dissolution **D**ynamic **N**uclear **P**olarization and **DNP** in liquids

methods for obtaining strongly polarized nuclear spins in solution

Increase in signal-to-noise ratio of >10,000 times in liquid-state NMR
J. H. Ardenkjær-Larsen, B. Fridlund, A. Gram, G. Hansson, L. Hansson, M. H. Lerche,
R. Servin, M. Thaning, K. Golman PNAS 2003, 100, 10158–10163

Dissolution DNP

The method uses

- low temperature,
- high magnetic field,
- dynamic nuclear polarization (DNP) to str polarize nuclear spins
- in the solid state (glass).

HyperSense - the in-vitro DNP Polariser
3.35 T magnet
sold by Oxford Instruments
can be coupled to any commercial superconducting NMR spectrometer
easy switch from normal to hyperpolarized mode
since 2012

Hyperpolarization signifies a spin polarization that deviates strongly from thermal equilibrium.

https://www.oxford-instruments.com/products/spectrometers/nuclear-magnetic-resonance-nmr/hypersense

DNP polarizer

superconducting magnet (3.35 T)

the sample is contained in a variable temperature insert, VTI, (inside the magnet cryostat, which was modified) cooled at 1.2 K by liquid helium (pumped below the inversion T).

placed in the resonator (microwaves at 94 GHz)

spectrometer

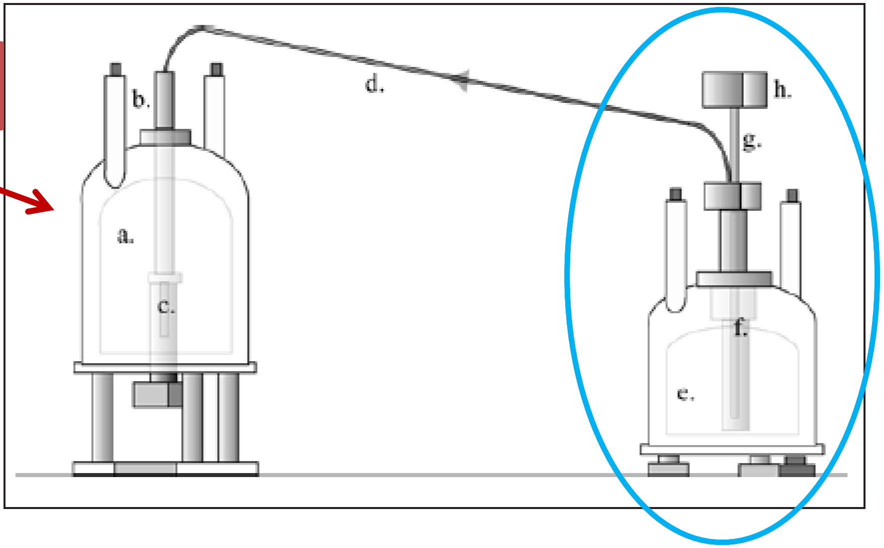
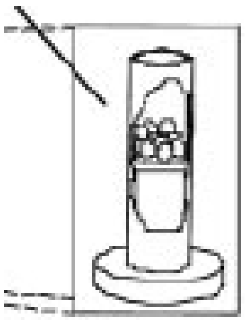


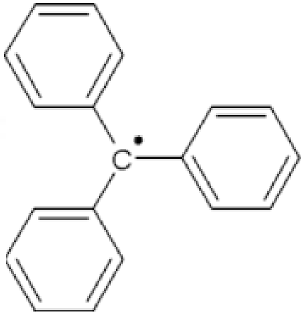
Fig. 4. Dissolution DNP setup, a) NMR cryomagnet (7.05 T); b) polarized solution injector; c) NMR tube sitting in the NMR probe; d) PTFE transfer line; e) DNP cryomagnet (3.35 T); f) cryostat; g) waveguide; h) microwave source.

