses
Length of 200 ns
Bandwidth of 200 MHz



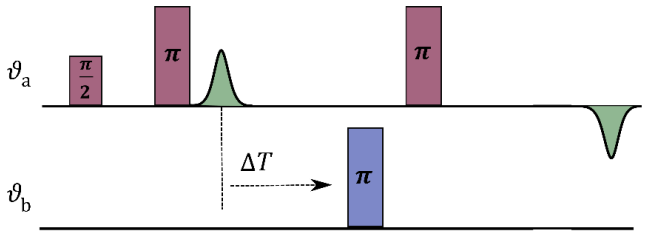


Angewandte (2014) JMR (2015)

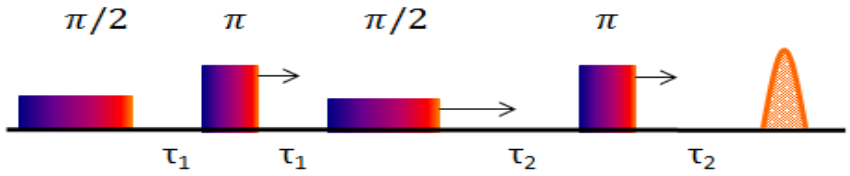
2D SIFTER experiment using broadband pulses

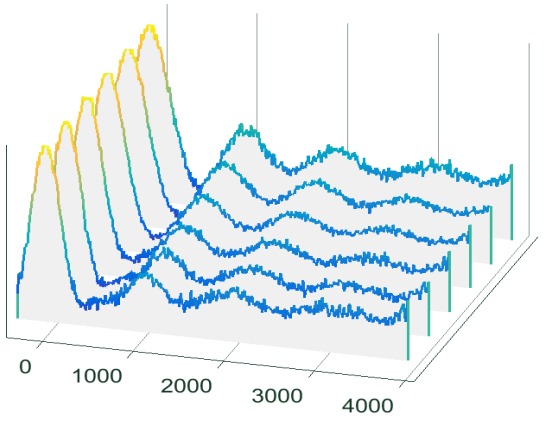
Doll, Jeschke PCCP 18, 23111 (2016)

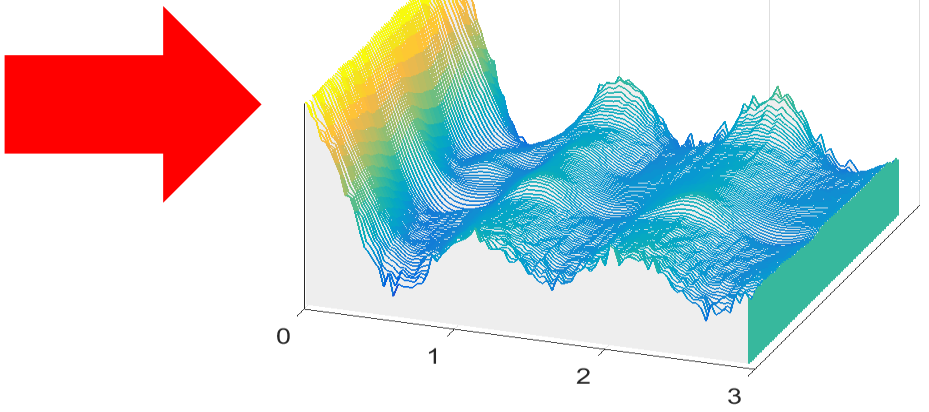
4 pulse PELDOR/DEER with several probe frequencies



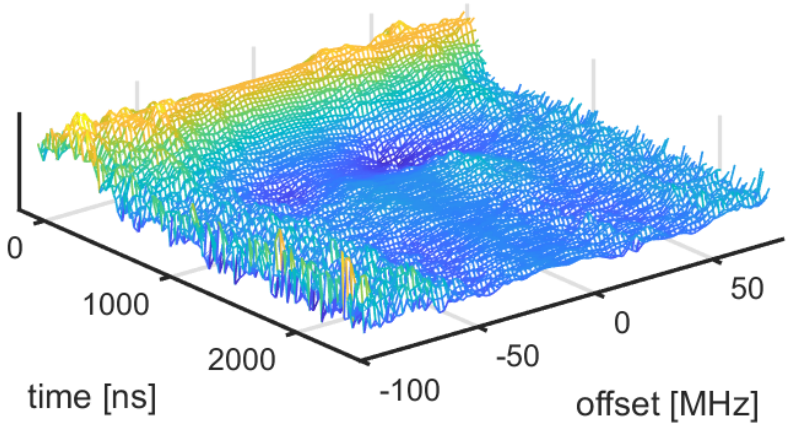
Broadband SIFTER with FT of Echo signal

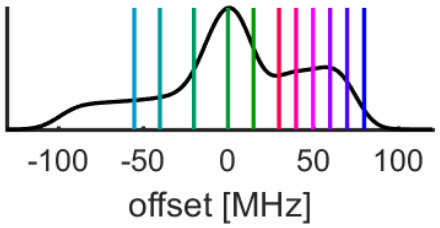


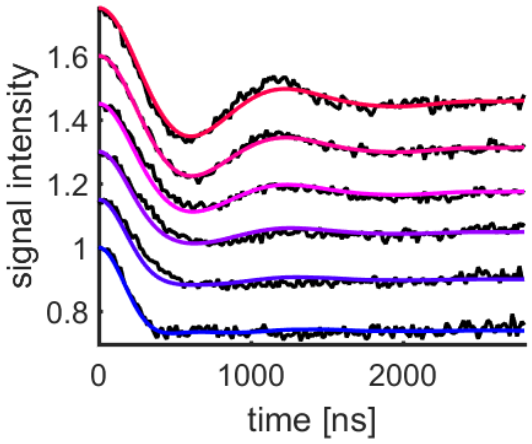


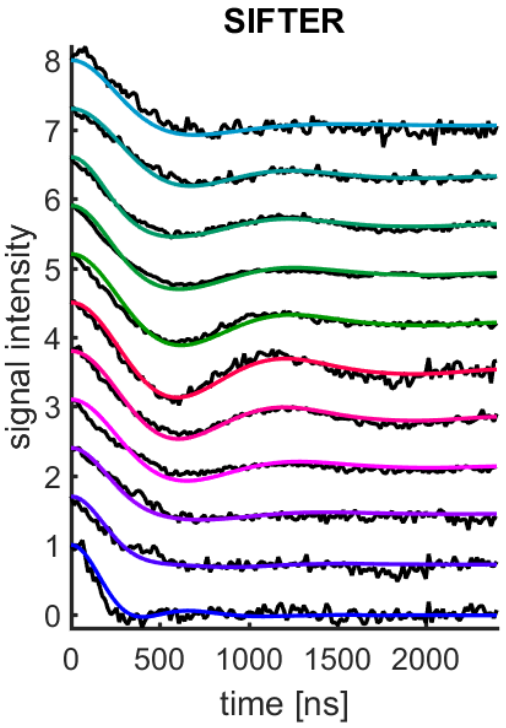


2D broadband SIFTER Experiment on dsDNA



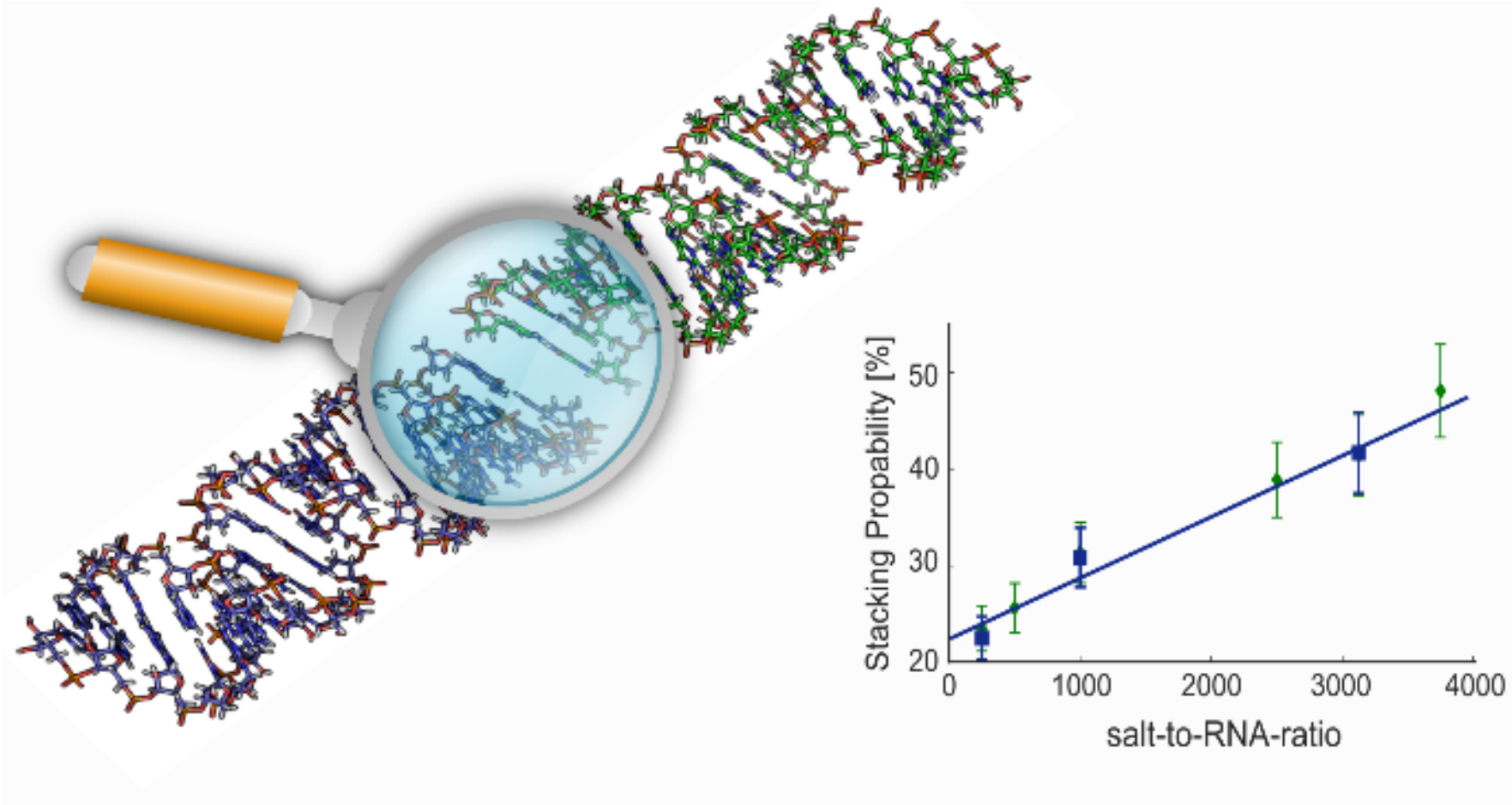




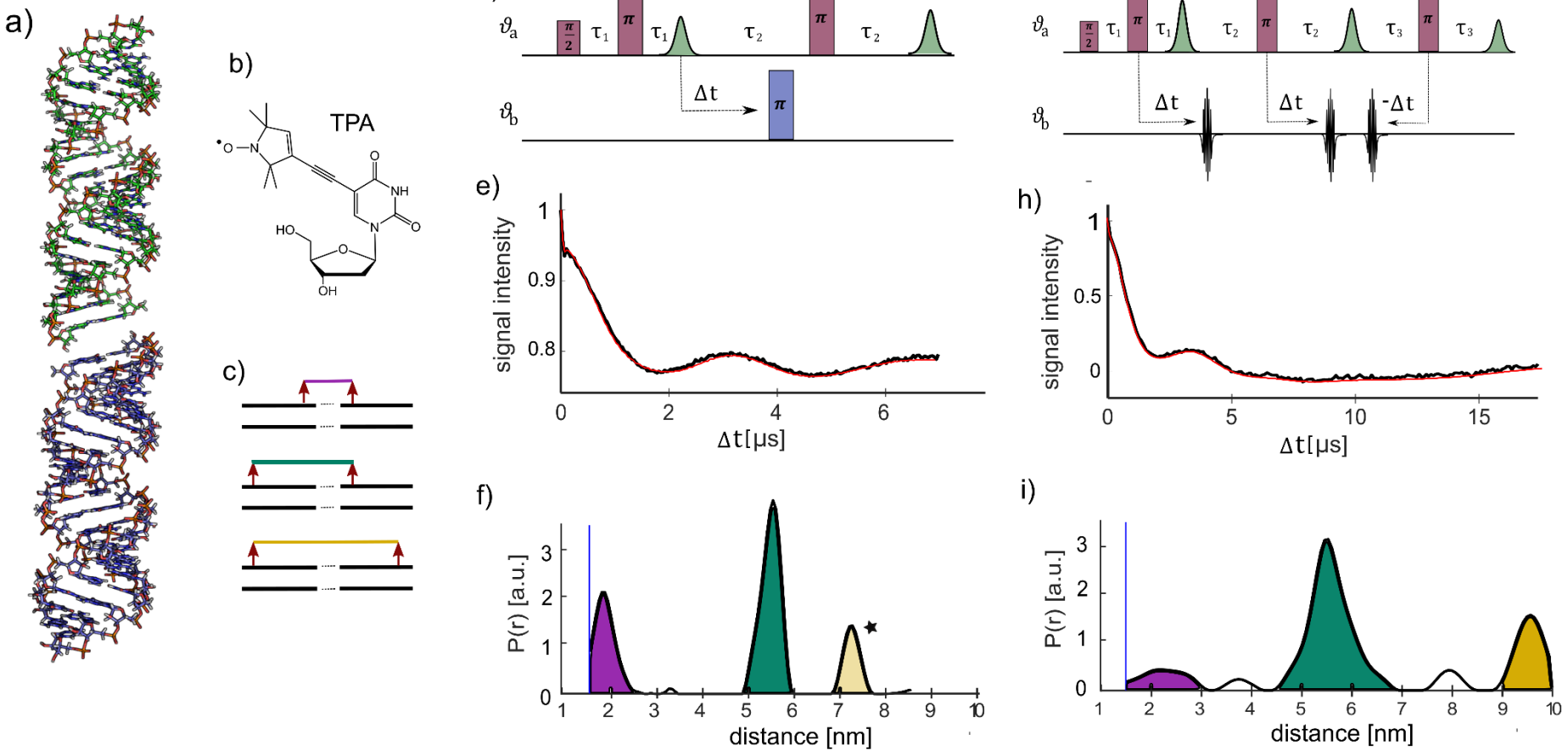


- Faster
- Higher resolution
- Over full spectum

Intermolecular Interactions of dsRNA

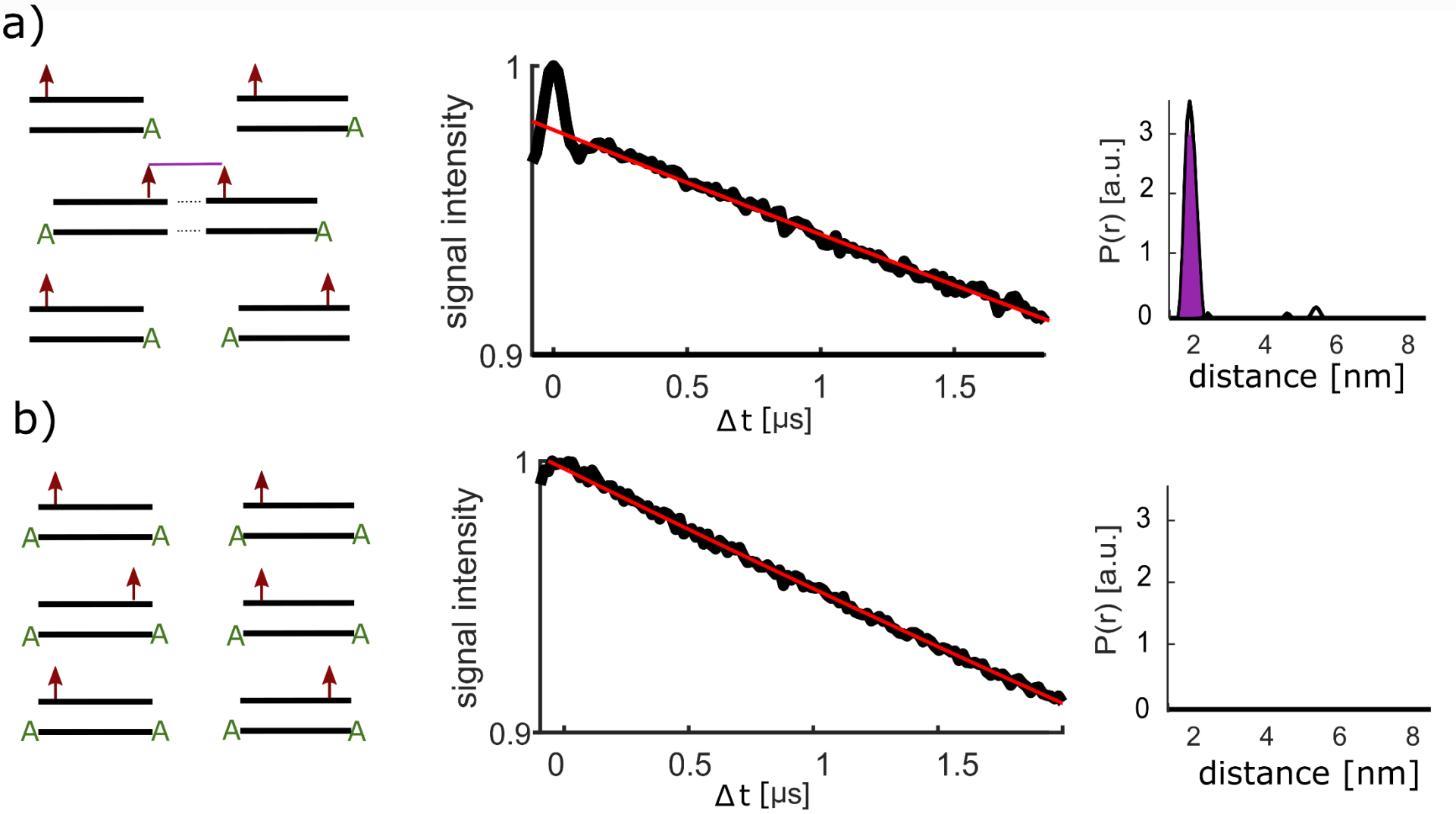


Conformational dynamics of RNA



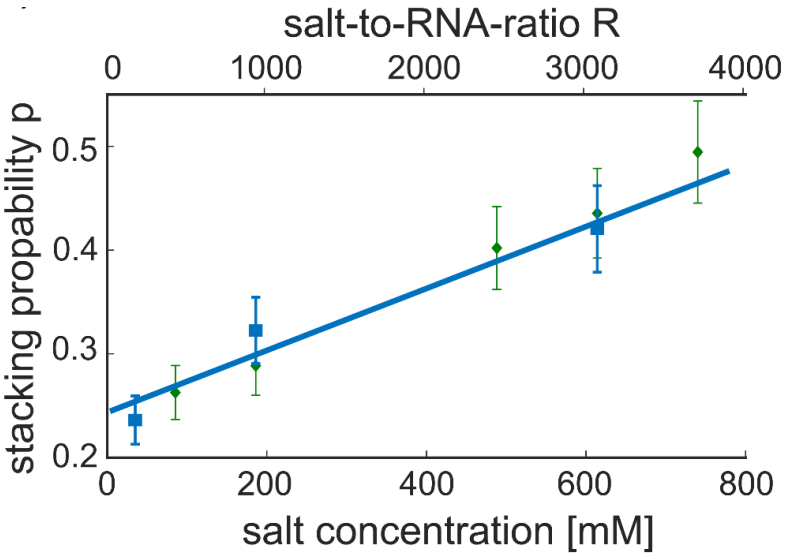
RNA (2018)

