NMR Signals Enhanced by Dynamic Nuclear Polarization

Application to Structural Biology and Material Science

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NMR signals enhanced by DNP –
Application to structural biology and material science

1. Nuclear magnetic resonance (NMR)
   and electron spin resonance (ESR or EPR)

2. Dynamic nuclear polarisation (DNP)

3. DNP-NMR in liquids

4. Solid-state DNP NMR

5. Applications

Nuclear Magnetic Resonance - NMR

Atomic nuclei (^{1}H, ^{13}C, …),
Intrinsic angular momentum (spin),
Magnetic moment,
External const. magnetic field.

Quantization of spins,
splitting of energy levels
(Nuclear Zeeman effect)
defines spin Larmor
frequency

Transition between
Zeeman levels.

If irradiation with an ac field
oscillating at Larmor frequency
(*radio frequency range*)
then resonance.

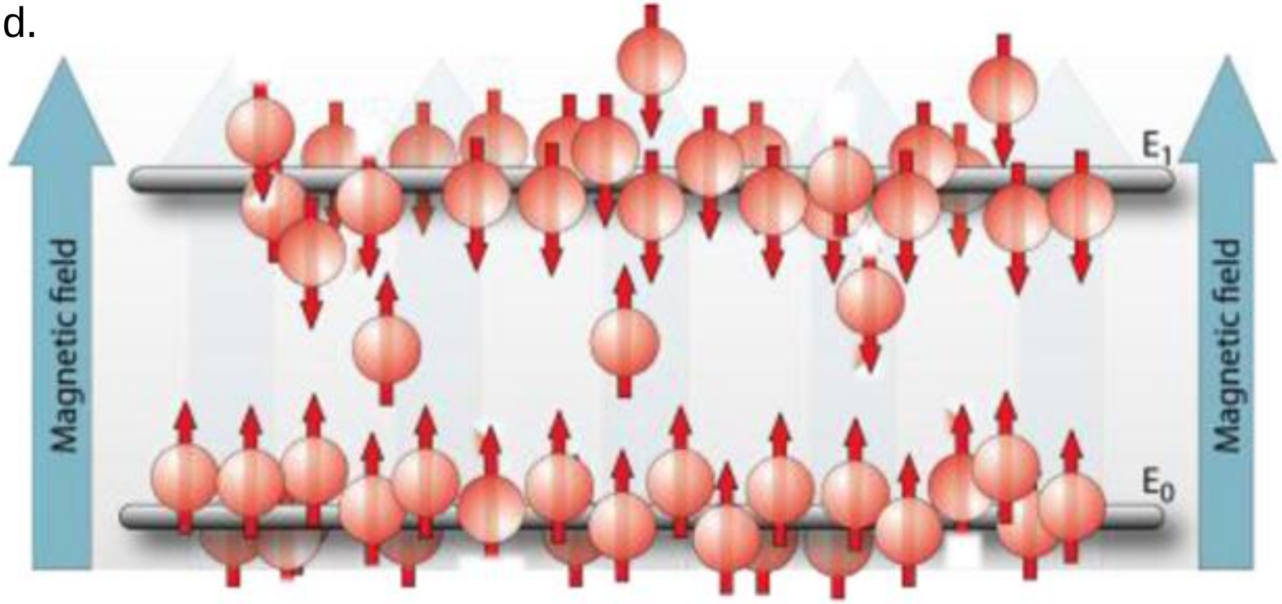


Figure courtesy of Sami Jannin, EPFL

Electron Paramagnetic Resonance - EPR

Analog for the electron spin:

Spin quantization
Energy level splittingirradiation

Transitions between
Zeeman levels.

If irradiation with an ac field oscillating
at electron Larmor frequency
(*microwave frequency range*)
then resonance.

Magnetic moment of electron
ca. 660 times larger than proton magnetic
moment

Electron spin resonance frequencies
ca. 660 times higher than those of ^{1}H NMR.