Sample

^{13}C urea solution in glycerol (29% wt/wt nearly saturated) and tritylradical (15/20 mM)

40–50 mg of the solution was dispensed as droplets into liquid nitrogen and transferred to the sample container as frozen pellet





sample container with sample beads

Loading the sample

1. the sample holder and container are pre-cooled in a nitrogen bath (the sample holder is a Teflon tube designed to hold the sample container in position in the magnetic field and subsequently to enable the elevation of the sample before dissolution)
2. the frozen pellets are placed in the container via an opening in the sample holder,
3. the sample holder is lowered into the variable temperature insert, into the liquid He
4. the VTI evacuated to 0.8 mbar to cool the sample to 1.2 K

Polarization

Dissolution of the sample

Dissolution-DNP is based on the notion that a 1 K "ice cube" can be dissolved in a fraction of a second or short on T_1 time scale

1. Soon after stopping mw irradiation by pressurizing the system the sample is raised 10 cm from the magnetic center to leave the liquid helium (magnetic field 3 T)
2. hot water is injected to dissolve and dilute the sample (inside the polarizer magnet)

Electron and Nuclear Polarization at Low Temperature

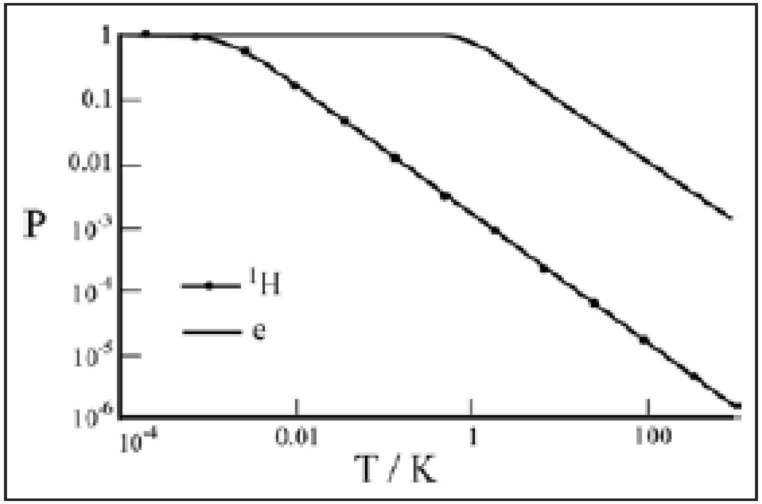


Fig. 1. Polarization of the 1/2 spins of the electron (plain line) and of the ^{1}H nucleus (line with dots) as a function of the temperature in Kelvin at 3.35 T.

$$P = \frac{exp\left(-\frac{1}{2}\gamma\frac{\hbar B_0}{kT}\right) - exp\left(\frac{1}{2}\gamma\frac{\hbar B_0}{kT}\right)}{exp\left(-\frac{1}{2}\gamma\frac{\hbar B_0}{kT}\right) + exp\left(\frac{1}{2}\gamma\frac{\hbar B_0}{kT}\right)}$$

For very low T the truncation of the series expansion is not allowed

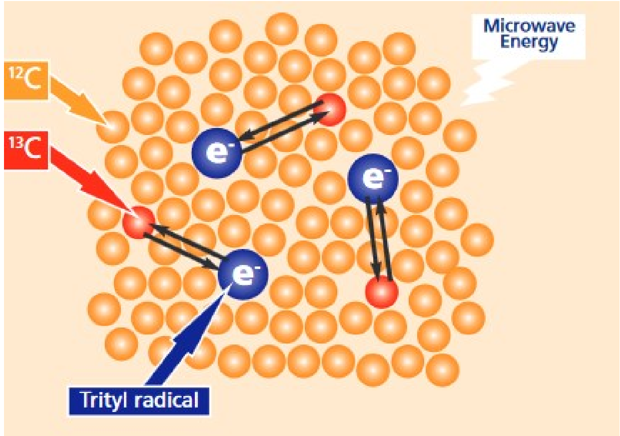
At 1.2 K e- polarization is full, that of nuclei not yet