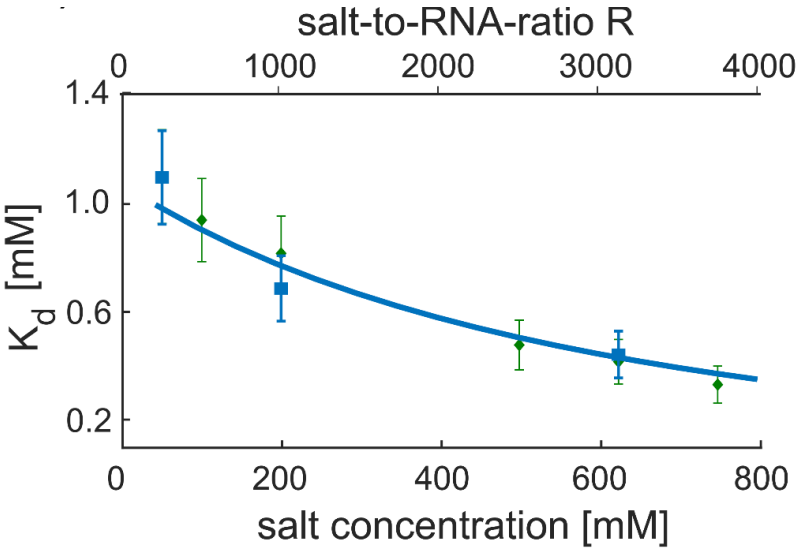
Conformational dynamics of RNA

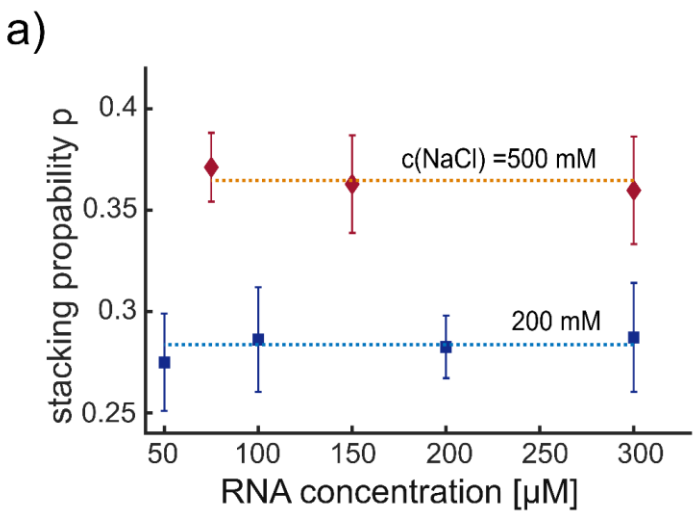


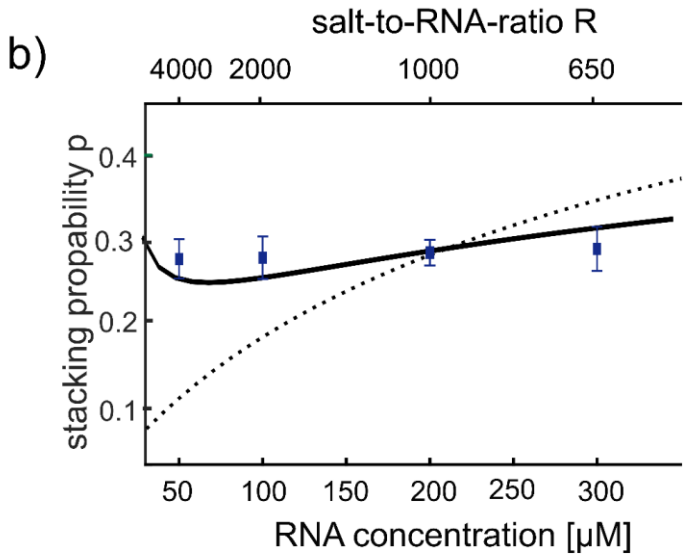
RNA (2018)

Conformational dynamics of RNA



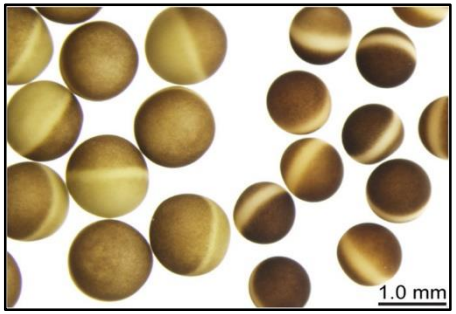




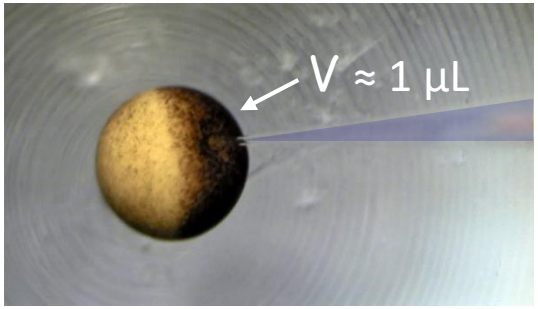


RNA (2018)

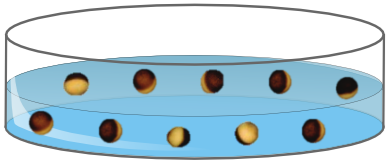
In-cell investigations of NAs



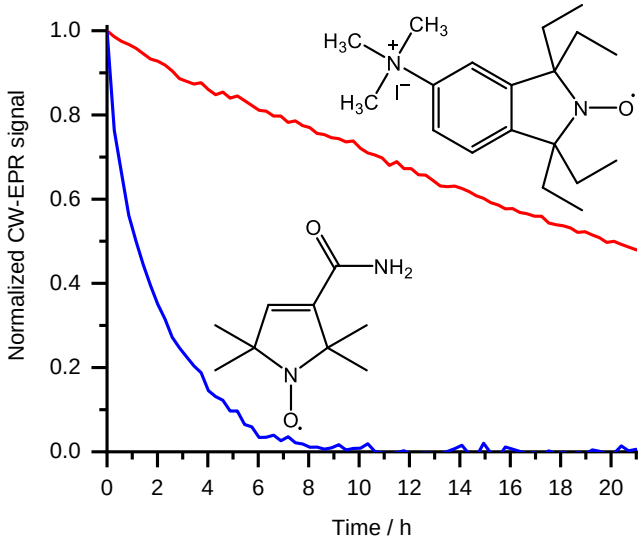
oocyte selection
by morphology



microinjection
(**1 mM, 50 nL** dsRNA)



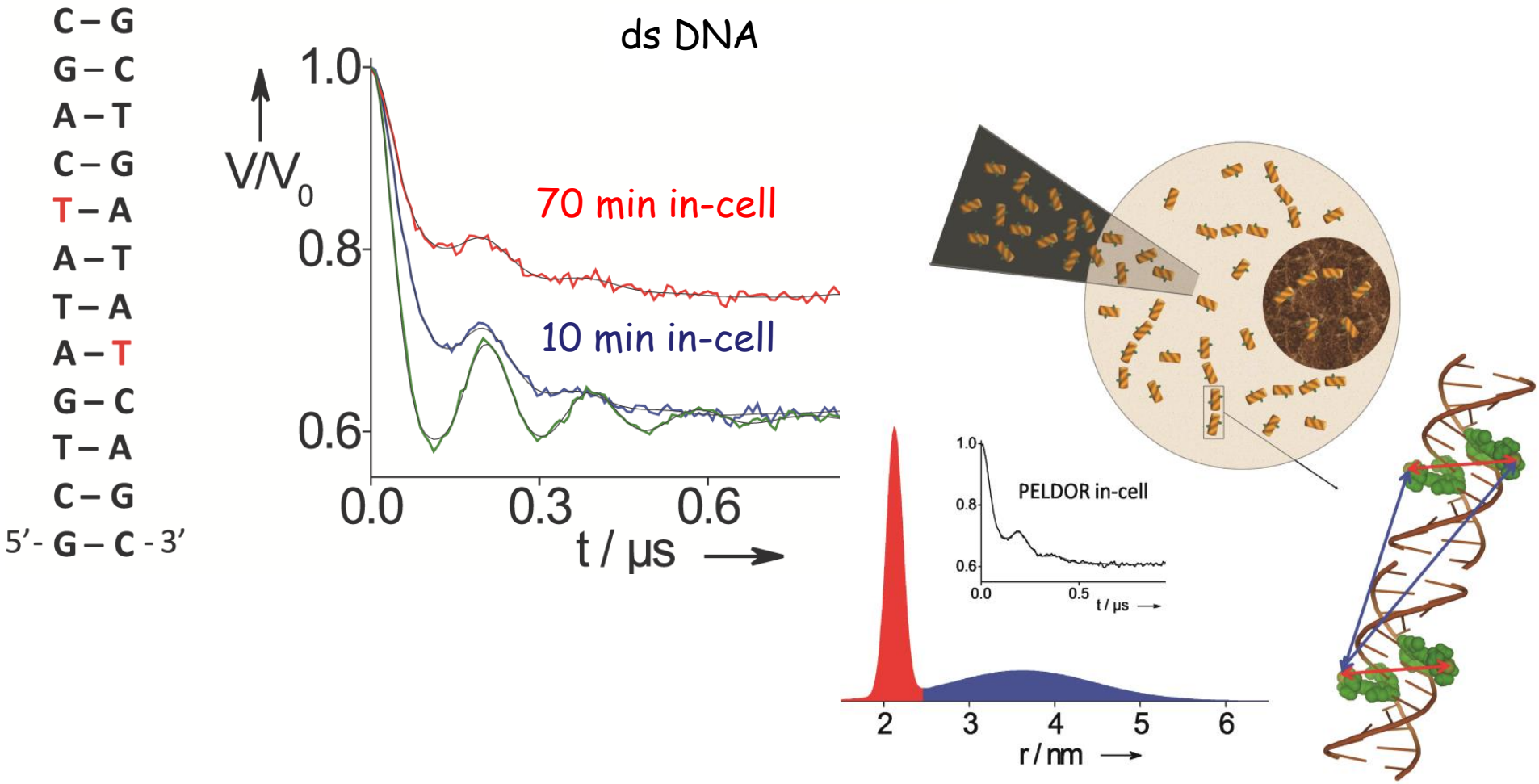
washing and incubation
(**60'**, T = 16°C)



Nitroxide Spin Labels have to be protected against reductions for applications in cells

Radical Res. (2014)

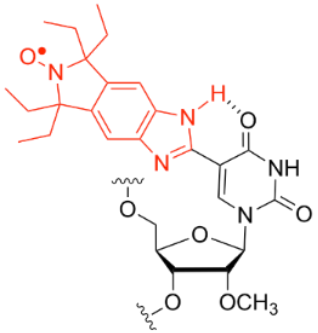
In-cell applications of PELDOR spectroscopy

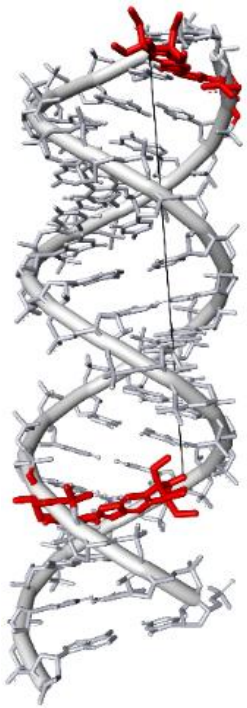


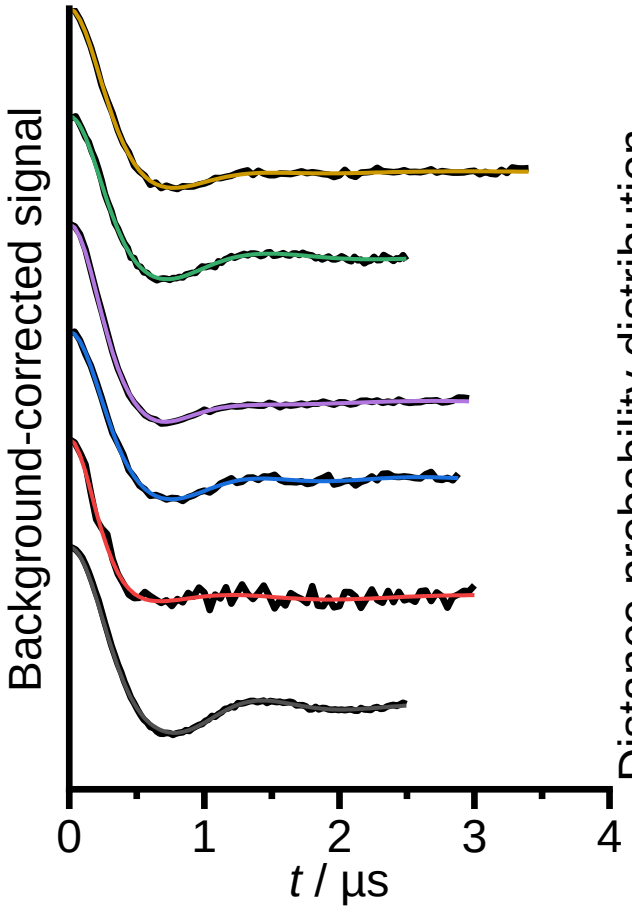
Second distance from ds-DNA stacking in-cells

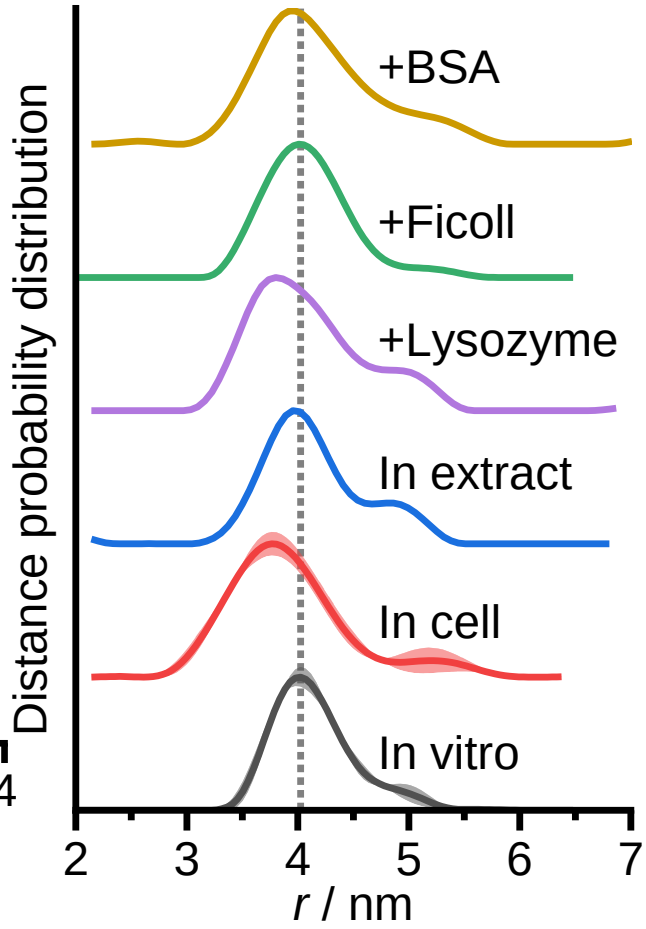
Angewandte (2011)