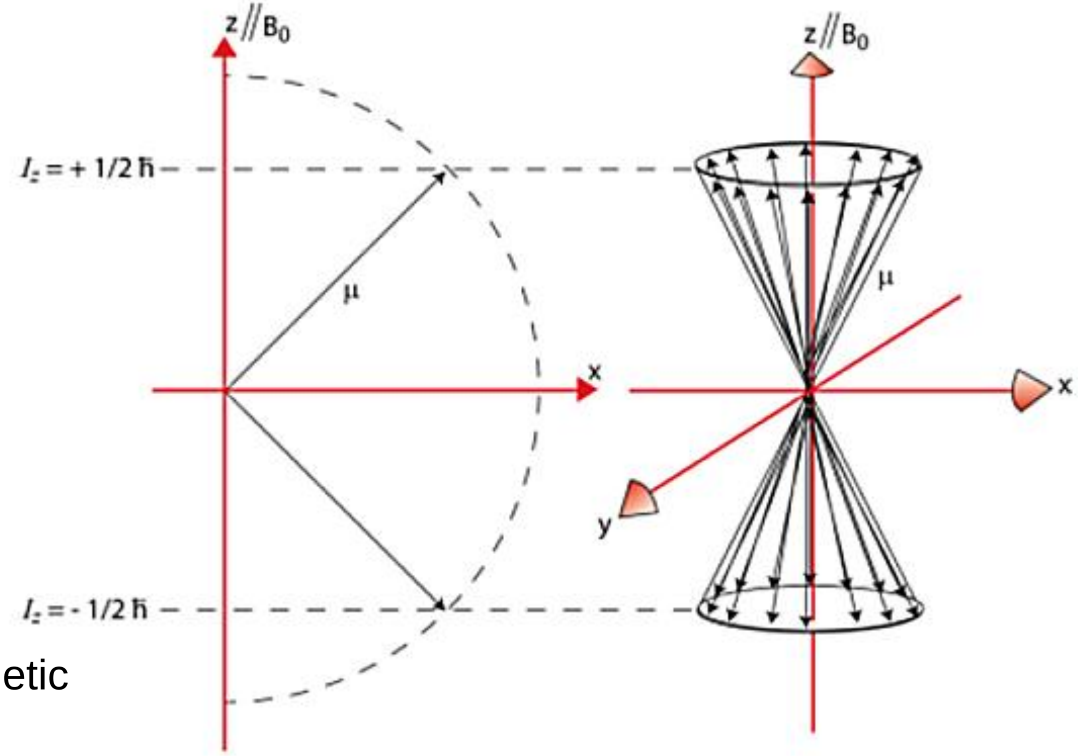
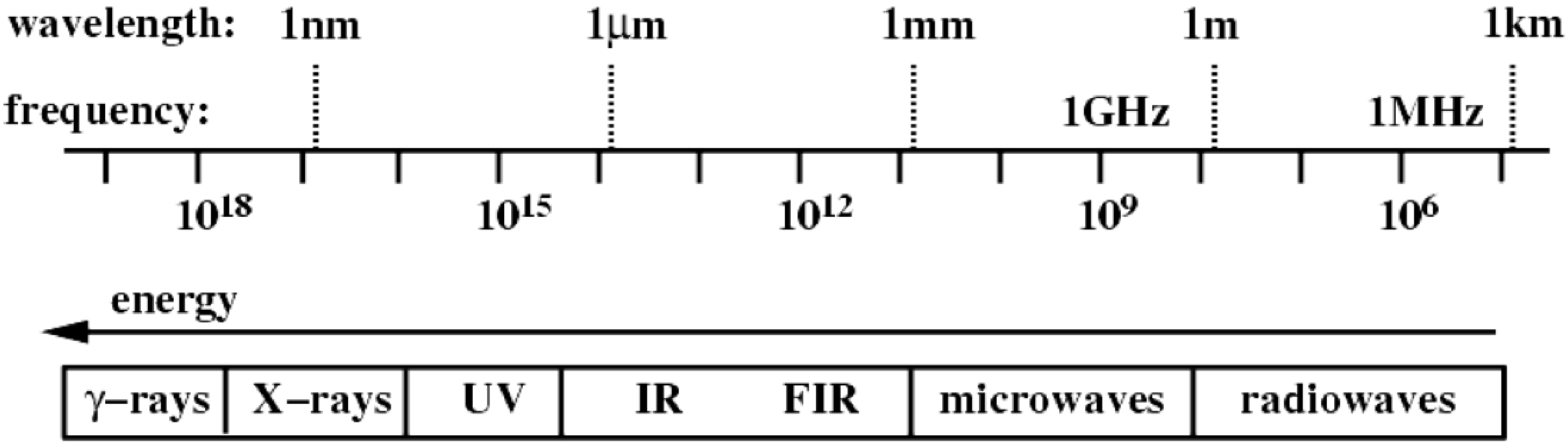


Figure courtesy of Sami Jannin, EPFL

The electromagnetic spectrum

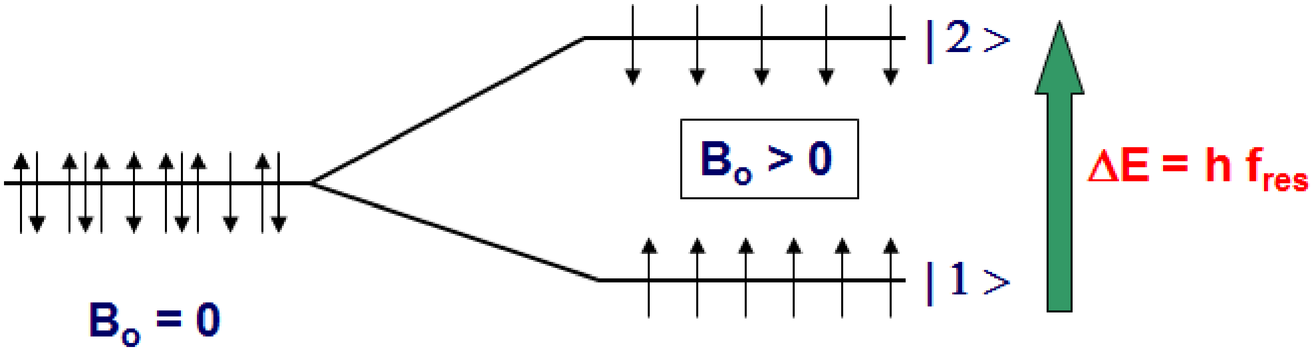


γ–rays	X–rays	UV	IR	FIR	microwaves	radiowaves
nucleus transitions	inner electrons	outer electrons	vibrations	rotations	e^- magnetic transitions	nuclear magnetic transitions
Moessbauer		optical spectroscopy			ESR	NMR

NMR of spin-1/2 nuclei

Nucleus	Natural abundance (%)	Sensitivity (abs.)	NMR frequency at 14.1T (MHz)	EPR frequency (GHz)
^{1}H	99.98	1	600	395
^{19}F	100	0.83	564.5	
^{31}P	100	0.066	242.9	
^{13}C	1.1	0.00018	150.9	
^{29}Si	4.7	0.00037	119.2	
^{15}N	0.37	0.0000039	60.8	

Zeeman coupling and chemical shift



$|2>$

$B_o > 0$

$\Delta E = h\, f_{res}$

$B_o = 0$

$|1>$

Resonance frequency: $f_{res} = \gamma_I B_0 / 2\pi$

γ_I: gyromagnetic ratio, constant for a given nucleus

- **Zeeman coupling:** Splitting of energy levels in external constant field B_0
- **Chemical shift:** Local variation of B_0 caused by magnetic shielding of nuclei by surrounding electrons, bond specific and dependent on spatial orientation of molecules relativ to B_0 (anisotropic)