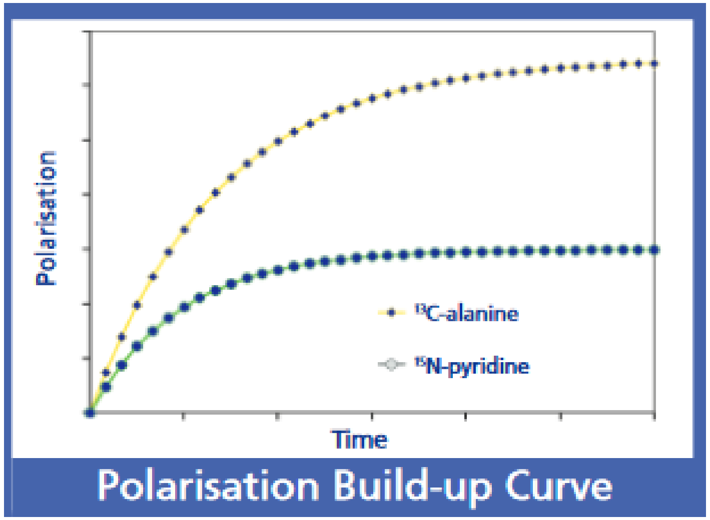
The maximum nuclear spin polarization is still only 1.5×10^{-3} at 1 K

NMR of Insensitive Nuclei Enhanced by Dynamic Nuclear Polarization
P. Miéville, S. Jannin, L. Helm, G. Bodenhausen Chimia 65 (2011) 260–263

Polarizing the Sample

The sample is irradiated
over 15 min to 4 hours
with microwaves at a frequency of 94 GHz
in the 3.35 T magnetic field
The build-up of the nuclear spin polarization
can be observed by 'small angle' pulsed NMR.





Polarisation Build-up Curve

polarization build up is
slow at low T

long relaxing nuclei are required
(quaternary carbons, ^{15}N)

https://www.oxford-instruments.com/OxfordInstruments/media/industrial-analysis/magnetic-resonance-pdfs/Dynamic-Nuclear-Polarisation-DNP-HyperSense.pdf