In-cell applications of PELDOR spectroscopy

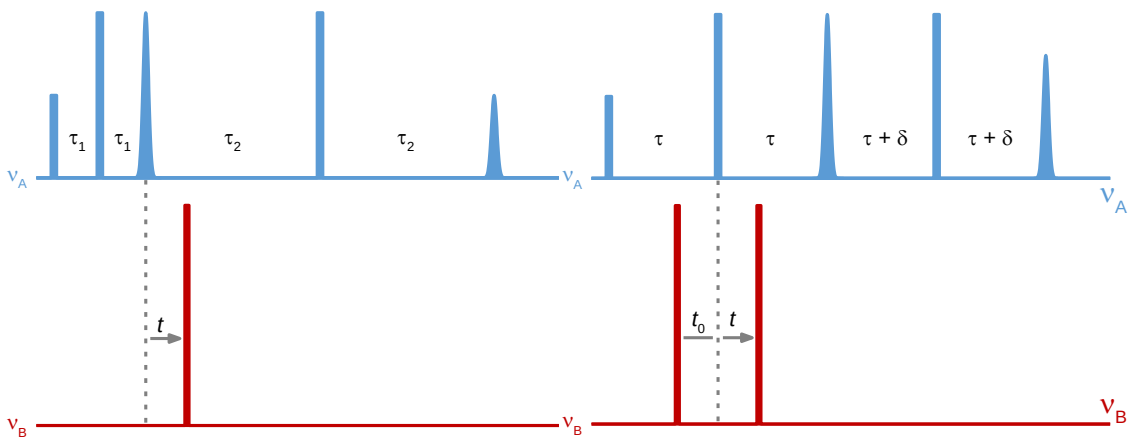


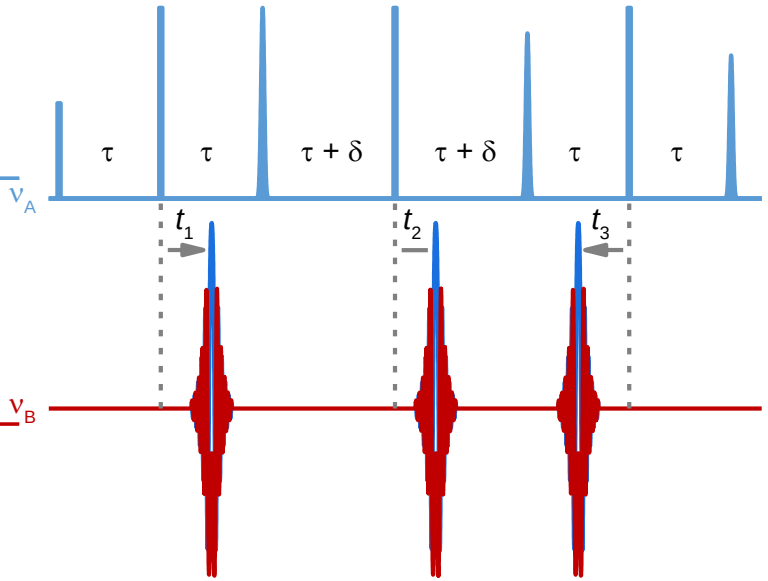


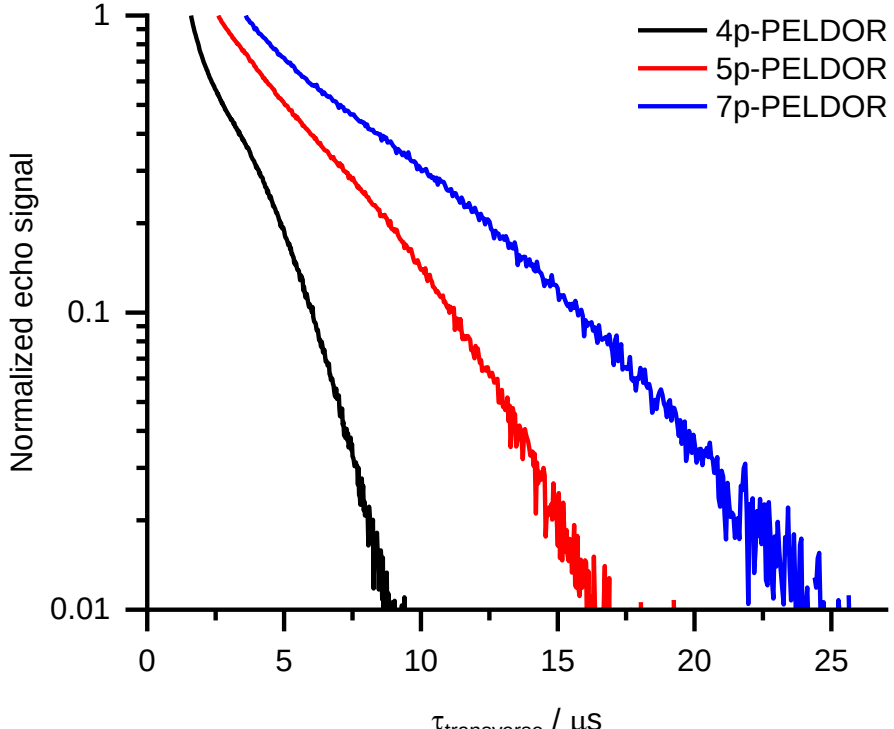


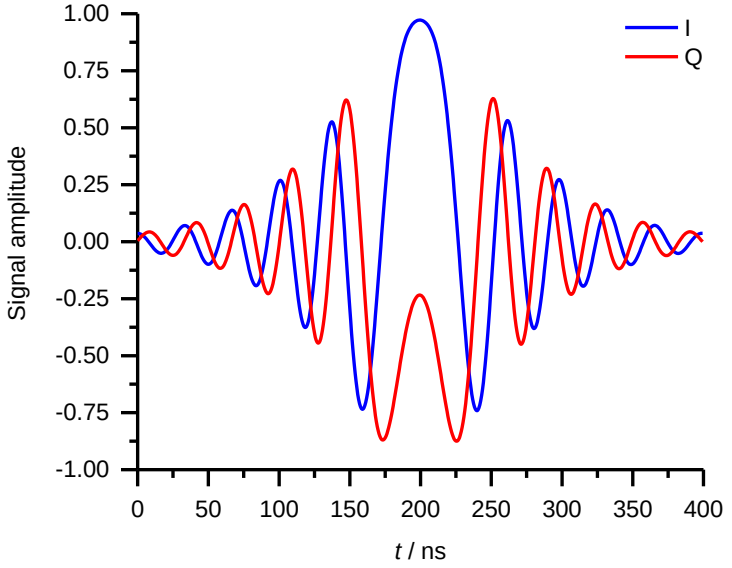


Prolonged observation time window by CP Sequence

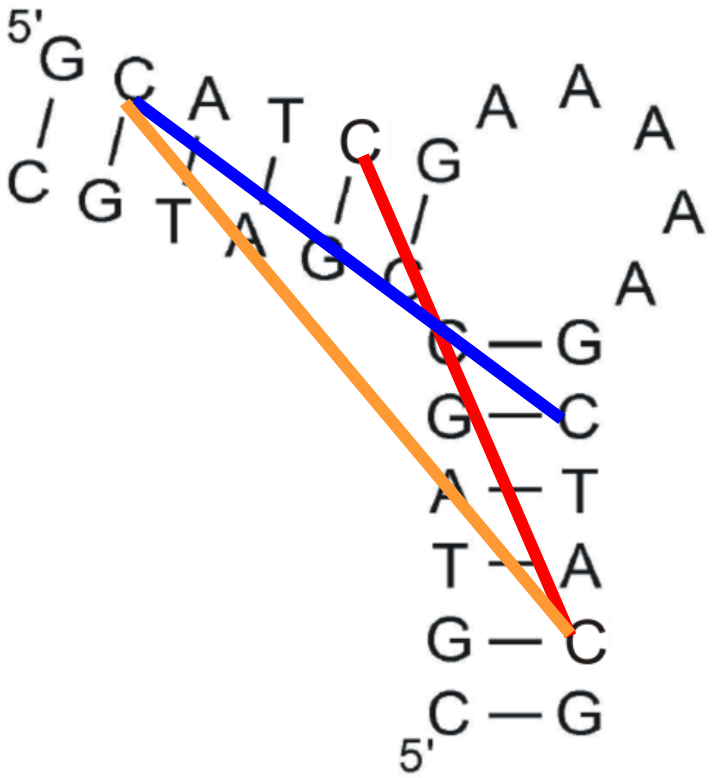


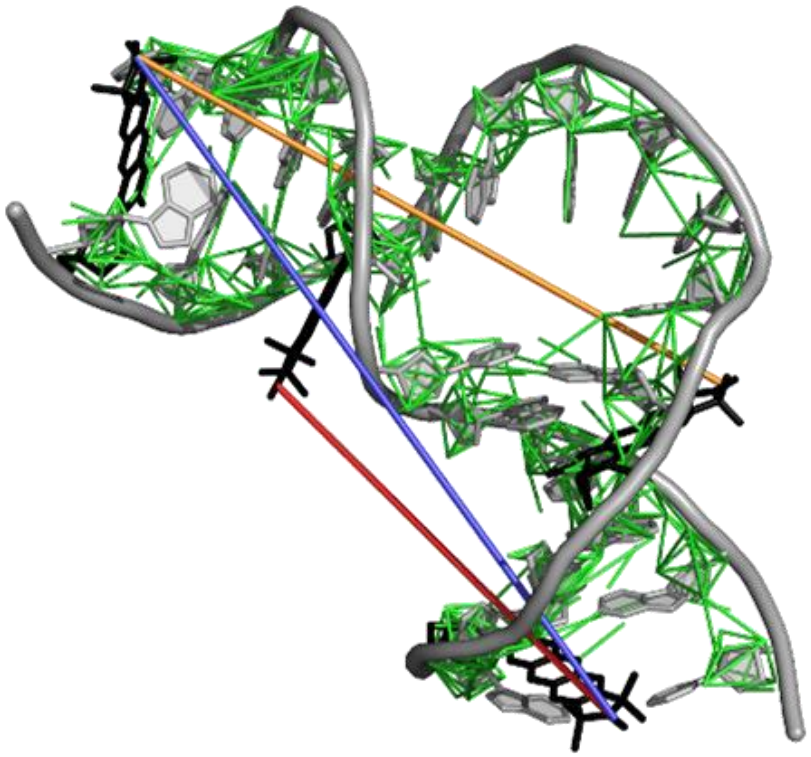






Investigation of DNA with A-buldge

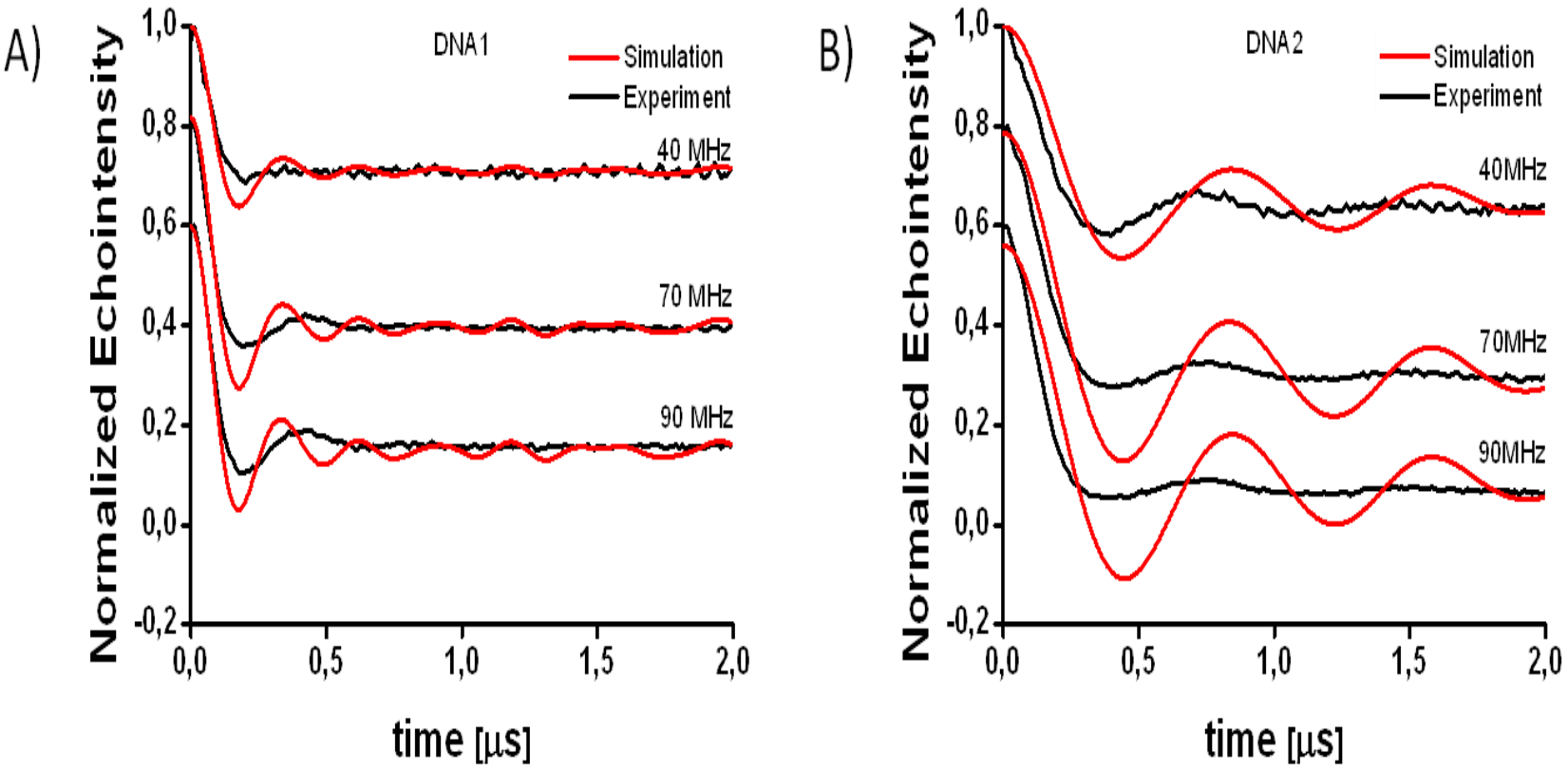




NMR restraints +
3 Pairs of EPR restraints

Comparison with predictions from NMR structure

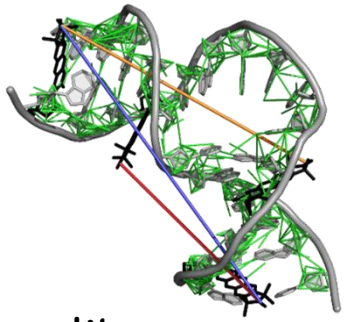
Dornberger et al.
Biochem. 38, 12860 (1999)

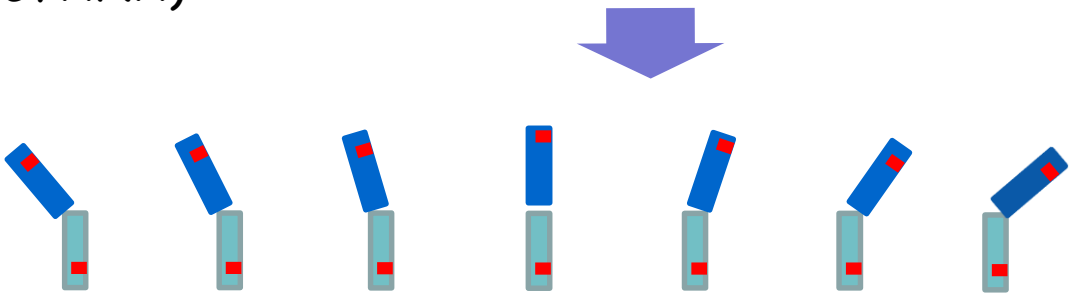


PELDOR time traces calculated from NMR structure
(20 best structures) do **not agree** well with PELDOR !