NMR of liquids

Sucrose

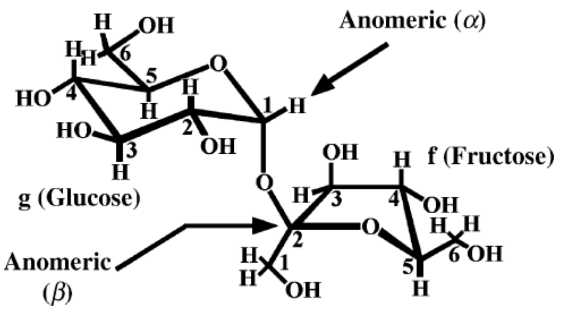
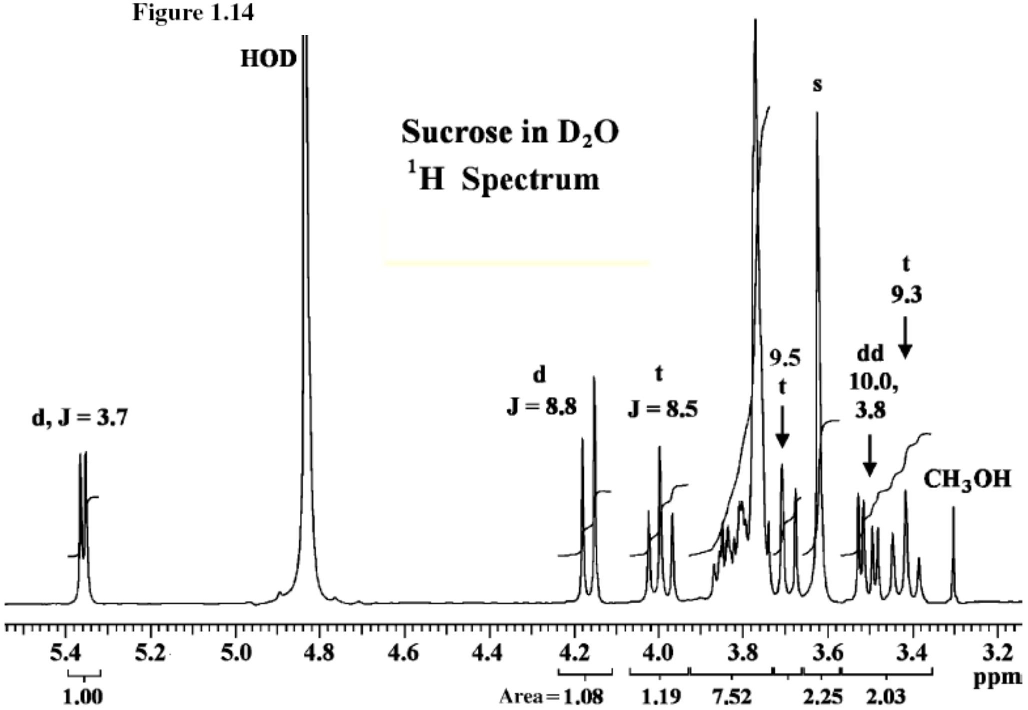
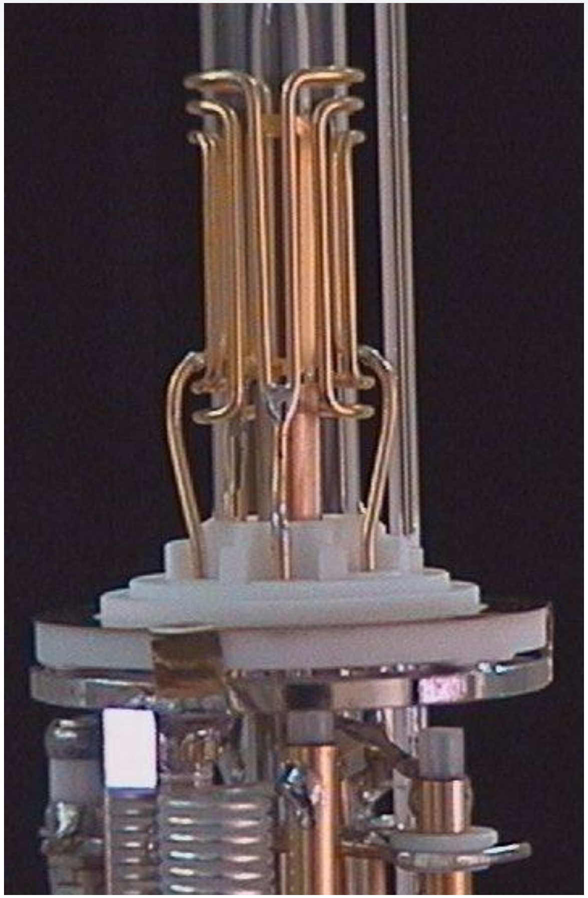