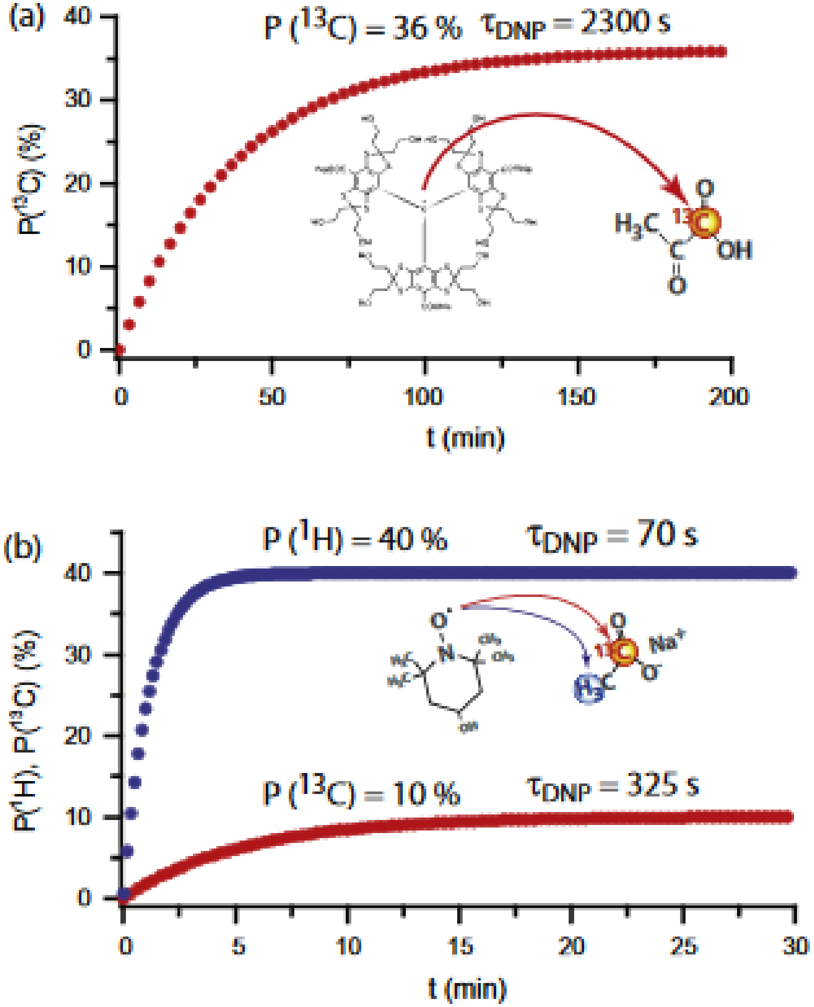


Fig. 1. (a) Typical DNP build-up of 1-^{13}C pyruvic acid with 15 mM trityl at 1.2 K and 3.35 T [9]. (b) Typical ^{1}H (blue) and ^{13}C (red) DNP build-up curves of 3 M 1-^{13}C acetate with 30 mM TEMPOL in D_2O:glycerol-d_8 (1:1) at 1.2 K and 3.35 T [29,30]. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Typical DNP build-up of 1-^{13}C pyruvic acid with 15 mM trityl at 1.2 K and 3.35 T

The build up is faster for ^{1}H

It is advantageous to polarize ^{1}H and then transfer the polarization to ^{13}C by means of cross-polarization

Optimizing dissolution dynamic nuclear polarization

A. Bornet, S. Jannin J. Magn. Reson. 2016, 264, 13-21

Overhauser DNP Enhancement

Overhauser DNP was theoretically predicted in 1953 [Overhauser] and subsequently experimentally observed in ammonia-dissolved alkali-metals [Slichter]

$$\frac{M_{ZI(ss)}-M_{ZI(0)}}{M_{ZI(0)}} = fs\left|\frac{\gamma_S}{\gamma_I}\right|(W_2\text{-}W_0)/(W_2+2W_{1I}+W_0)$$

- I: nucleus
- S: electron

max: -359 for pure dipolar interaction:
0.5* ratio of gyromagnetic ratios

- $0 \leq s \leq 1$: **saturation factor** describes the efficiency of microwave pumping, depends on microwave power and e- relaxation
- f: **leakage factor** $R_{1para}/(R_{1para}+R_{1dia})$ reflects the relative importance of the relaxation of the nucleus by the interaction with the e- with respect to the overall relaxations pathways of the nucleus
- the correlation time may be the e- relaxation time

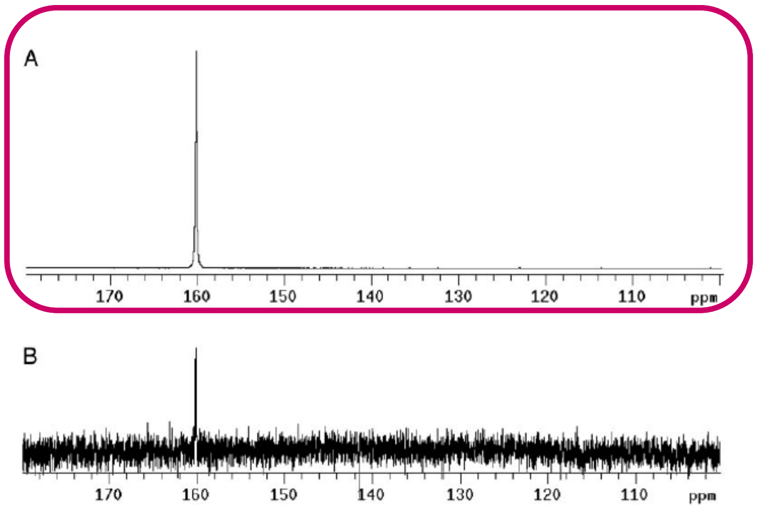
Basic facts and perspectives of Overhauser DNP NMR, E. Ravera, C. Luchinat, G. Parigi
J. Magn. Reson. 2016, 264, 78-87

Transfer to the NMR Spectrometer

The dissolution process effectively preserves nuclear polarization.
The resulting hyperpolarized liquid sample is transferred to a high-resolution NMR spectrometer, where an enhanced NMR signal can be acquired, or it may be used as an agent for *in vivo* imaging or spectroscopy

NMR Measurement of the Hyperpolarized Sample

The polarization is often created ex situ, and therefore that cannot be recreated to the initial state. It is a game of “use it (smart), or lose it”.