Procedure to determine conformational ensemble

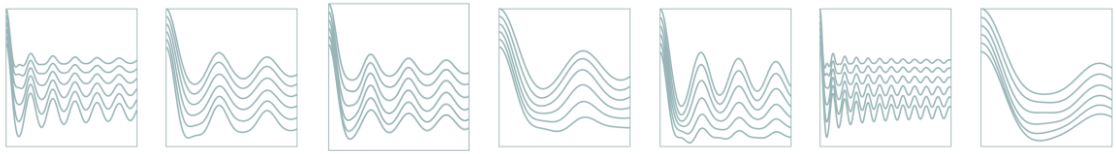
1. Step: Modeling with NMR restraints & PELDOR R's (CYANA)





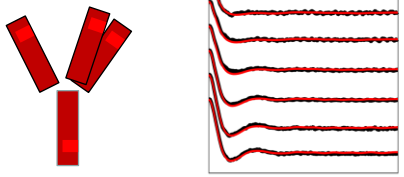
structure library
~15.000 conformers

2. Step: Simulation of PELDOR datasets for each conformer



PELDOR
time trace library

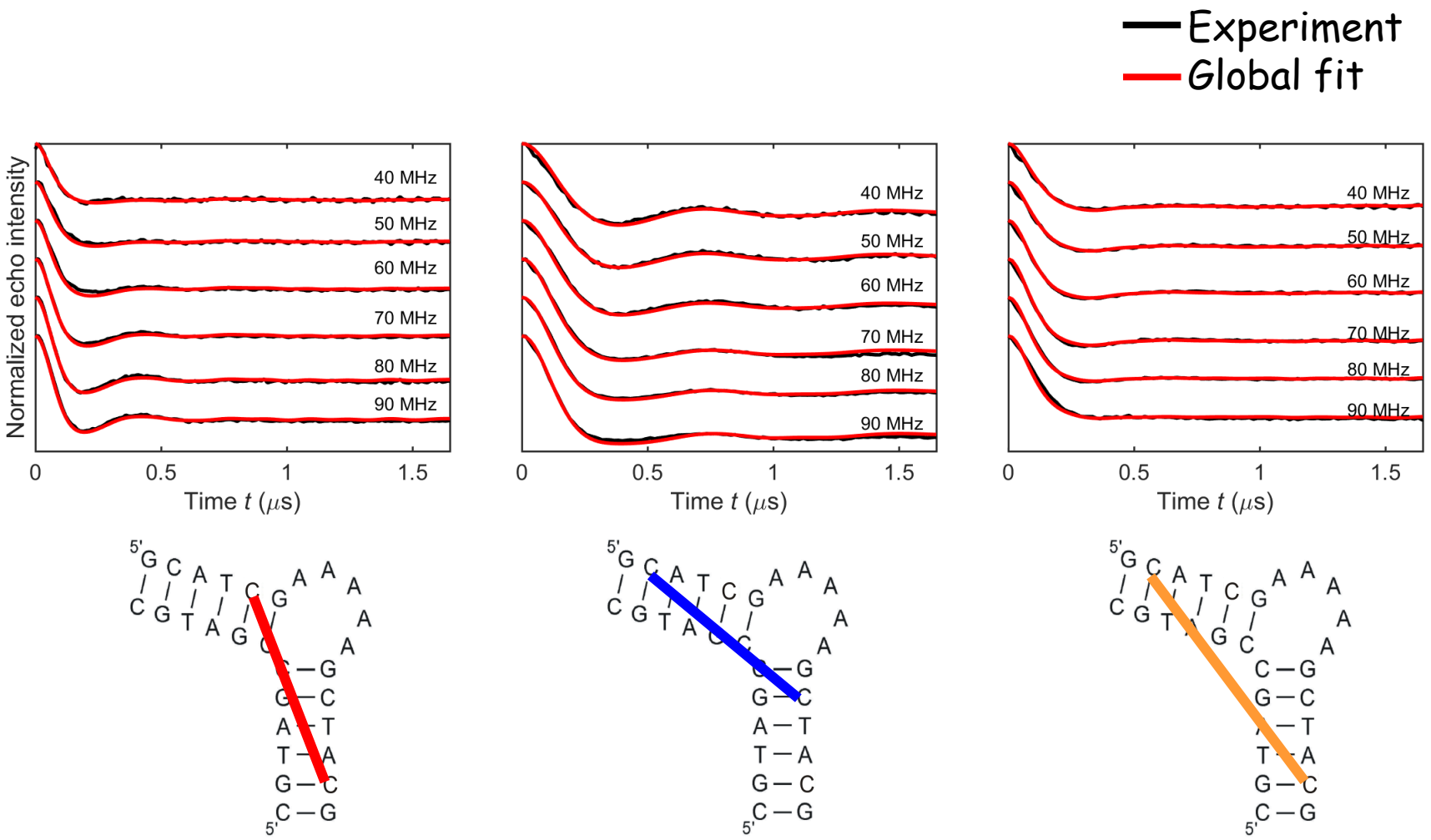
3. Step: Iterative fit of experimental PELDOR dataset by PELDOR library



Conformational ensemble
in agreement with all
NMR and PELDOR restraints

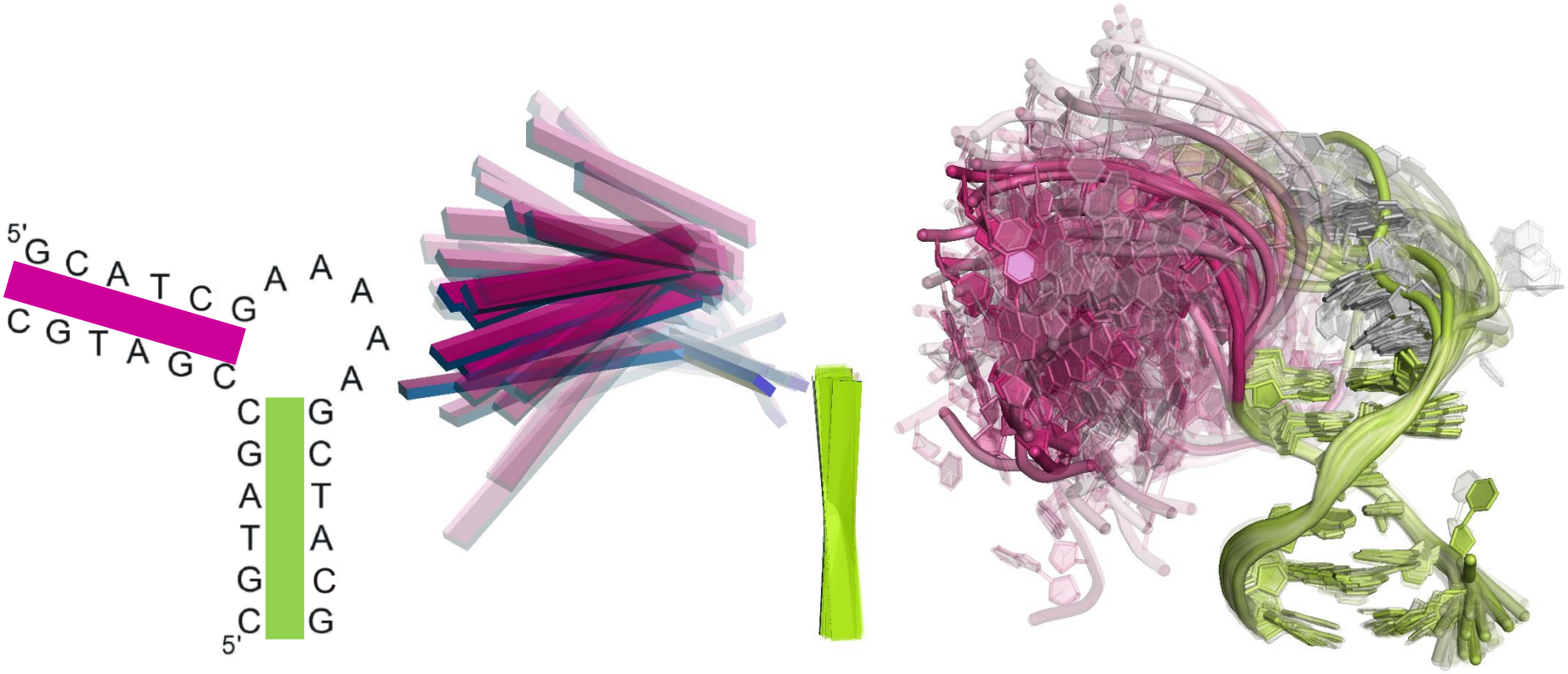
PCCP (2017)

Simultaneous Fit of 3 PELDOR datasets



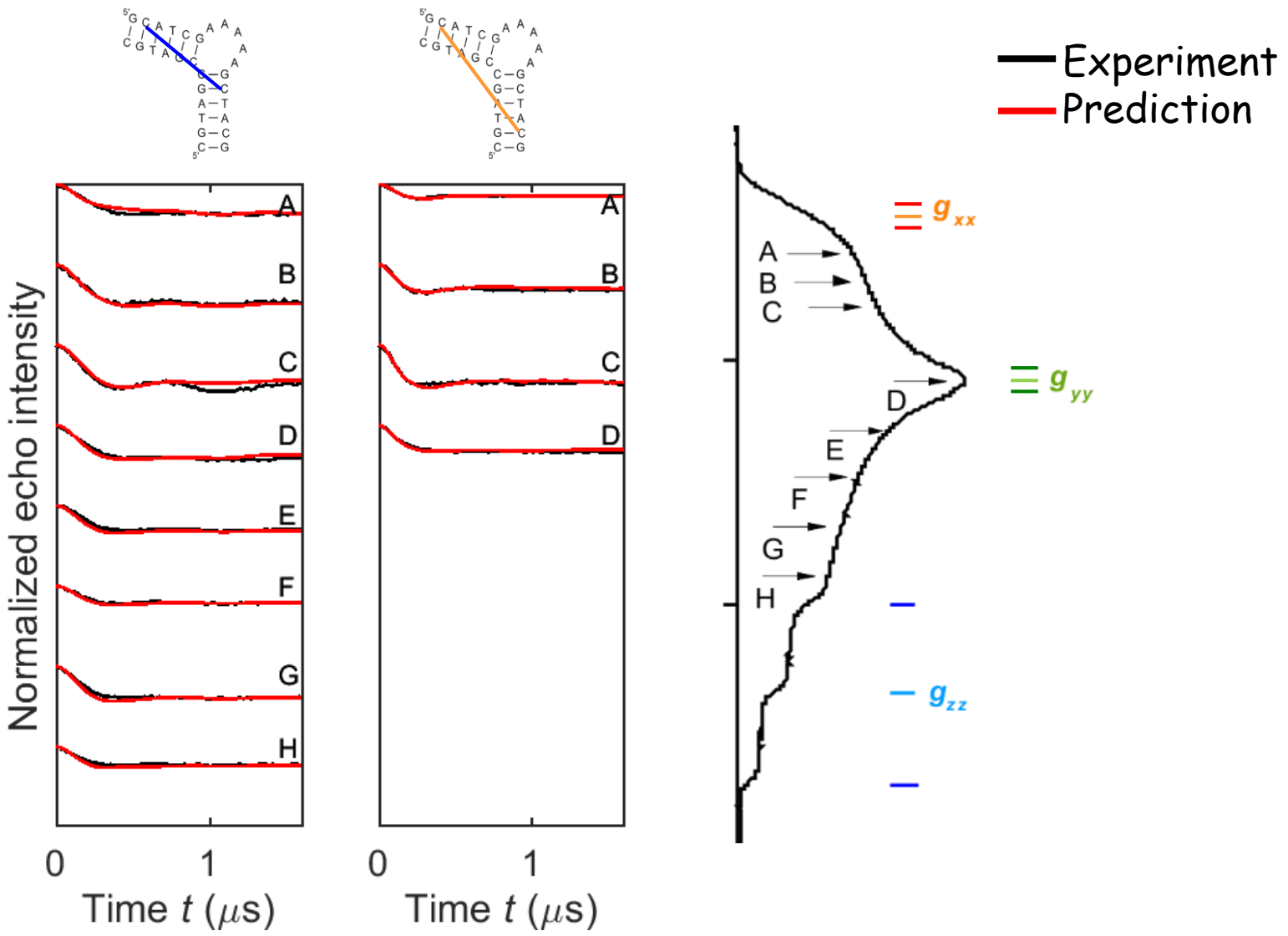
PCCP (2017)

Conformational bundle describing bend DNA



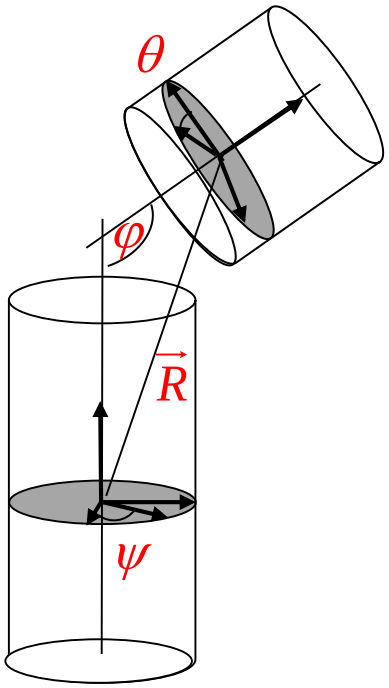
PCCP (2017)

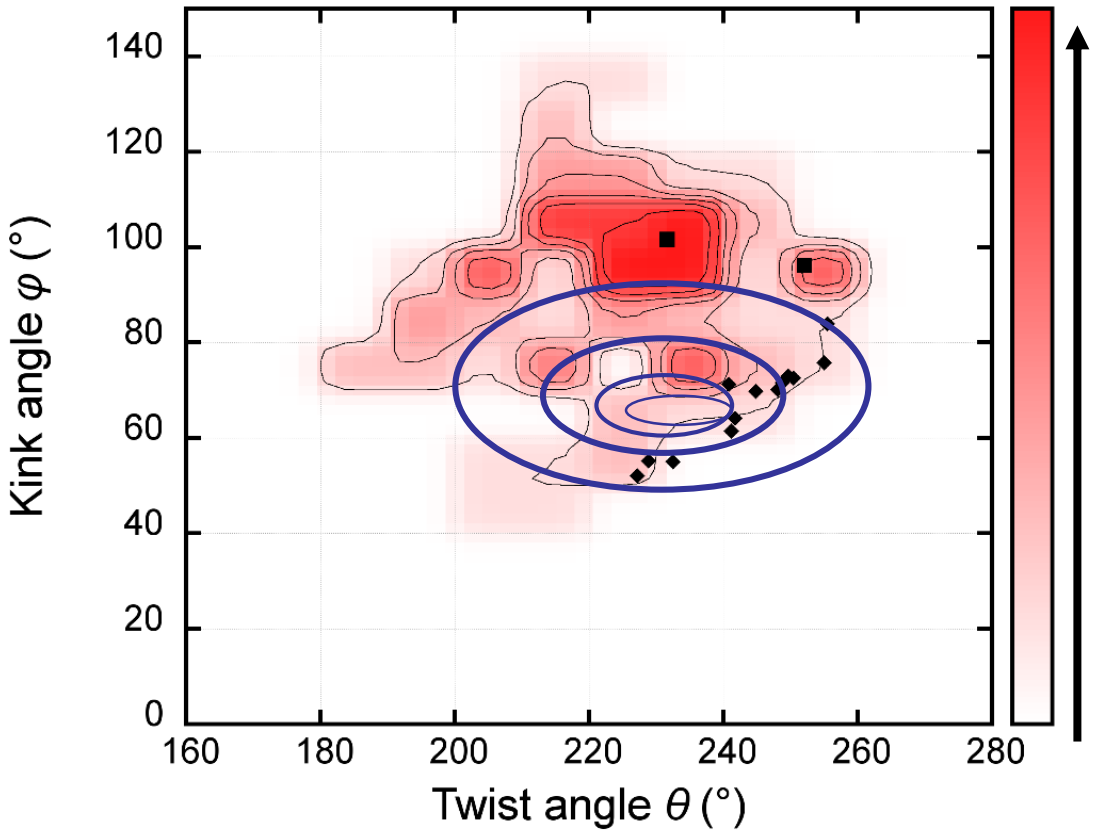
Evaluation of solution by G-band PELDOR data



PCCP (2017)

Comparison with NMR alone and FRET



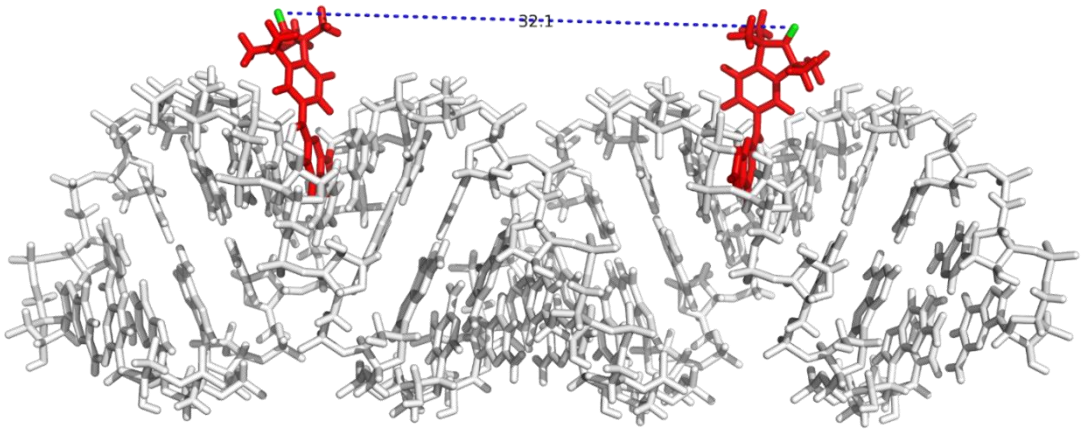


EPR/NMR approach
NMR
FRET

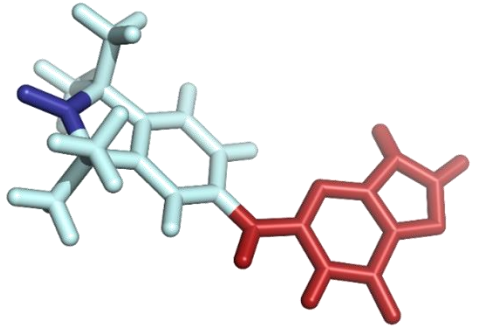
PCCP (2017)

*Wozniak, A. K., et al. (2008). PNAS **105**: 18337-18342.*

Non-covalent labels for nucleic acids



Collaboration with Sigurdsson (Iceland)