Figure 1.14





120 µl flow NMR probe

Overview

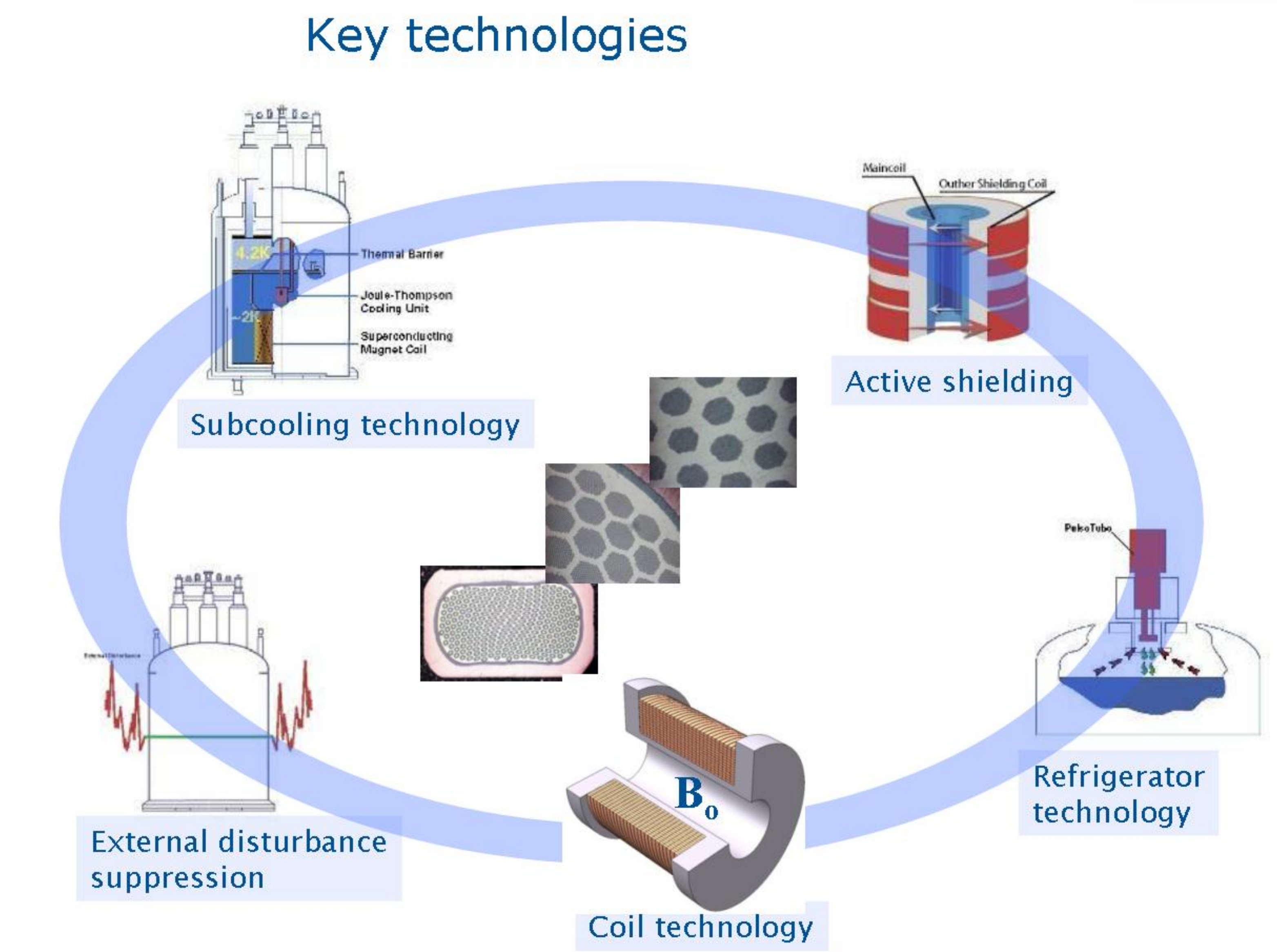
Magnets - B_0

NMR console - B_1 + NMR signal

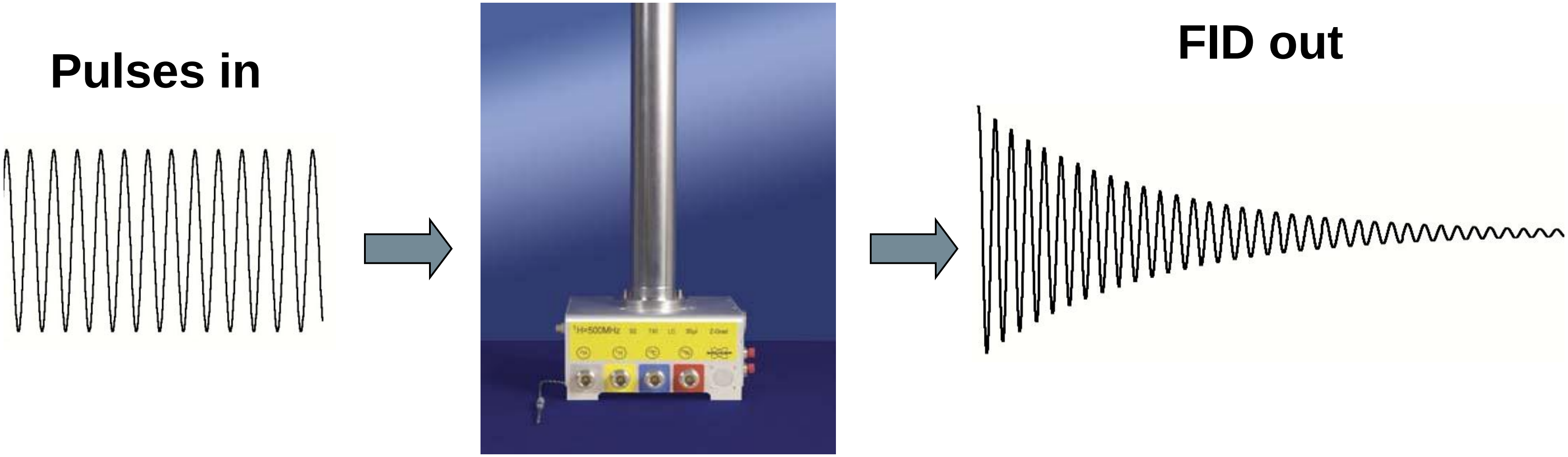
Probes - B_0 + B_1 + sample + NMR signal

Accessories - additional functionality
for example, for DNP

NMR magnets


Key technologies
4.2K
~2K
Thermal Barrier
Joule-Thompson Cooling Unit
Superconducting Magnet Coil
Subcooling technology
Maincoil
Outher Shielding Coil
Active shielding
PulsoTube
Refrigerator technology
External Disturbance
External disturbance suppression
B0
Coil technology

NMR Experiment

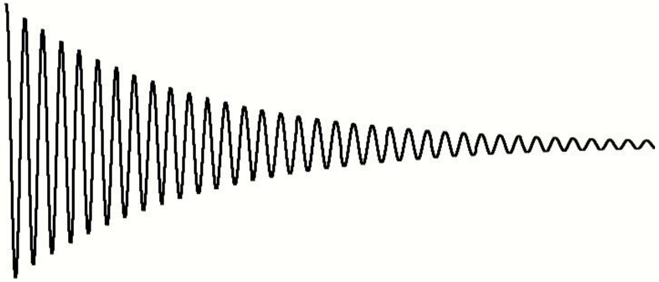


Pulses in

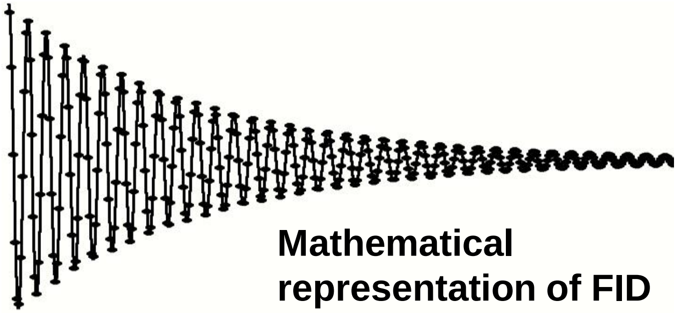
FID out

Digital FID

Analog FID out / Receiver



Conversion ⬇ to digital: ADC



Mathematical representation of FID

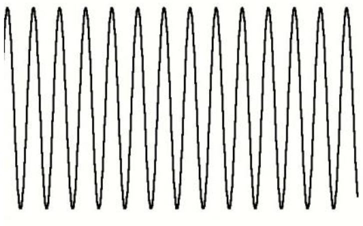
⬇ **FFT**

Spectrum

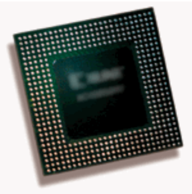
Digitization of a properly filtered (anti-aliasing) FID gives the exact mathematical representation of the NMR signal.