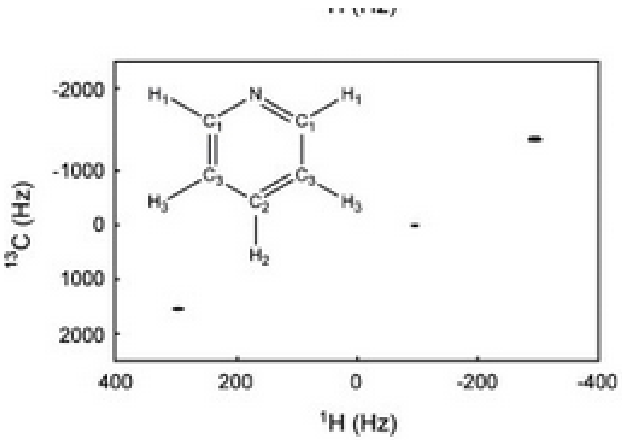


^{13}C natural abundance urea that was polarized to 20%: a 24,000-fold enhancement over 9.4 T.
The thermal spectrum on the same sample took 65 h to acquire with a thousand-fold lower SNR. 27 years of averaging would have been required to reach the same SNR.

It is possible a kinetic study using short pulses, e.g. 10°

Hyperpolarization is suited to directed detected heteronuclear correlation

2D NMR Ultrafast Experiments: 2D hetero-correlations in a **single scan**



pyridine 3 mM

HyperSPASM NMR: A New Approach to Single-Shot 2D Correlations on DNP-Enhanced Samples
K. J. Donovan, L. Frydman J. Magn. Reson. 2012, 225, 115-119

07.12.16 - EPFL's Institute of Chemical Sciences and Engineering (ISIC) has installed an

NMR SYSTEM WITH THE HIGHEST SENSITIVITY AND RESOLUTION IN THE WORLD

https://actu.epfl.ch/news/epfl-installs-world-unique-nmr-system-6/

In Situ Overhauser DNP at High Magnetic Fields

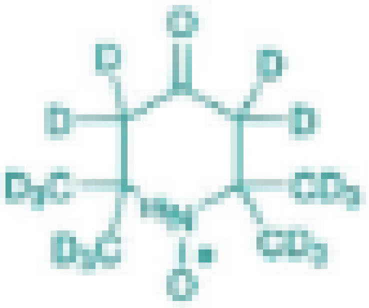
- The polarization transfer from small radical is optimal at low magnetic field (0.33 T, 9 GHz for e-) owing to the favourable modulation by translational motion of the e-nucleus dipolar interaction
- By increasing the field the efficiency decays as $1/B_0^2$
- because we becomes much faster than the motions modulatig the e-n dipolar interaction
- Dielectric losses of the solvent (e.g. H_2O) are strong for $\lambda \leq 1$ mm. Heating of the sample
- **gyrotron**: μW high power source for low λ

In Situ Overhauser DNP at High Magnetic Fields

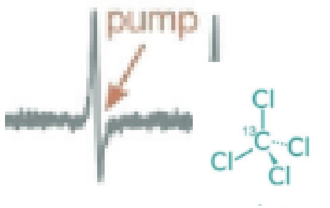
- To minimize dielectric loss the E and B field must not superpose
- not easy at high magnetic field (e.g. 9.4 T, 400 MHz for 1H and 268 GHz for e-) λ= 1.12 mm
- the resonator can hold only tiny samples (100 nL)
- the sample must be placed in the position of minimum E and Max B of the stationary μW radiation

DNP in liquids

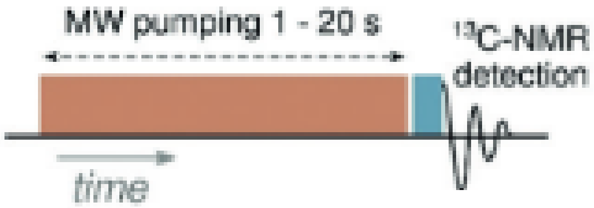
polarizing agent; nitroxide radical
MW irradiation (seconds to 1 min: time ion the order of T_1 nucleus)