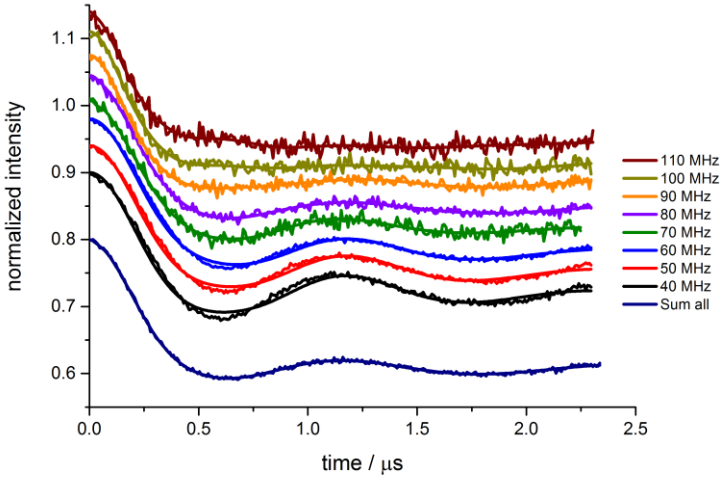
G Spin-label

5′-CGAC-GFA-UCG-CGC-GAU-CCG-UCG-3′

3′-GCUG-CCU-AGC-GCG-CUA-FGC-AGC-5′

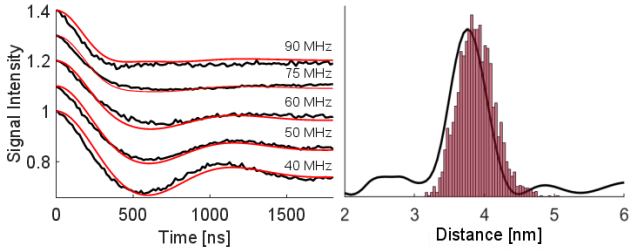
High selectivity (abasic sites) high binding affinity

Rather rigidly incorporated in double stranded RNA

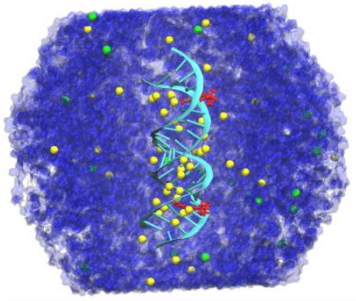


Combining MD simulations with PELDOR

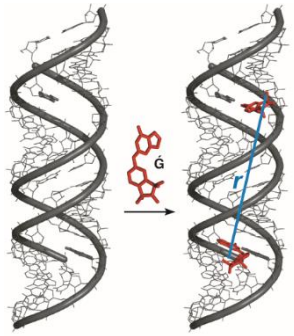
Collaboration with Hummer (MPI Biophysics)



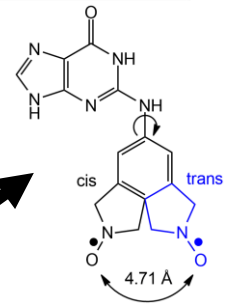
Quantitative comparison between PELDOR and MD



Incorporation of spin label

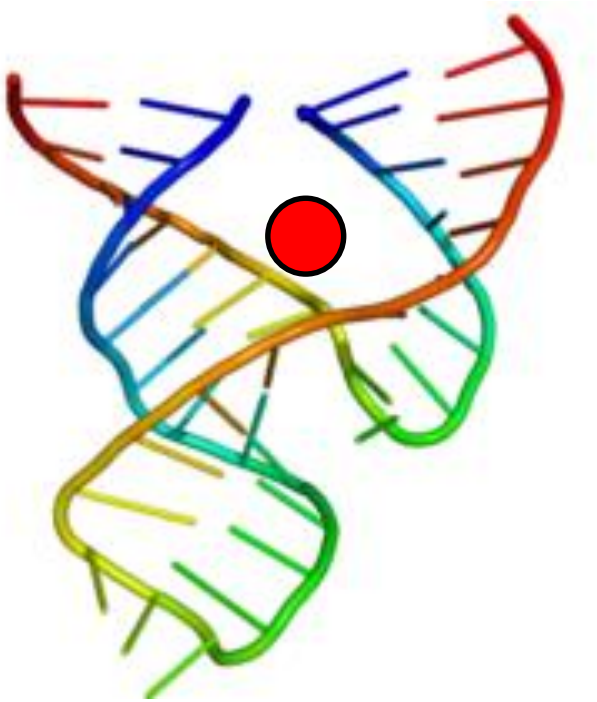


Spin label dynamics



A synergistic approach to conformational dynamics and flexibility of RNA in the 1-10 nm range

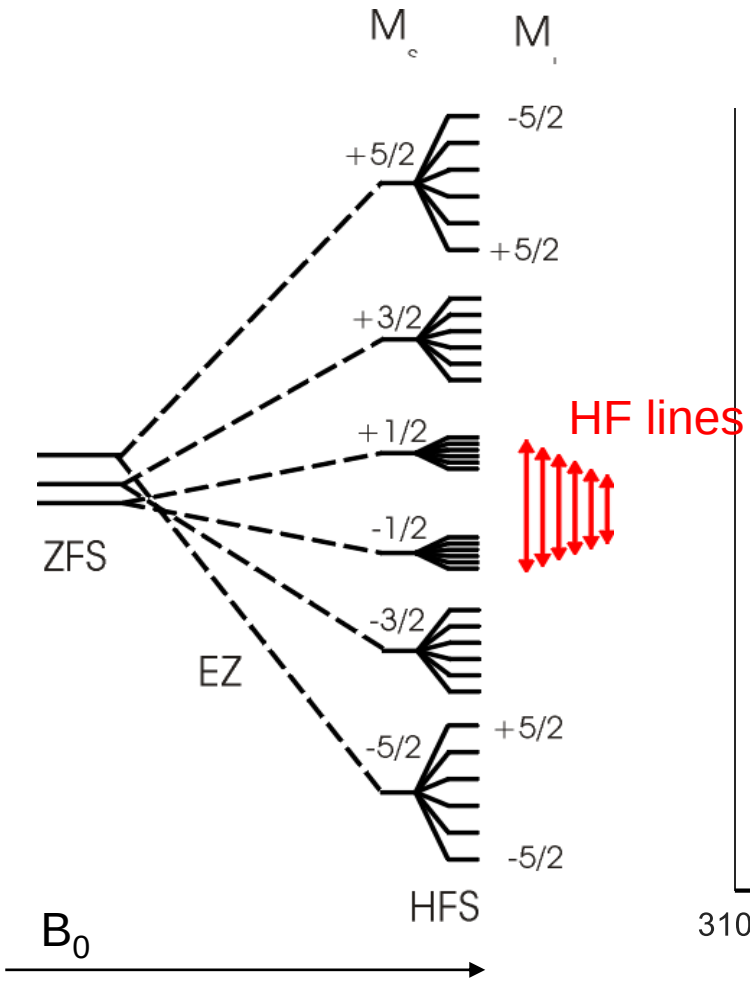
Mg ion binding sites in RNA motives



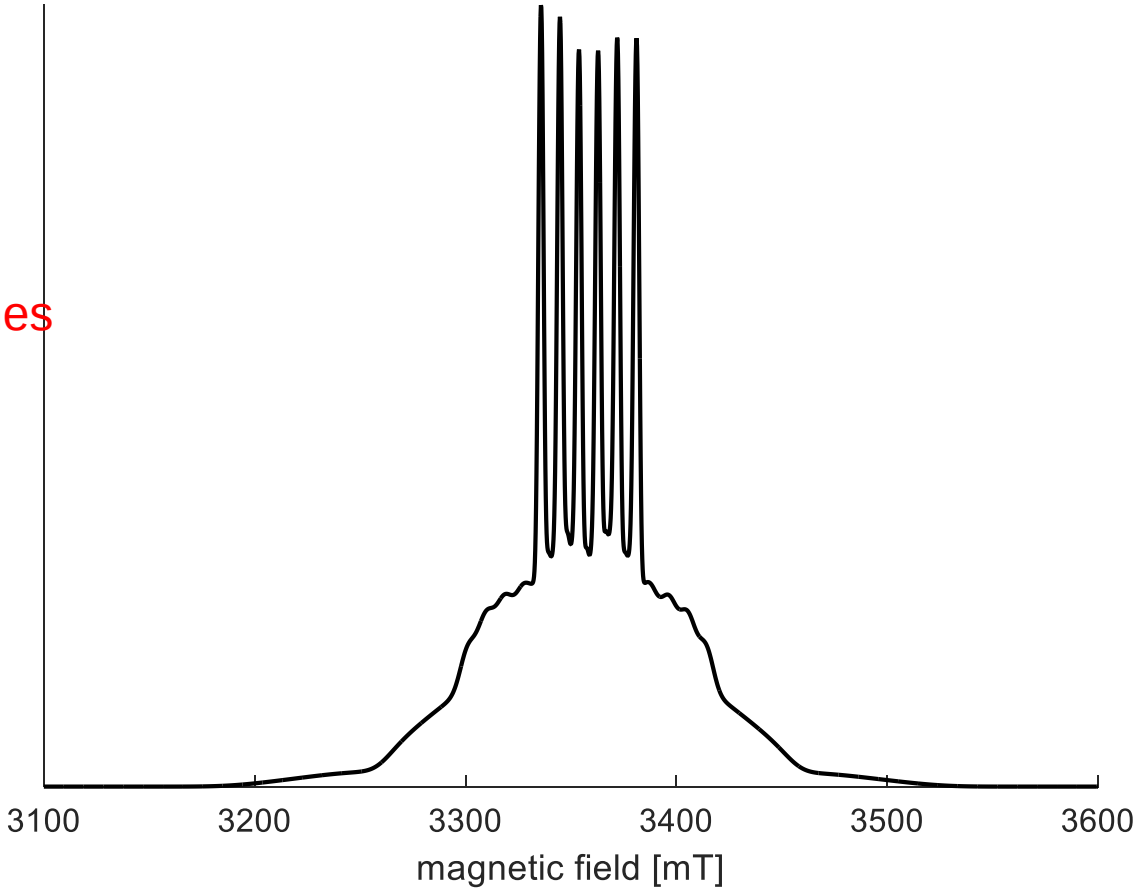
Important for tertiary structure
in
Ribozymes, riboswitches, …

Replacement of
diamagnetic Mg^{2+}
by
paramangnetic Mn^{2+}

High spin Mn ion (S=5/2, I=5/2)

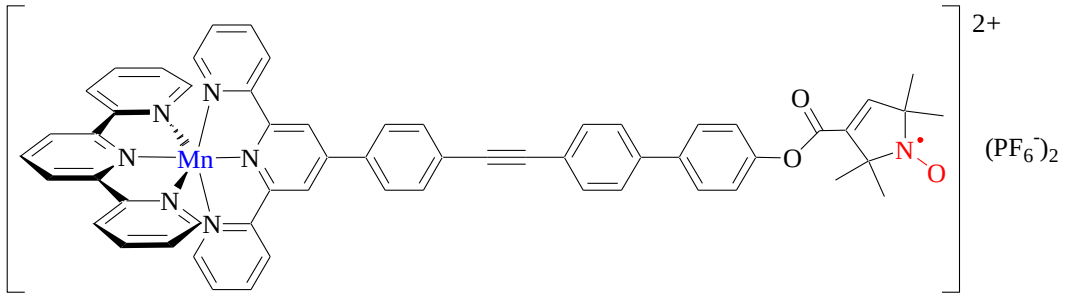


HF lines



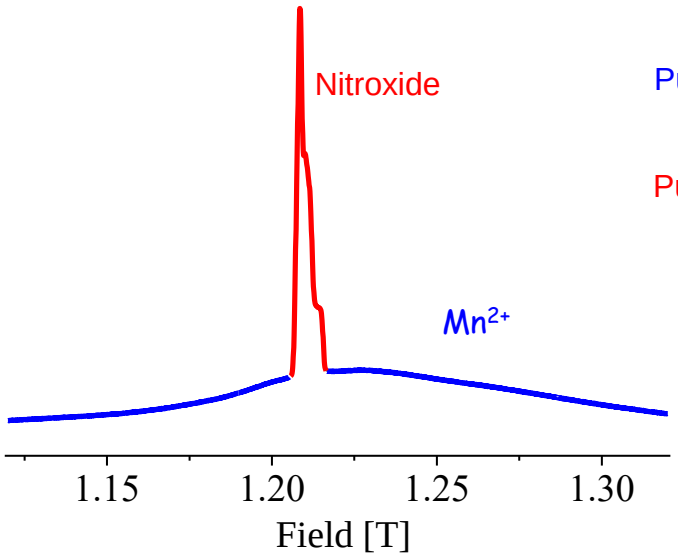
High magnetic fields (>> D) necessary to obtain spin system easy to analyze
Narrowing of central Mn transition at high fields (D^2/B_0)

PELDOR on Mn^{2+} -Nitroxide Model-Compound



Mn-bis(terpy)-nitroxide with an estimated distance R (Mn^{2+}-$NO^{\bullet}$) ≈ 2.7 nm

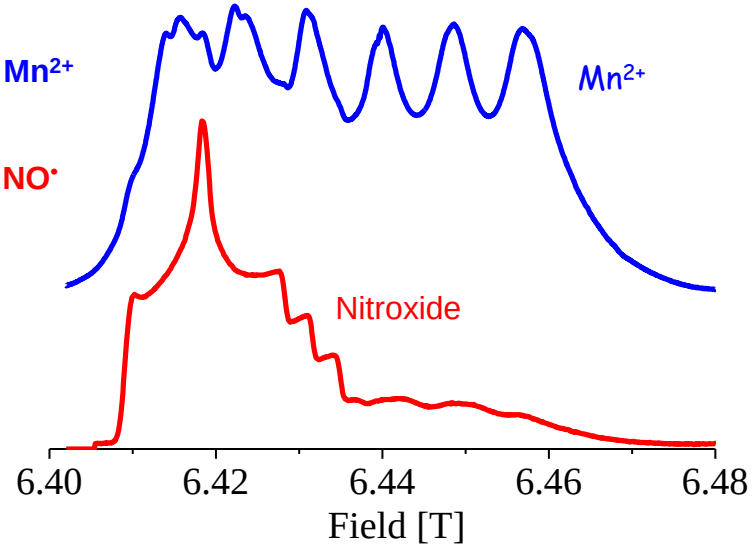
Q-band frequencies (34 GHz)



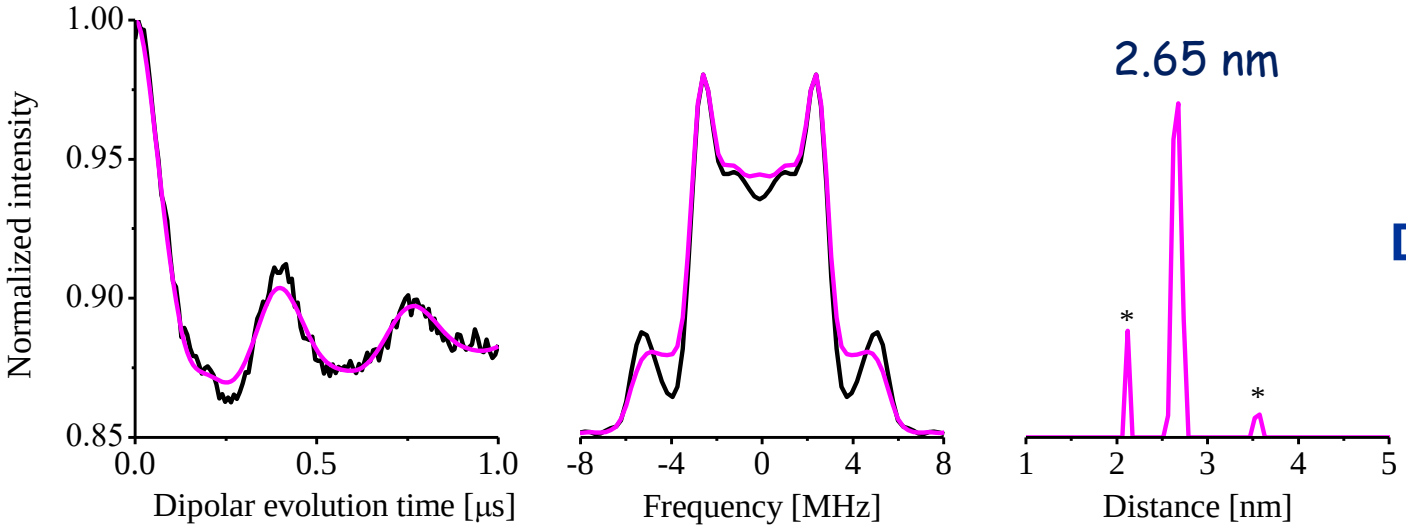
Pulses optimized for **Mn^{2+}**
(15 /30 ns)

Pulses optimized for **$NO^{\bullet}$**
(40/80 ns)