Digital Pulse Sequence

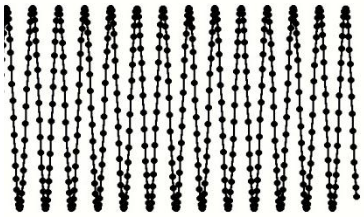
Pulses in / Transmitter



Conversion to analog: DAC



Numerically Controlled Oscillator



Mathematical representation of RF

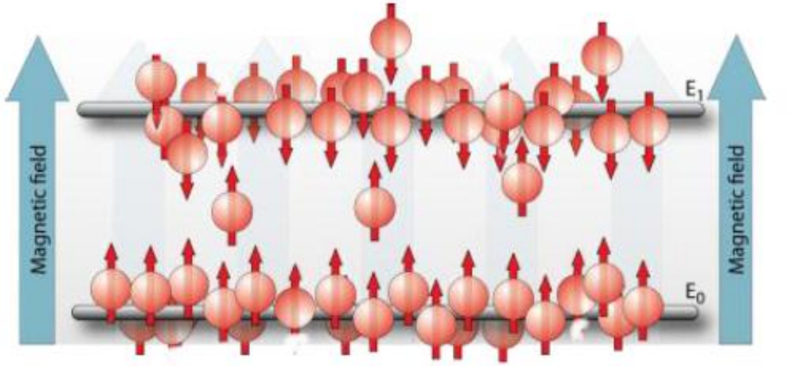
Pulse Sequence (Theory)

A mathematically exact representation of the excitation signals can be converted to real distortion-free RF using an D/A converter

Some basic issues …

Spin polarization calculation

The polarization is the ratio between the difference of population and the total population :



Spin polarization calculation $P = \frac{\Delta N}{N_{tot}} = \frac{N_\alpha - N_\beta}{N_\alpha + N_\beta}$

Boltzmann equilibrium $\frac{N_\alpha}{N_\beta} = e^{\left(\frac{\gamma h B_0}{k_B T}\right)} = e^{\left(\frac{\hbar\omega}{k_B T}\right)}$

$N\alpha$ spins at lower energy level
$N\beta$ spins at upper energy level

Figure courtesy of
S. Jannin, DNP course EPFL 2012

Some basic issues …

Polarization and magnetic resonance signal

For a thermally polarized sample :

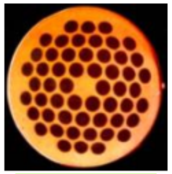
$$P=\frac{N_\beta\cdot e^{\left(\frac{\gamma hB_0}{k_BT}\right)}-N_\beta}{N_\beta\cdot e^{\left(\frac{\gamma hB_0}{k_BT}\right)}+N_\beta}=\frac{e^{\left(\frac{\gamma hB_0}{k_BT}\right)}-1}{e^{\left(\frac{\gamma hB_0}{k_BT}\right)}+1}\cdot\frac{e^{\left(-\frac{\gamma hB_0}{2k_BT}\right)}}{e^{\left(-\frac{\gamma hB_0}{2k_BT}\right)}}=\frac{e^{\left(\frac{\gamma hB_0}{2k_BT}\right)}-e^{\left(-\frac{\gamma hB_0}{2k_BT}\right)}}{e^{\left(\frac{\gamma hB_0}{2k_BT}\right)}+e^{\left(-\frac{\gamma hB_0}{2k_BT}\right)}}=\tanh\left(\frac{\gamma hB_0}{2k_BT}\right)$$

The NMR signal is proportional to P through :

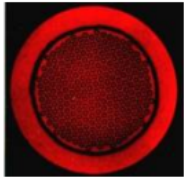
$$S(t)\propto M_{xy}(t)=P\cdot N_{tot}\cdot e^{-\frac{t}{T_2}}\cdot e^{-i\omega t}=\tanh\left(\frac{\gamma hB_0}{2k_BT}\right)\cdot N_{tot}\cdot e^{-\frac{t}{T_2}}\cdot e^{-i\omega t}$$