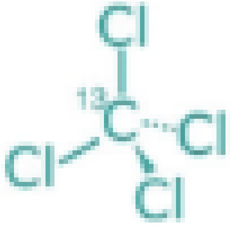
34 GHz EPR Spectrum 1.2 Tesla



^{13}C NMR 13 MHz



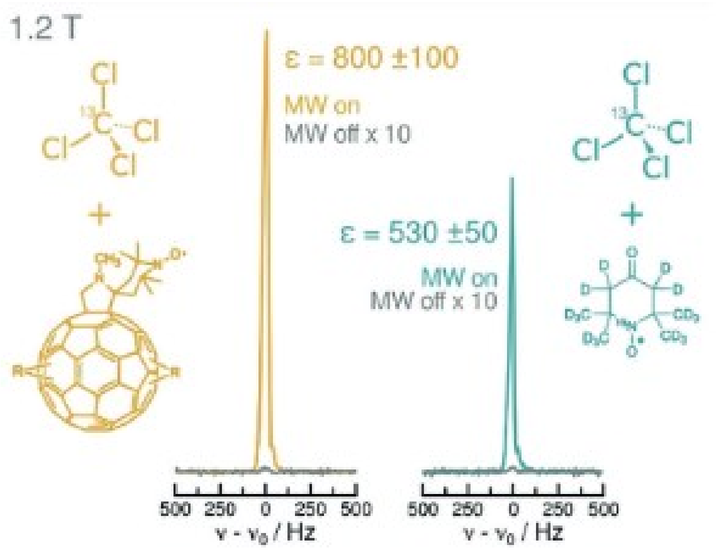
perdeutero 4-oxo-2,2,6,6-tetramethylpiperidine ^{15}N-oxide 10 mM



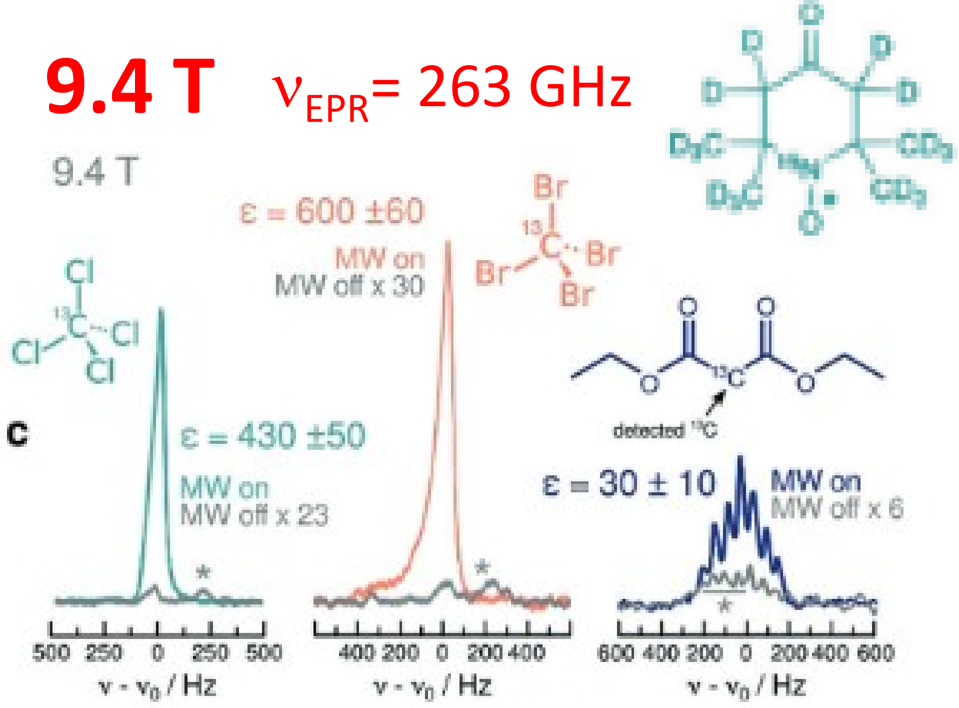
$^{13}\mathbf{C}Cl_4$

Dynamic Nuclear Polarization of ^{13}C Nuclei in the Liquid State over a 10 Tesla Field Range M. Bennati et al. Angew. Chem. Int. Ed. 2019, 58, 1402-1406