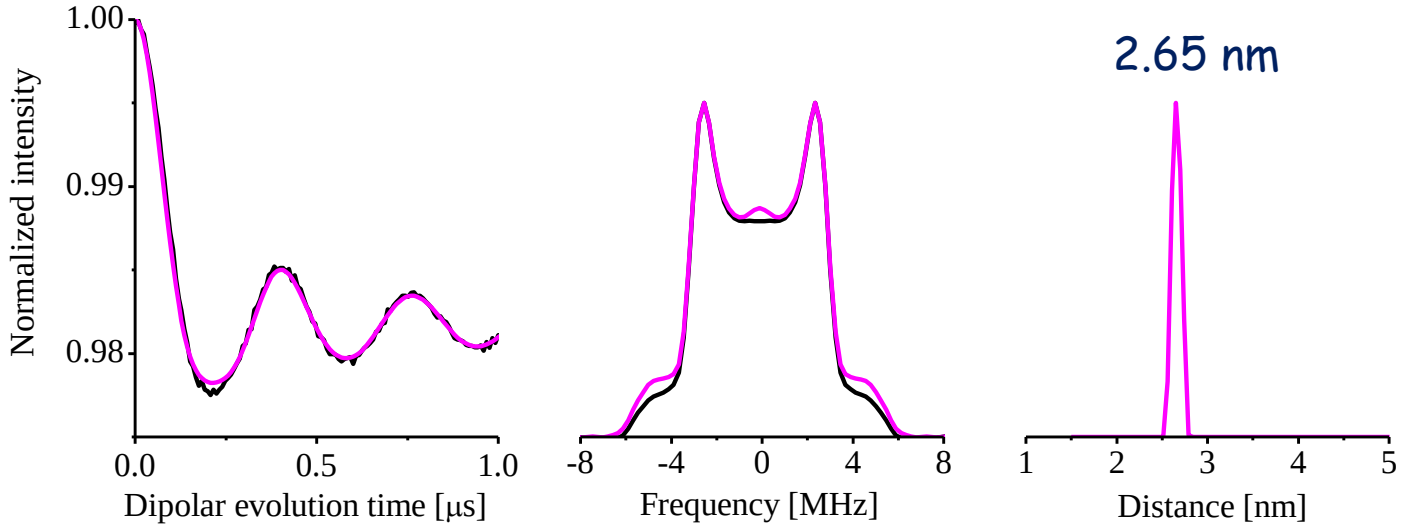
G-band frequencies (180 GHz)



Q-band PELDOR on Model compound



Pump on NO•
Detect on Mn^{2+}



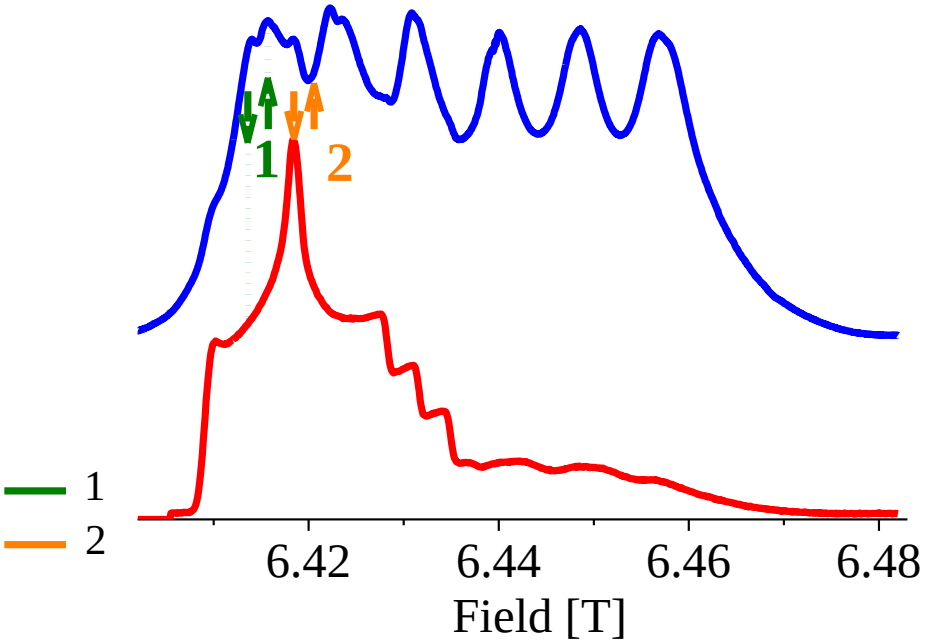
Pump on Mn^{2+}
Detect on NO•

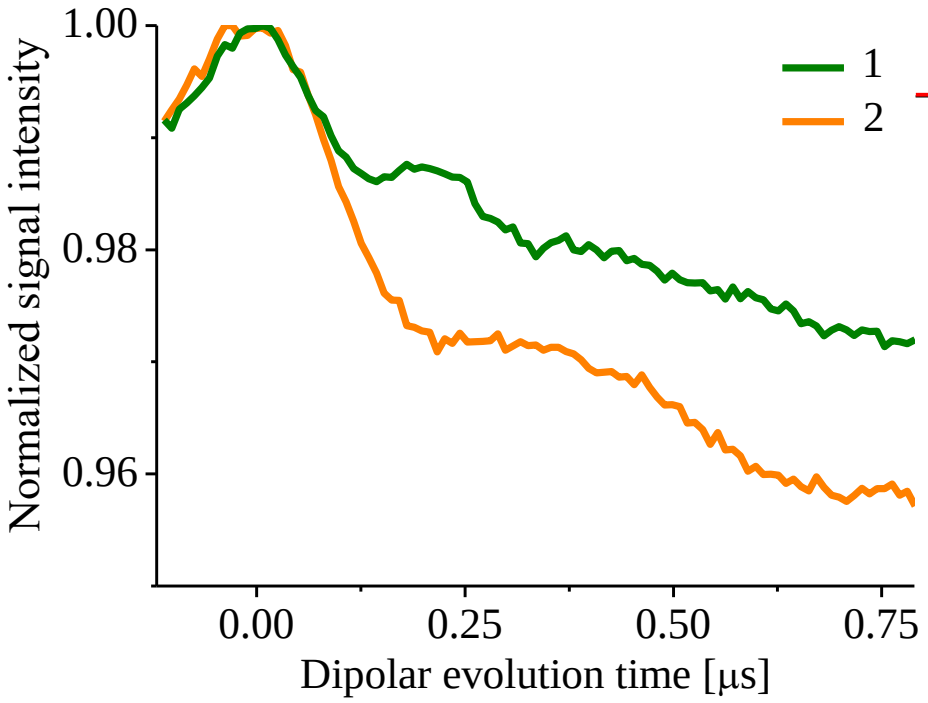
Akhmetzyanov et al *PCCP (2015)*

G-band PELDOR on Mn-bis(terpy)-nitroxide

T = 10 K

Different dipolar oscillations observed depending on the pump/probe position (1 / 2)

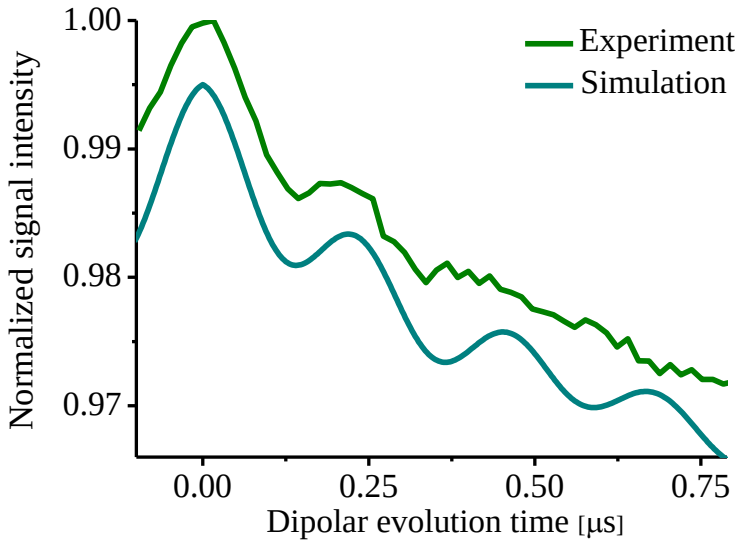


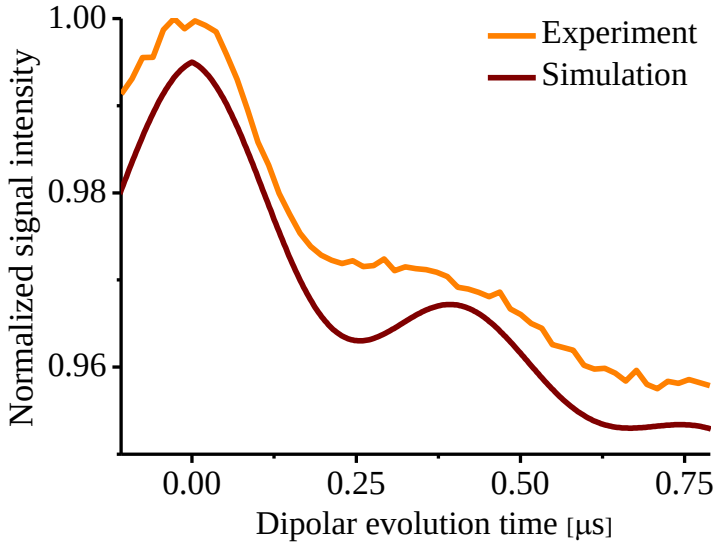


→ Orientation selection effects related to the anisotropic g tensor of the nitroxide radical at high magnetic fields

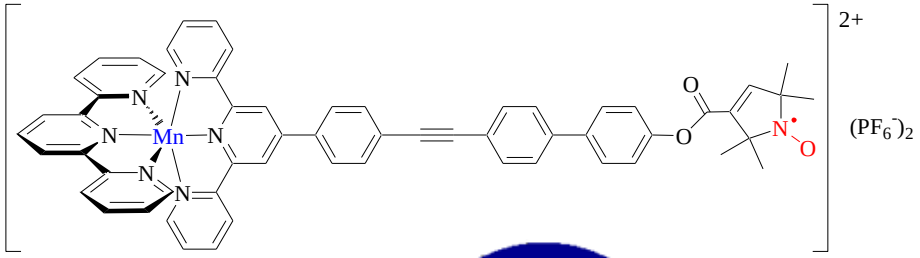
Akhmetzyanov et al *PCCP (2015)*

Simulation of orientation effects

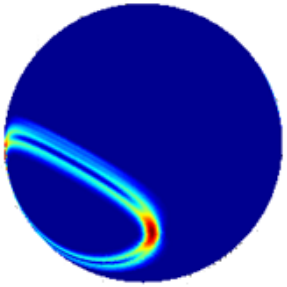


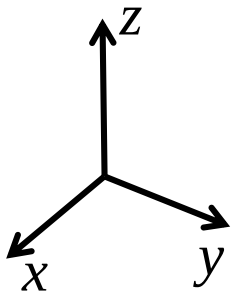


Akhmetzyanov et al *PCCP (2015)*

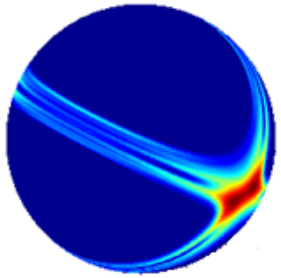


Position 1 (‘close to x’)



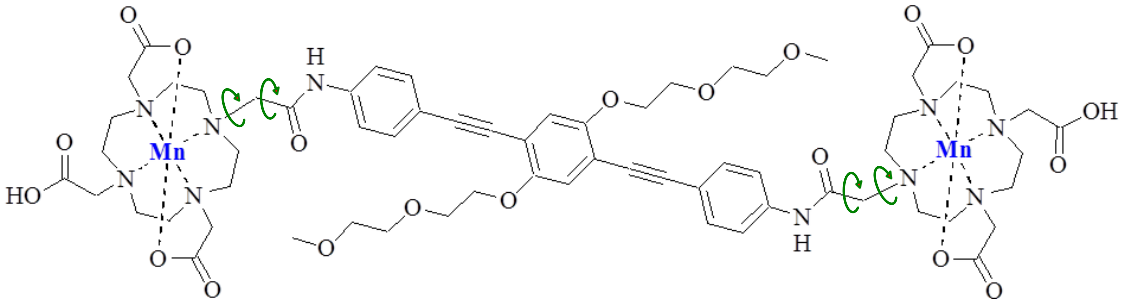


Position 2 (‘y’)

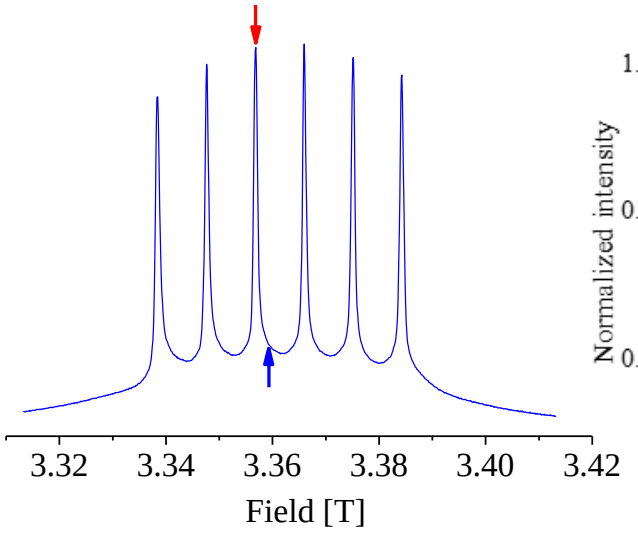


Excited orientations of the **NO˙**

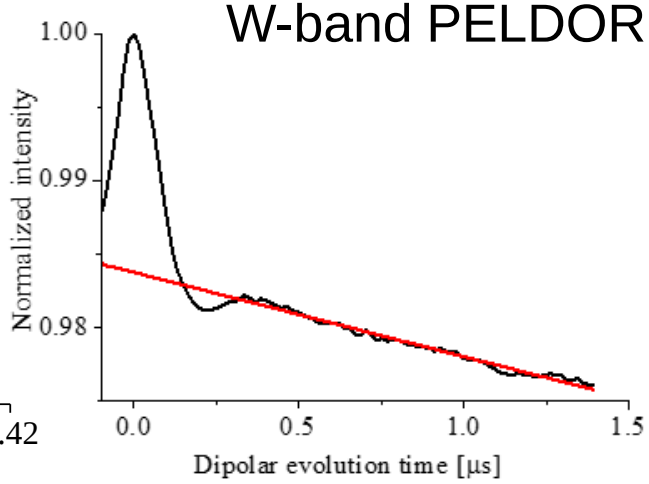
Bis-Mn-DOTA complex

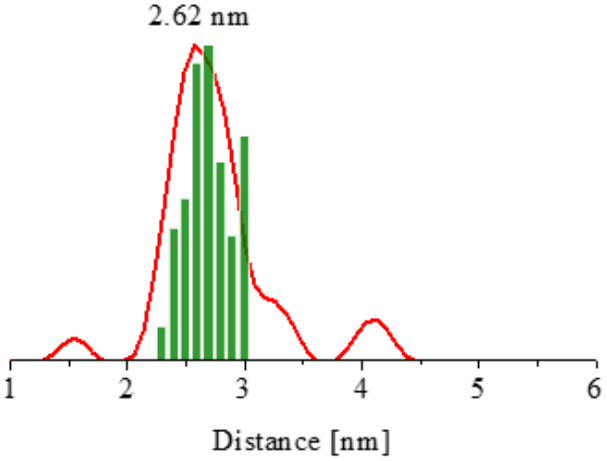


Collaboration with
C. Policar (ENS Paris)
S. Un (CEA Sacley)



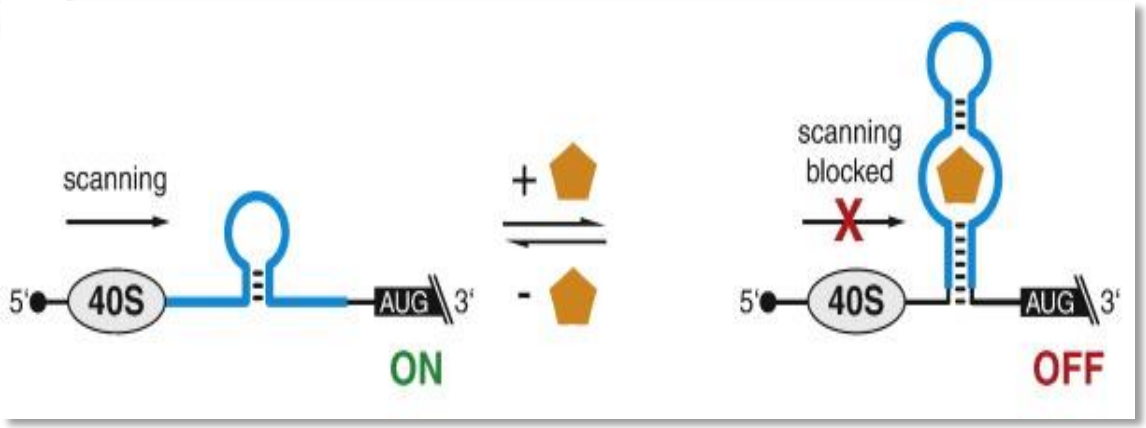
W-band PELDOR





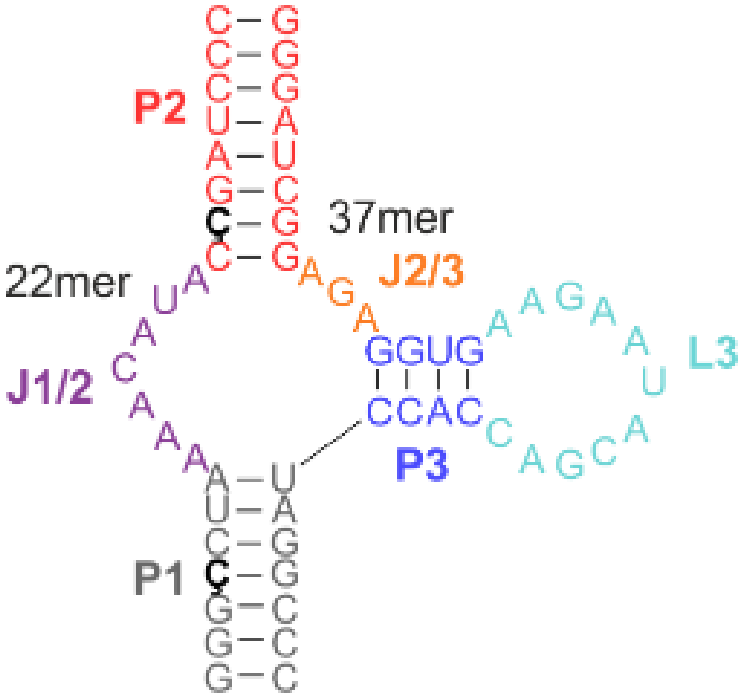
Ching et al, *PCCP*, **2015**, 17, 6760.
Demay-Drouhard et al*, ChemPhysChem*, **2016**