NMR magnets

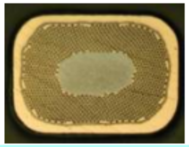
NbTi Single



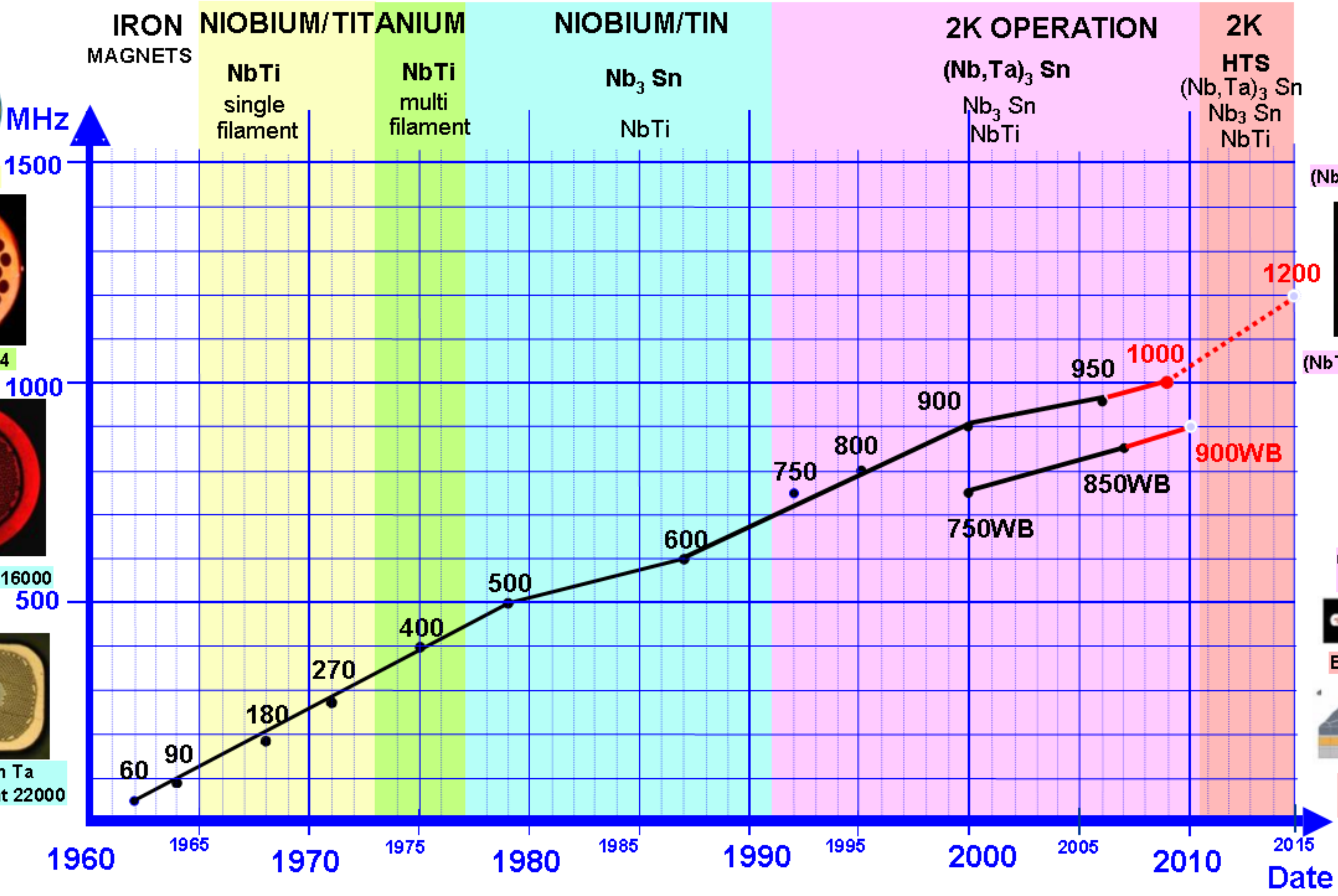
NbTi Multi 54

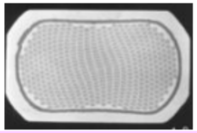


Nb_3Sn Round 16000



Nb_3Sn with Ta Reinforcement 22000



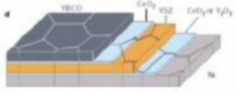


$(Nb,Ta)_3$ Sn Square 50000

$(NbTaTi)_3$ Sn Square 64000

$(NbTaTi)_3$ Sn Bundles, 256 each

Bi-2223 Tape Multi 121



YBCO Substrate Conductor

Magnets with full NMR specifications and working in „persistent mode“ operation

Some basic issues …

Electron polarization vs. nuclear spin polarization

Thermal equilibrium polarization at 1.2 K and 3.35T for a spin $I = \frac{1}{2}$

$$P = \frac{\langle I_z \rangle}{I_z^{\max}} = B_I\left(\frac{\hbar\omega I}{k_B T}\right) = \tanh\left(\frac{\hbar\gamma B}{2k_B T}\right) = \begin{cases} \text{95\% for electron} \\ \text{0.28\% for } {}^{1}\text{H} \\ \text{0.072\% for } {}^{13}\text{C} \end{cases}$$

Some basic issues …

What is DNP?

Transfer of magnetization (polarization) of a (macroscopic) system of electron spins to a (macroscopic) system of nuclear spins.

Which tools do we need in oder to fully understand DNP?

- Equilibrium and non-equilibrium thermodynamics

- Electron and nuclear spin relaxation

- Quantum mechanics and quantum statistics

- Radical chemistry, chemistry of the sample

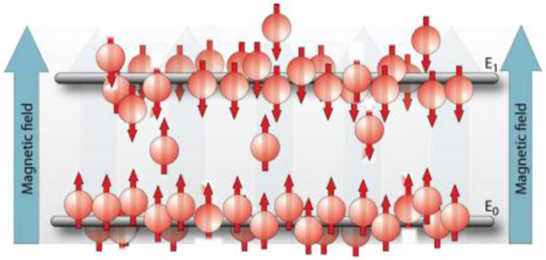
- Theory and practice of microwave/sub-THz interaction with matter

A very concise history of DNP

1953	- Overhauser experiment in metal - Slichter experimental proof
1956	- Slichter DNP in other media (^{1}H)
1960 - 1980	- Liquids and Solids (0.3300 T, ~10 GHz) - Hauser, Mueller-Warmuth, Richards, and others Solids - Abragam, Goldman, Atsarkin, Provotorov, and others . Nuclear magnetic ordering, polarized targets for particle physics, liquids at low fields
-1980 - today	- Griffin , DNP-MAS coupled with a Gyrotron - Golman, Hyperpolarized MRI

Hyperpolarization techniques

Hyperpolarization:
increase of spin polarization M_I above the Boltzmann polarization by establishing a coupling between a low-polarized spin system I with a spin system S of high polarization or coherence order.



DNP
(µ waves)