1.2 T



9.4 T ν_{EPR}= 263 GHz



^{13}C diethylmalonate

$$\varepsilon = 1 - \xi f s \frac{|\gamma_e|}{\gamma_{13C}}$$

$$\xi = \frac{w_2^{dip} - w_0^{dip} - w_0^{scalar}}{\left(w_0^{dip} + 2w_1^{dip} + w_2^{dip}\right) + w_0^{scalar}}$$

ε: DNP Overhauser enhancement factor

f: leakage factor

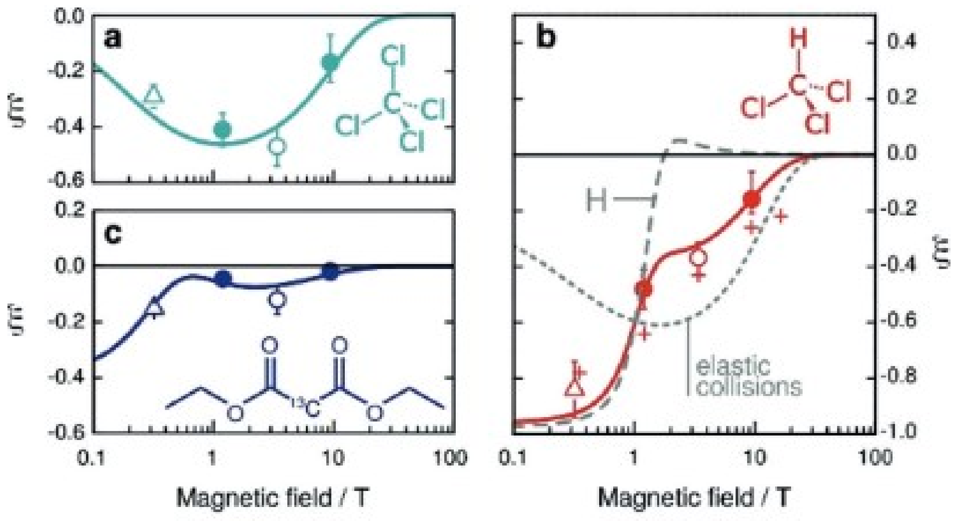
s: saturation factor s~0.6

$|\gamma e|/\gamma_{13C}$~ 2600

^{13}C DNP coupling factor, ξ, contain contributions from scalar and dipolar relaxation, which are counteracting

w_2 negligible at high fields

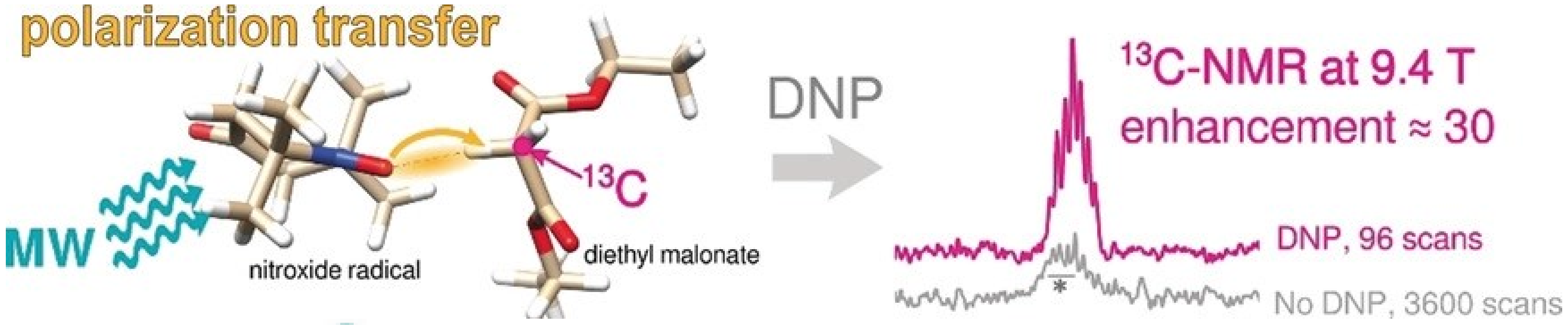
B_0 Dependence of DNP ^{13}C Coupling Factor