Tetracyclin-binding Aptamer



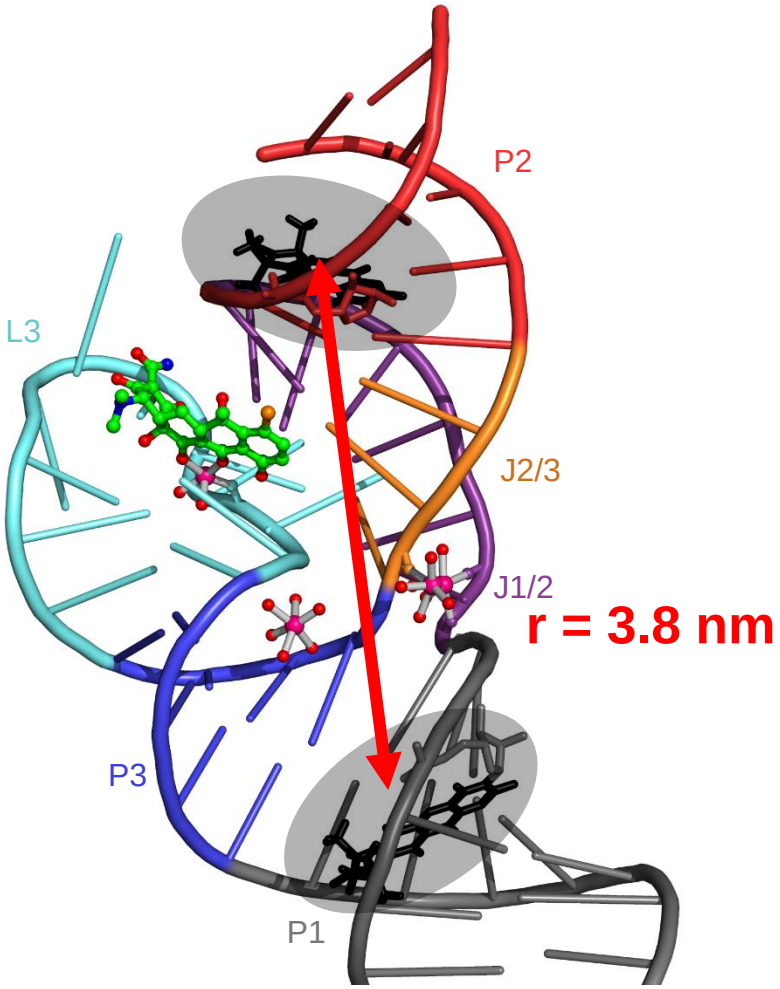
Groher & Suess BBA 2014

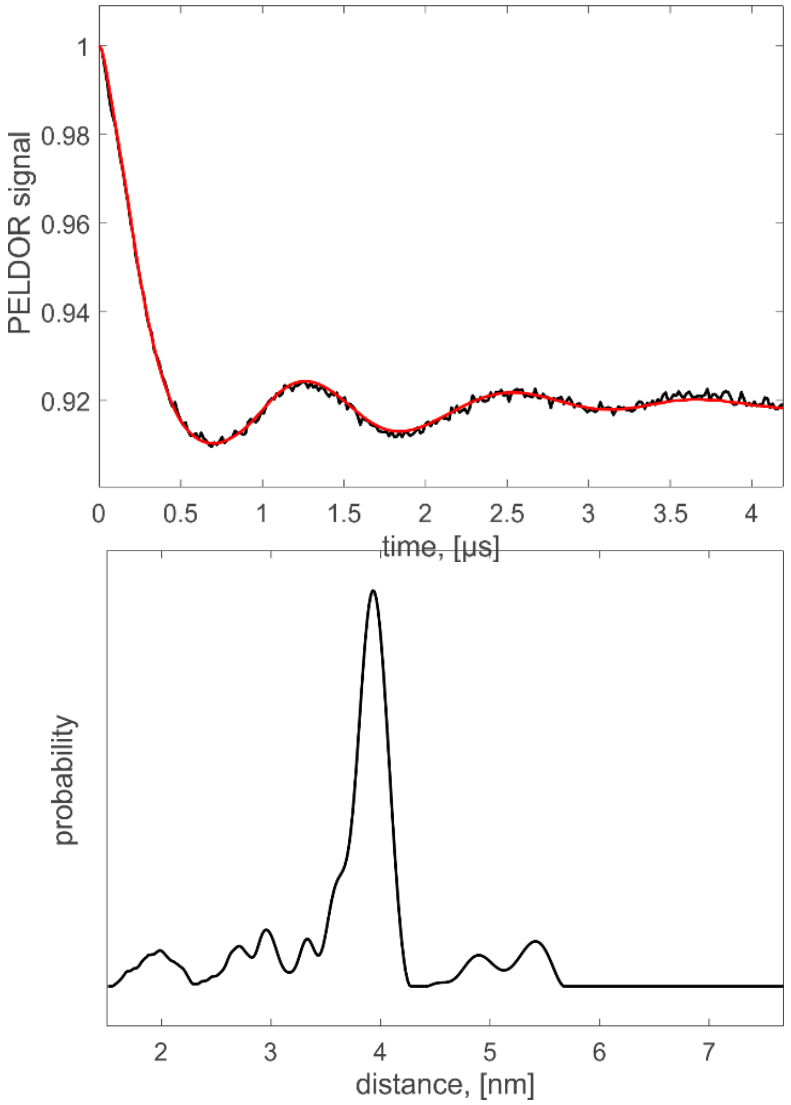
Binds TC with K_d = 5 nM



Application to Tetracyclin Aptamer

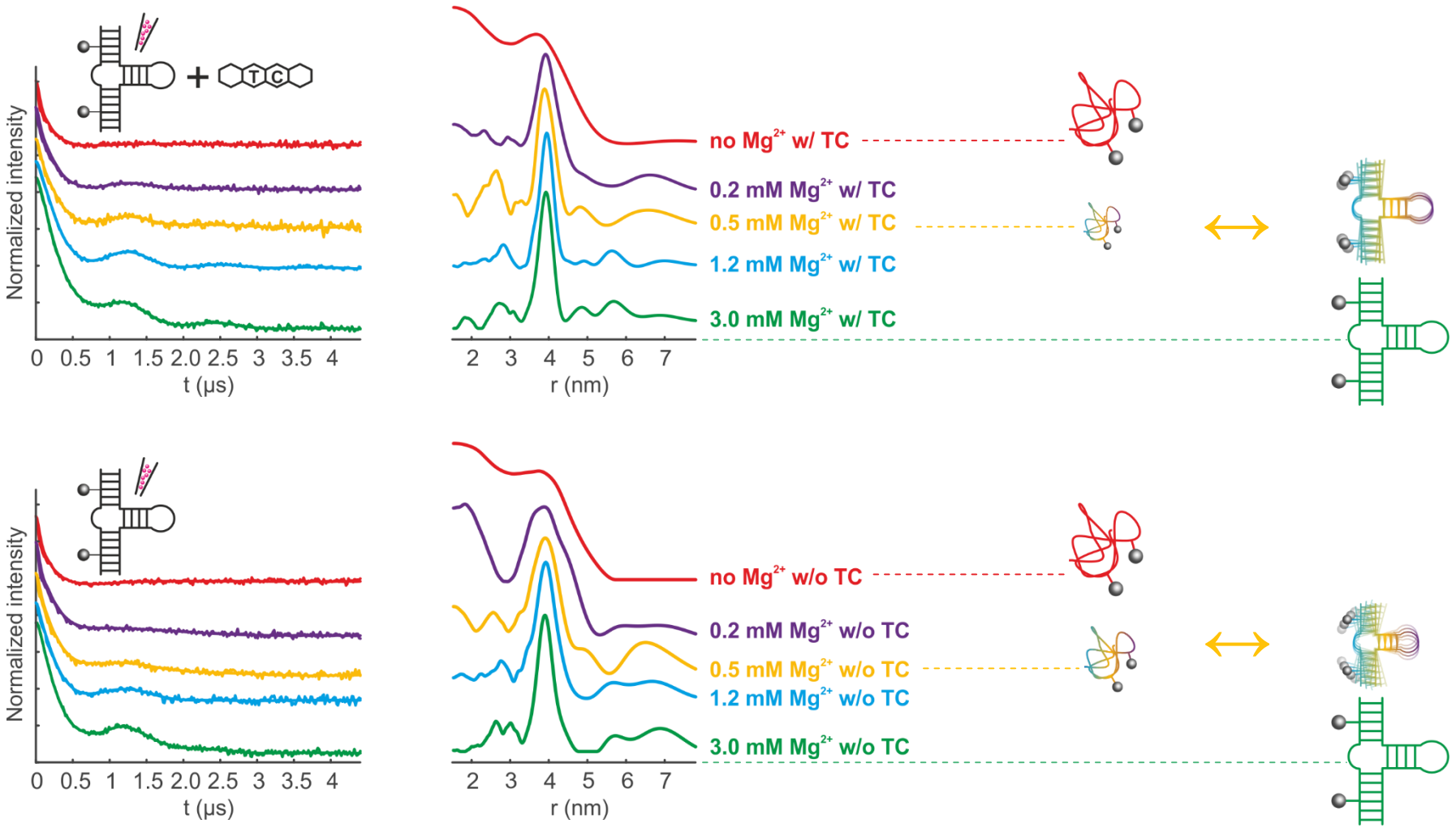
Xiao et al. Chem. Biol. 2008





Collaboration with Beatrice Suess (TU Darmstadt)

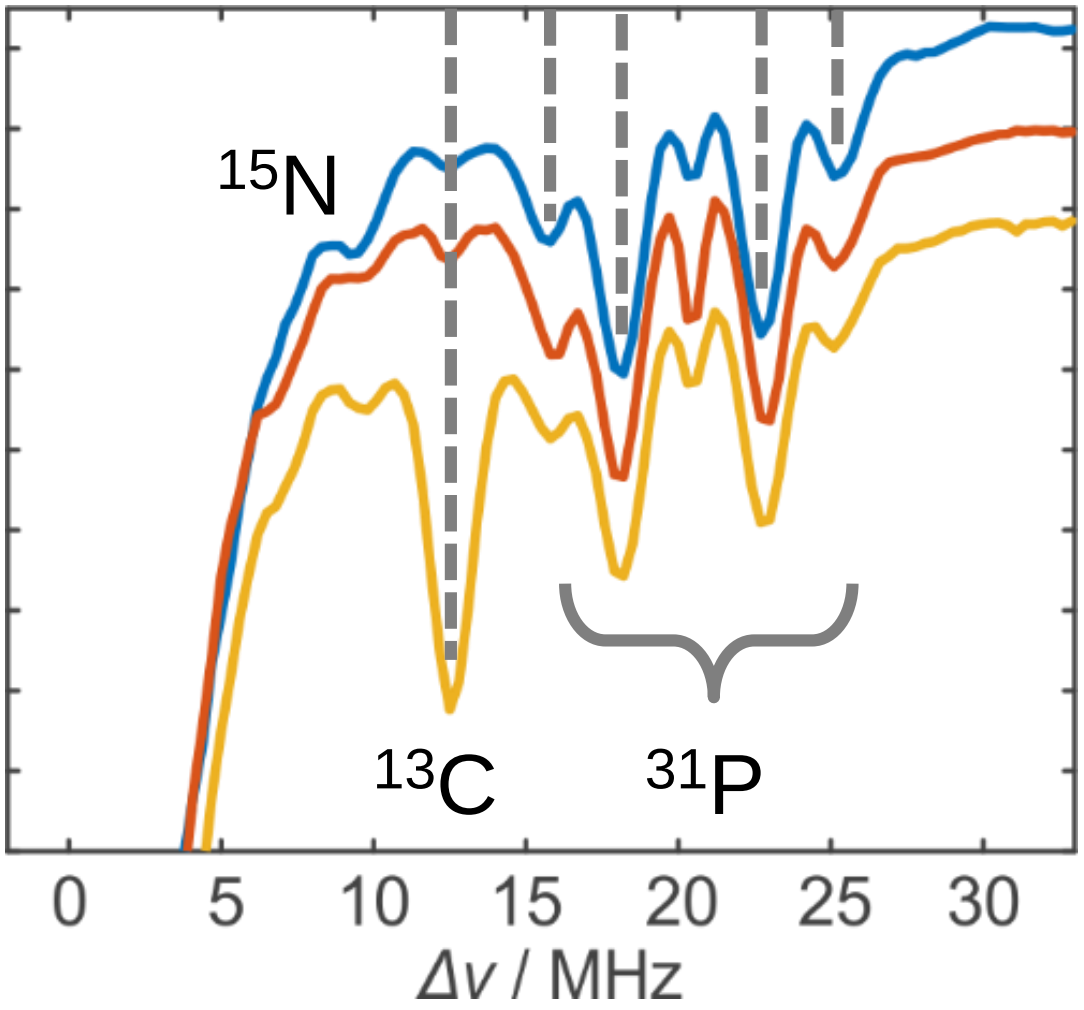
Stabilization of teriary structure by Mg^{2+}