CIDNP

ODNP

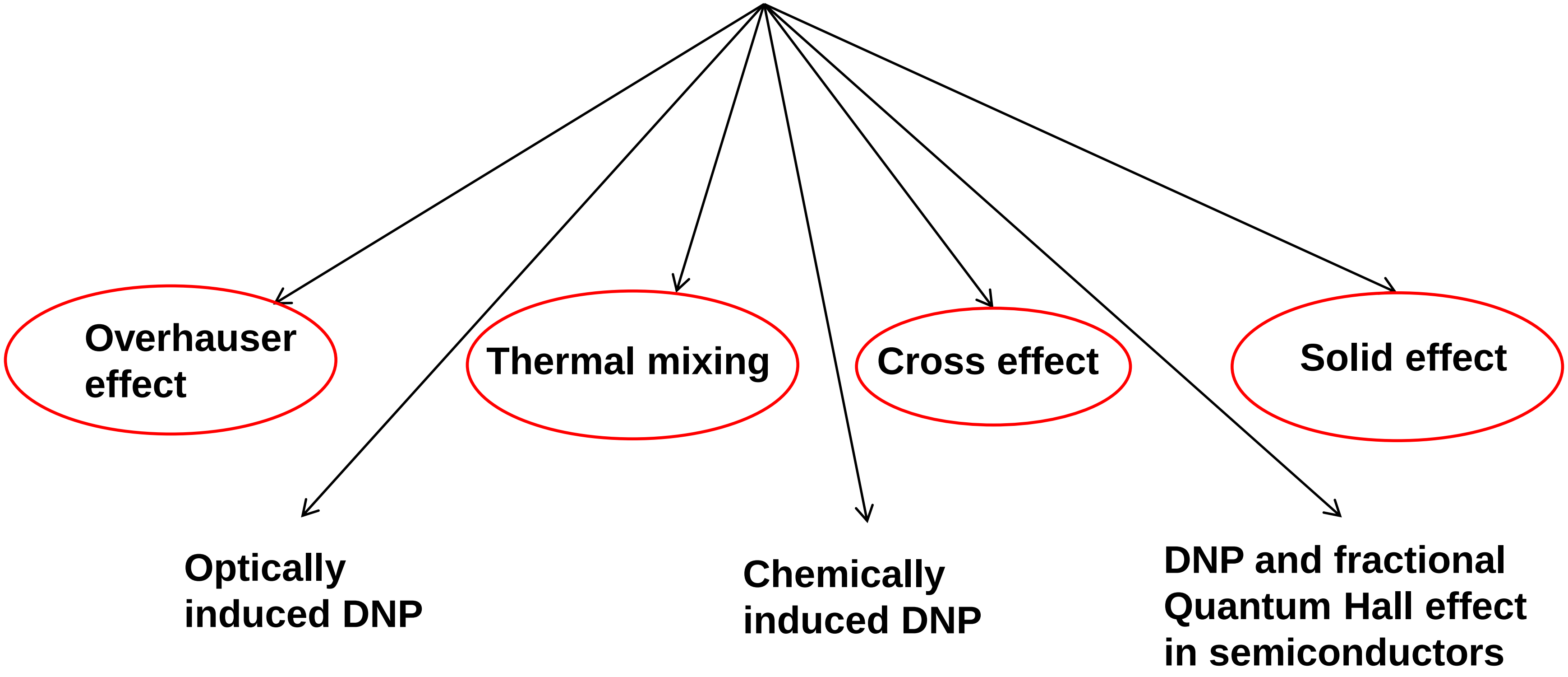
Hyperpolarization

CP

PHIP

DNP mechanisms

How to transfer the polarization of the electron spin system to the nuclear spin system?



Overhauser effect

Thermal mixing

Cross effect

Solid effect

Optically induced DNP

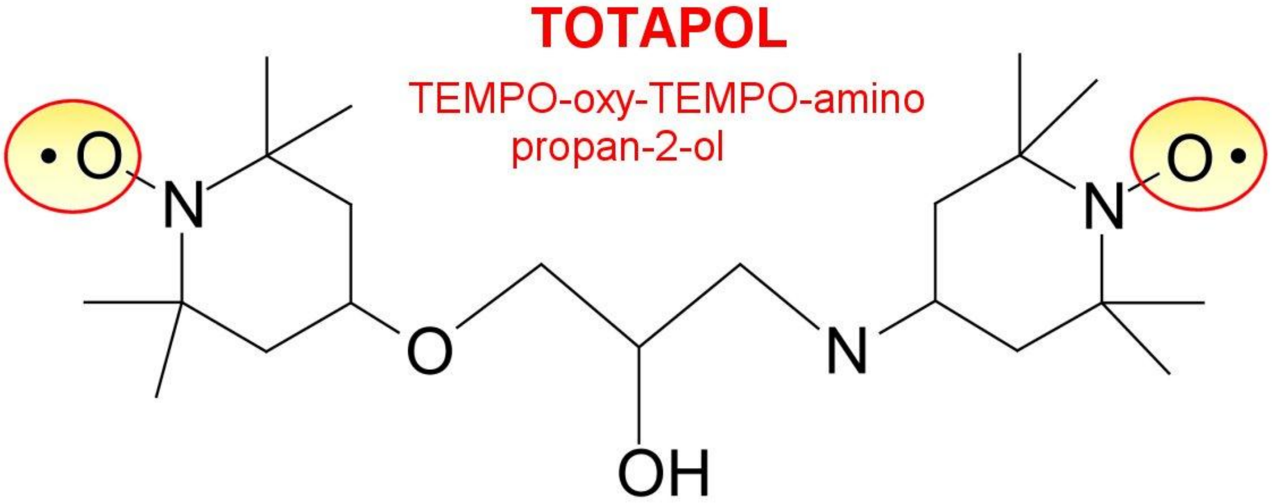
Chemically induced DNP

DNP and fractional Quantum Hall effect in semiconductors

Some basic issues

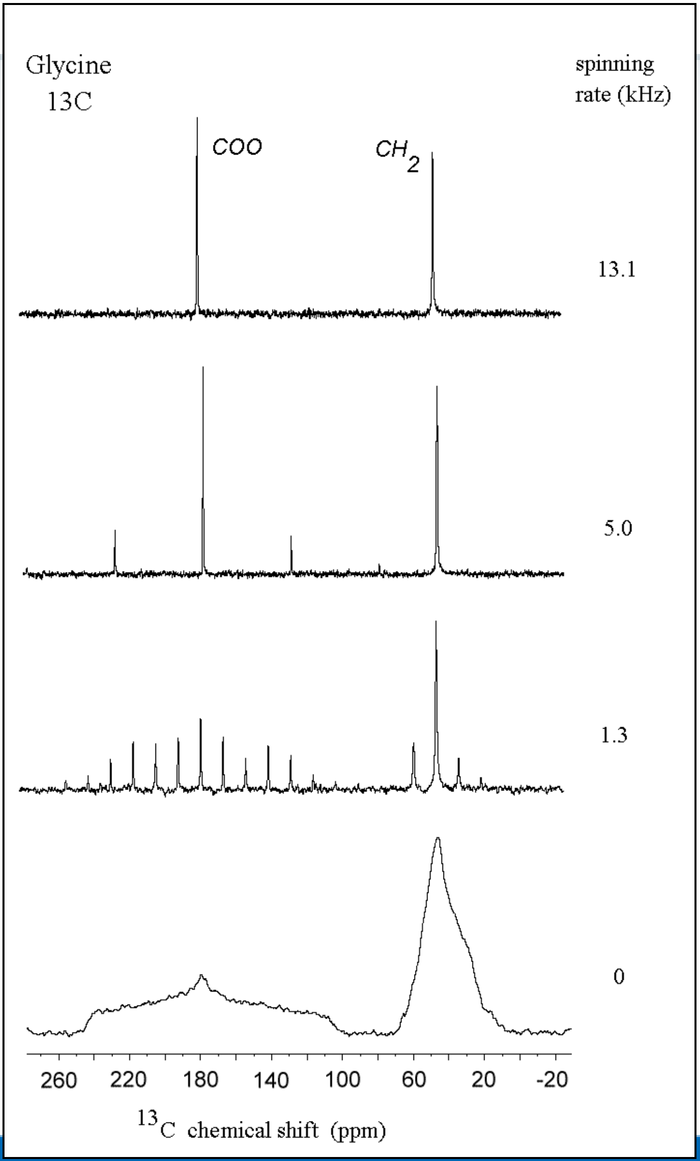
From where do we get the electron spins?
What is a radical?