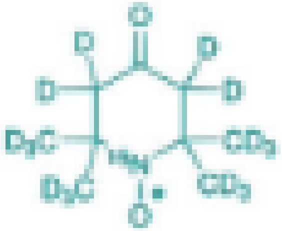


w_2 negligible at high fields

$$\xi_{\text{high field}} \approx -\, w_0^{\text{scalar}} / \left(2w_1^{\text{dip}} + w_0^{\text{scalar}}\right)$$

DNP enhanced ^{13}C NMR spectra of organic compounds at 9.4 T

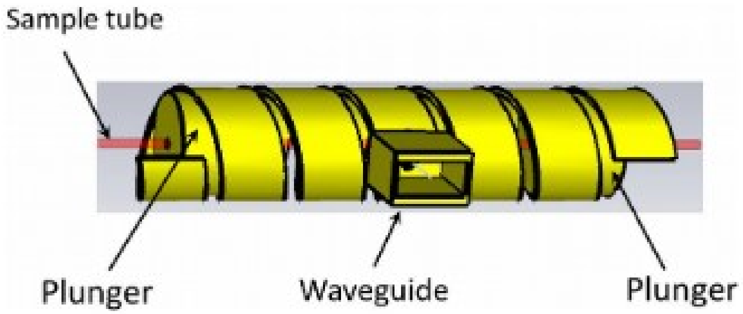




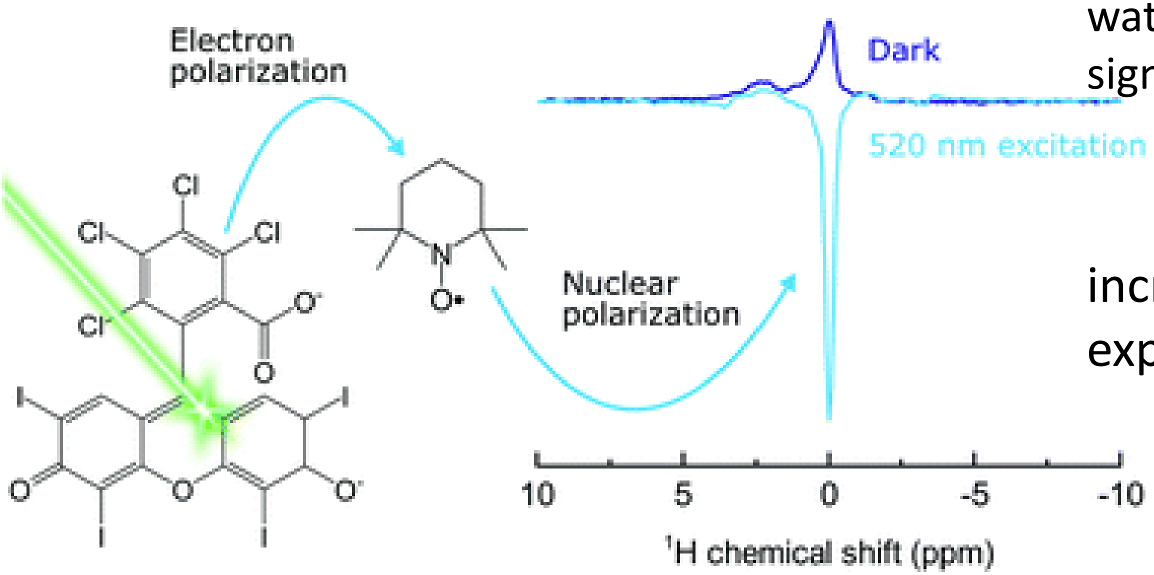
active sample volume 35 **nL**

continuous μW irradiation during the experiment

The resonance structure consist of a cylindrical cavity made of copper tape forming a helical six-turn coil of 1.5mm inner diameter, which serves also as rf coil. One of the silver coated plungers is movable for tuning the resonant cavity



Optically Generated Hyperpolarization



water ^{1}H NMR signal

increment: 8 x experimental

Photogenerated triplet state in a diradical by green laser radiation
Coupling of the e- triplet spin state with a e- doublet spin state gives a quartet and a doublet

Optically generated hyperpolarization for sensitivity enhancement in solution-state NMR spectroscopy M. W. Dale, C. J. Wedge Chem. Commun. 2016, 52, 13221-13224