Tetracycline binding structure is already preformed

Hetzke et al *RNA (2018)*

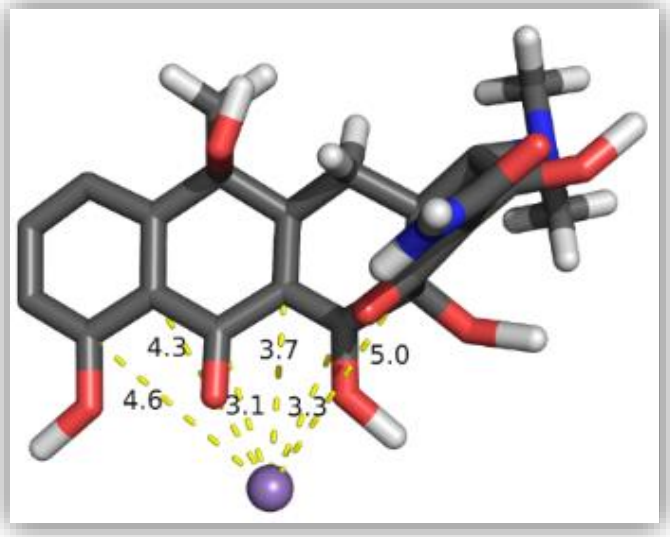
ELDOR detected NMR on TC aptamer



RNA/Mn^{2+}

RNA/Mn^{2+}/TC

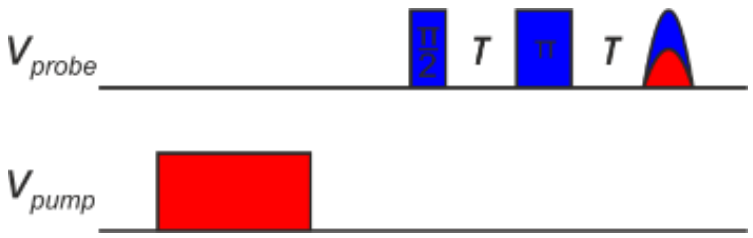
RNA/Mn^{2+}/^{13}C-TC



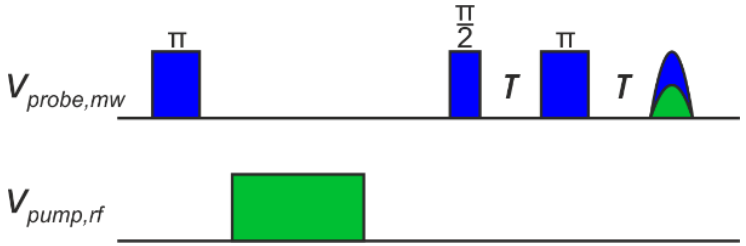
Mn binds to TC and to Aptamer

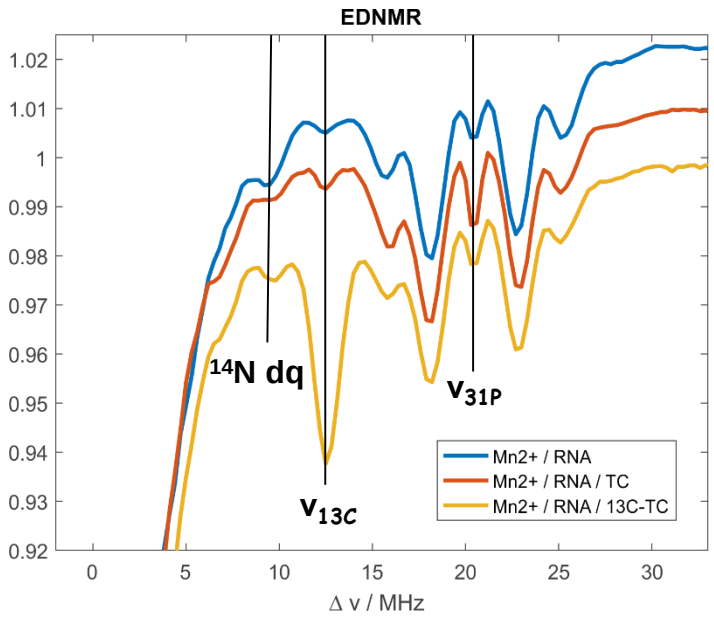
Comparison EDNMR & ENDOR

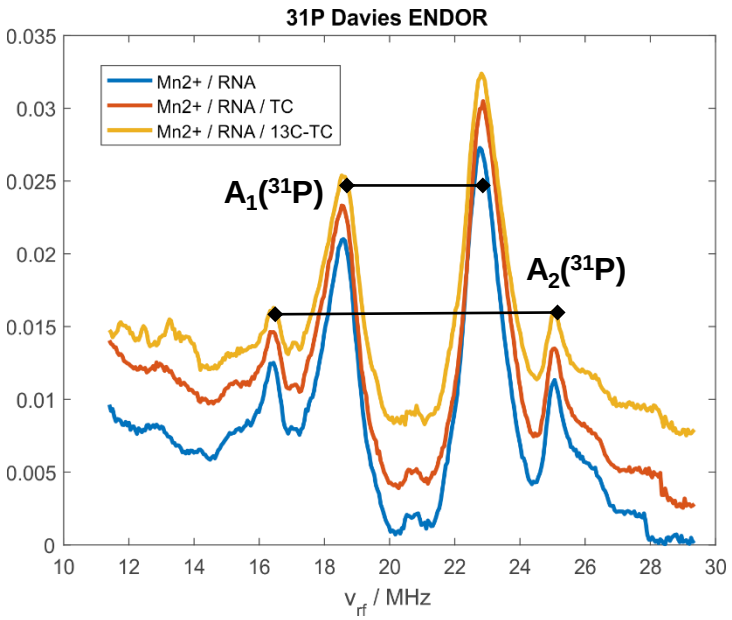
EDNMR



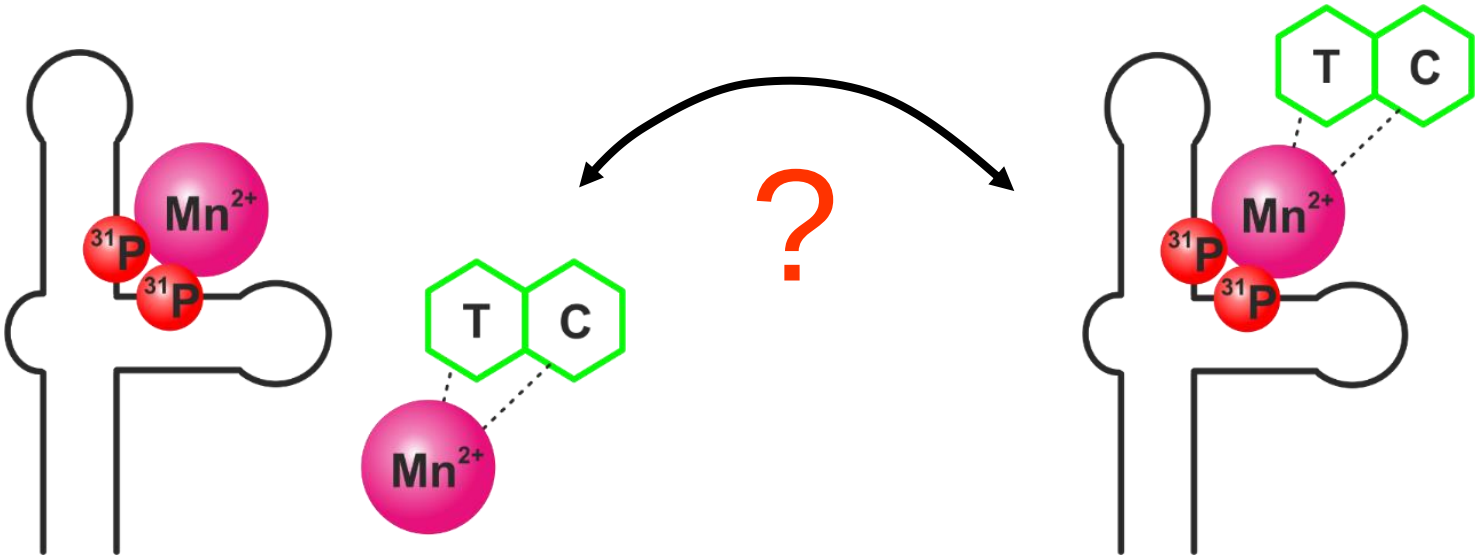
Davies-ENDOR



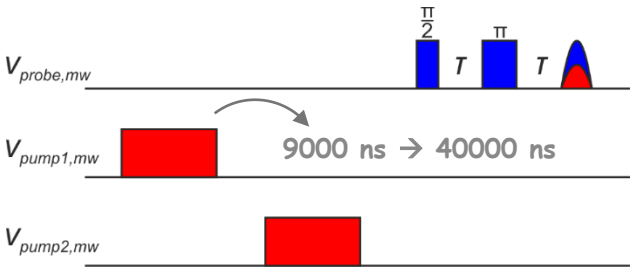




Comparison EDNMR & ENDOR

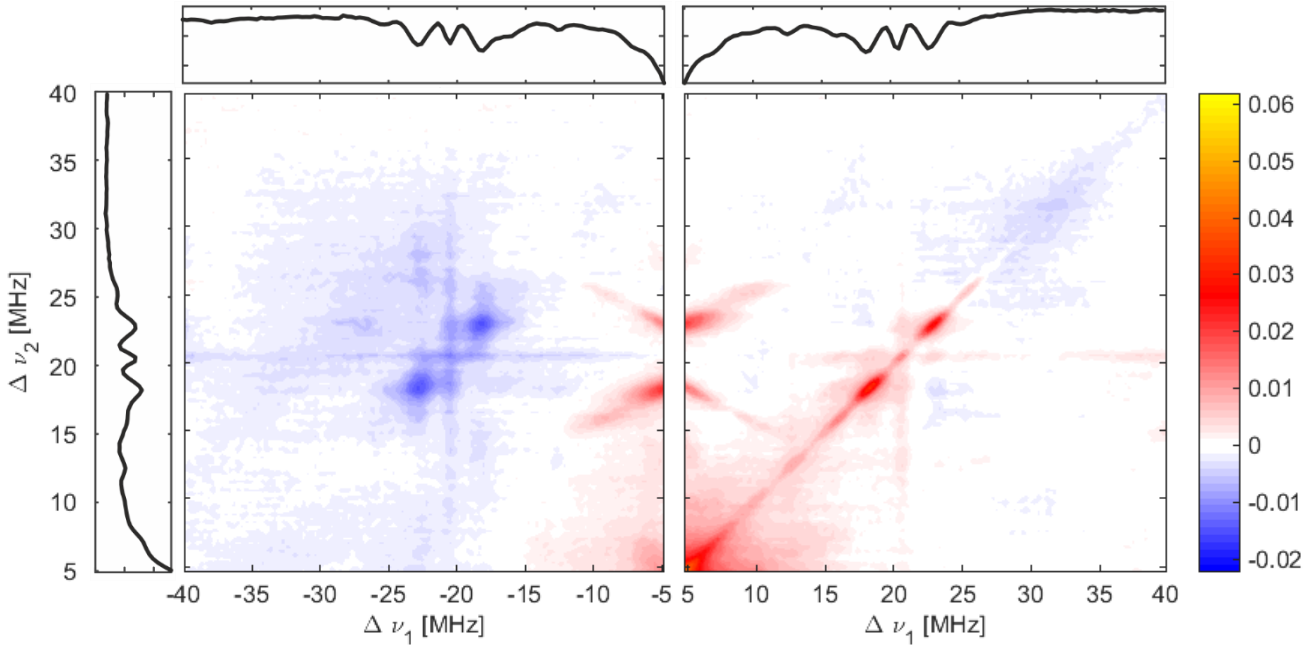


2D-EDNMR applied to TC aptamer

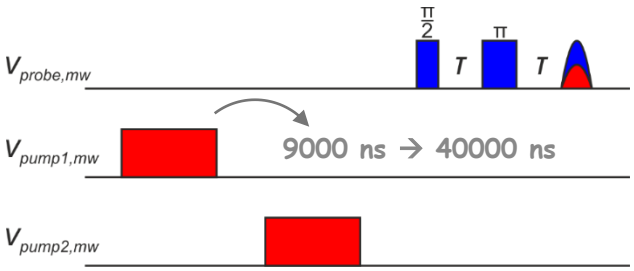


2D-EDNMR

Bg-corrected 2D-EDNMR 1mM RNA/Mn^{2+}/TC

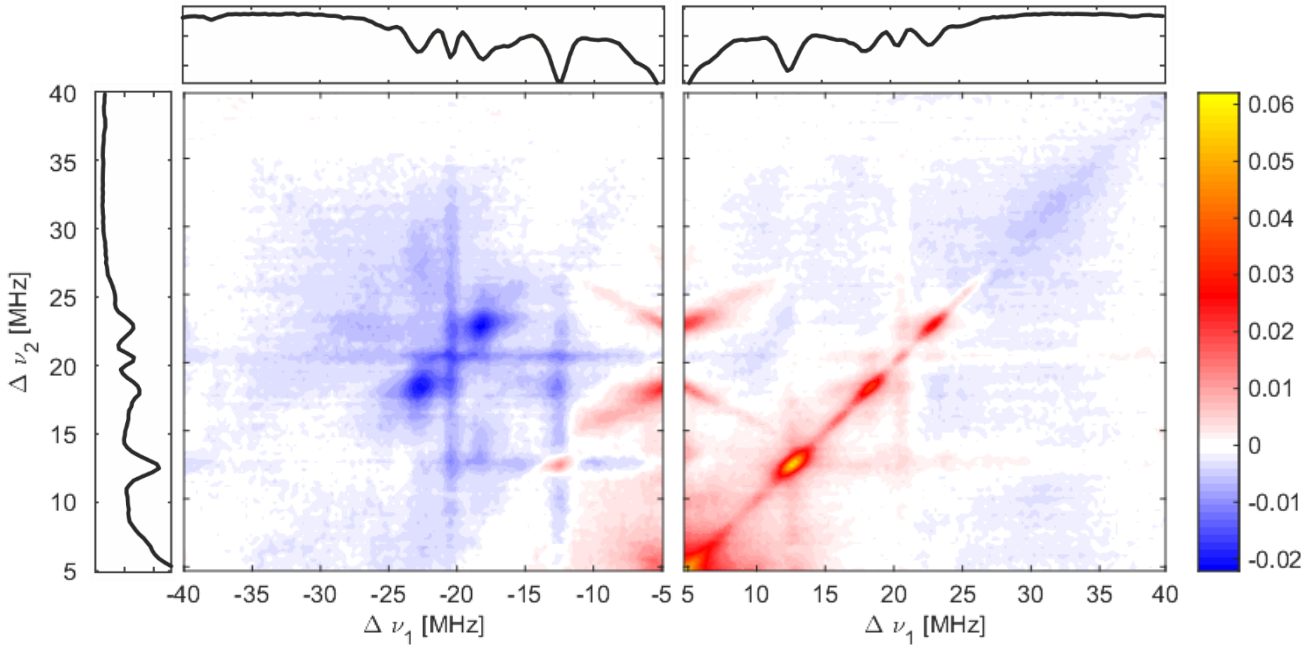


2D-EDNMR applied to TC aptamer

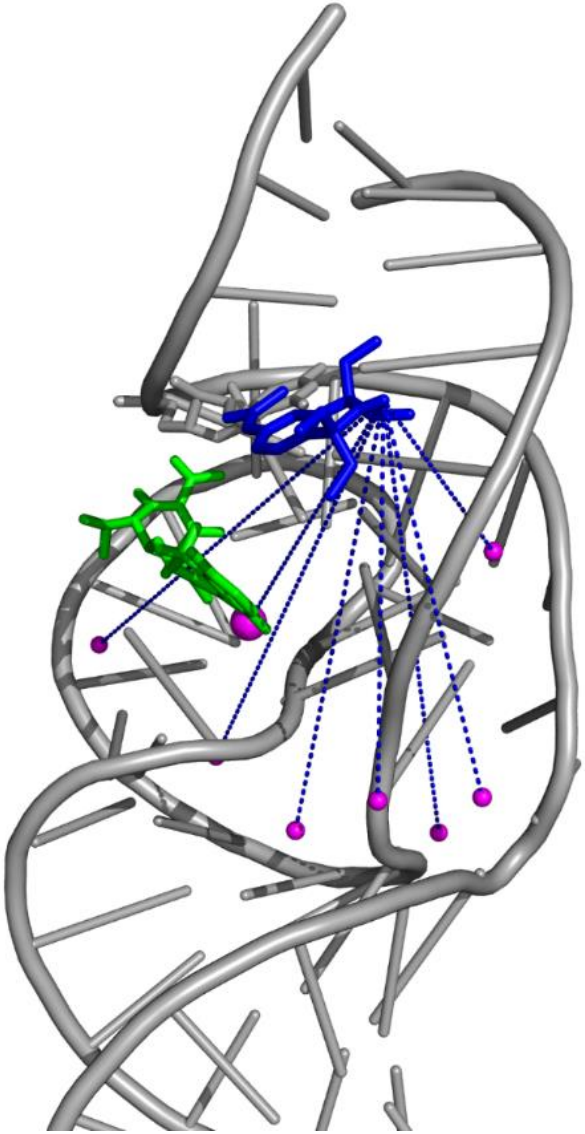


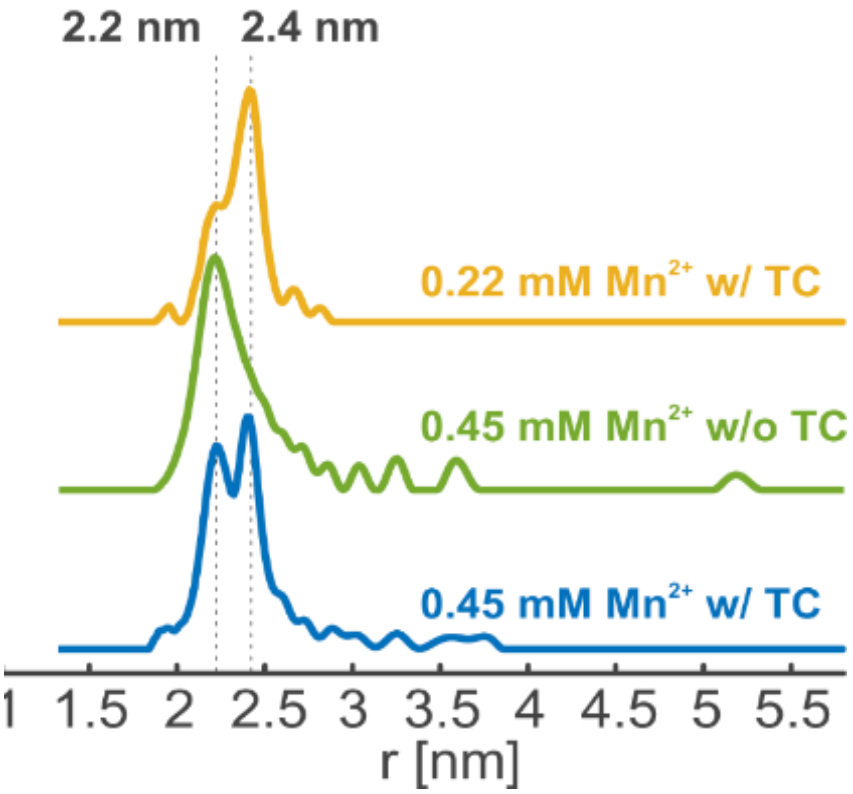
2D-EDNMR

Bg-corrected 2D-EDNMR 1mM RNA/Mn^{2+}/^{13}C-TC



PELDOR from Cm spin label to Mn^{2+}





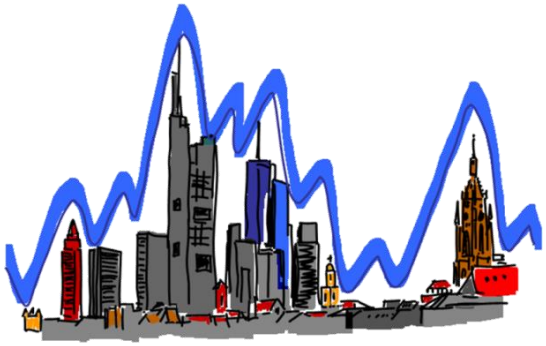
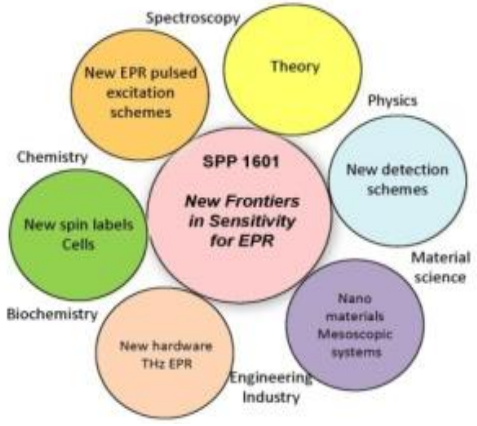
2 Mn binding sites can be detected

Acknowledgements

Thilo Hetzke, Claudia Grytz, Alberto Collauto,
Markus Gränz, Nicole Erlenbach

Philipp Spindler, Philipp van Os, Alice Bowen

Burkhard Endeward, Vasyl Denysenkov



Snorry Sigurdsson (University of Iceland)
Gerhard Hummer (MPI Biophysik Frankfurt)
Peter Güntert (Biophysical Cchemistry Frankfurt)
Steffen Glaser (TU Munich)
Elena Bagryanskaya (Novosibirsk)