The electrons in atomic or molecular orbitals are often spin paired (spin-up and spin down) yielding a net e spin of zero. In radicals this “rule“ is violated and at least a single electron spin is not paired resulting in a paramagnetic center.

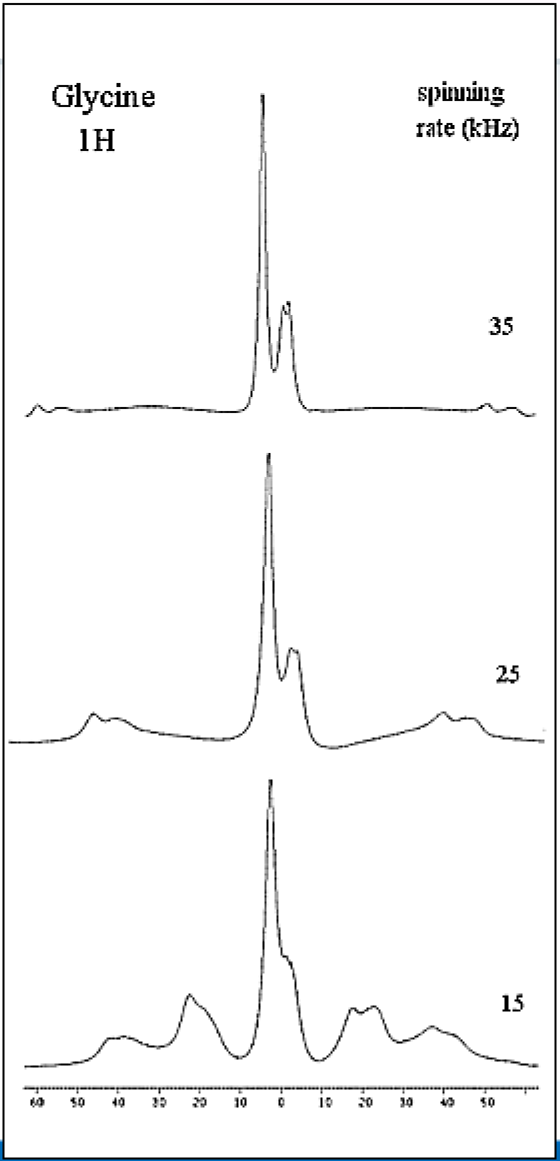


NMR of solids
Magic angle spinning - MAS

^{13}C CSA



^{1}H-^{1}H DD

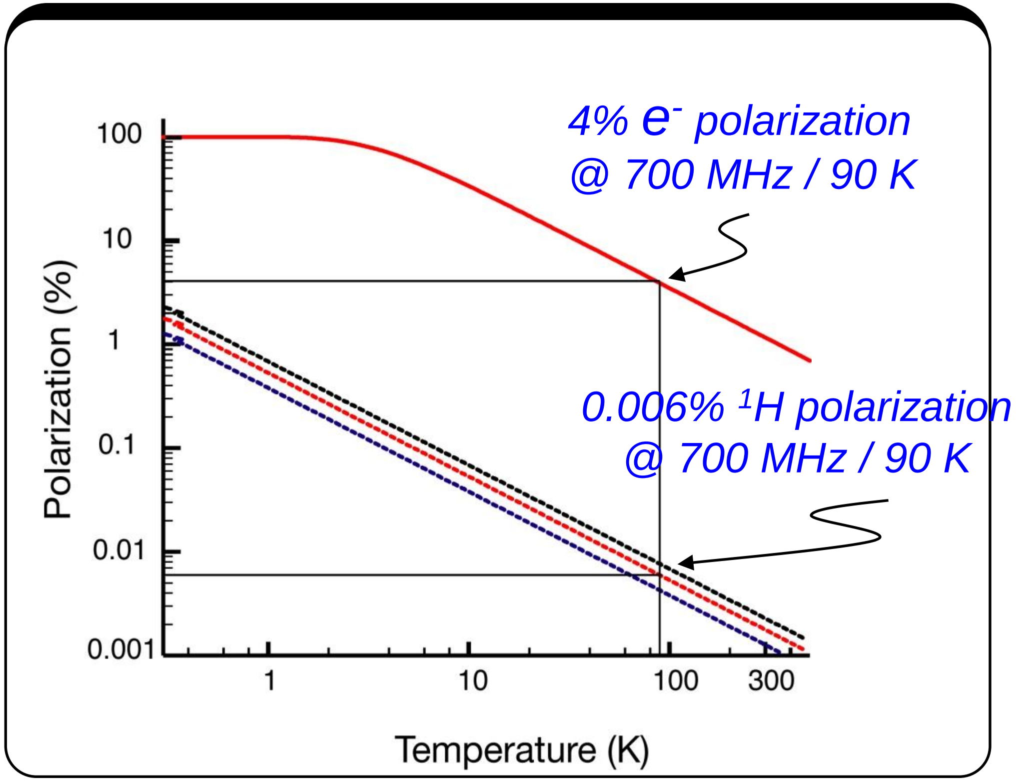


Dynamic Nuclear Polarization

(µw) DNP

- Enhance nuclear polarization by transfer from electron spins
- Principles known for 50 years
- Potential gains: γ_e / γ_N
  - For protons: ~660
  - For carbons: ~2600

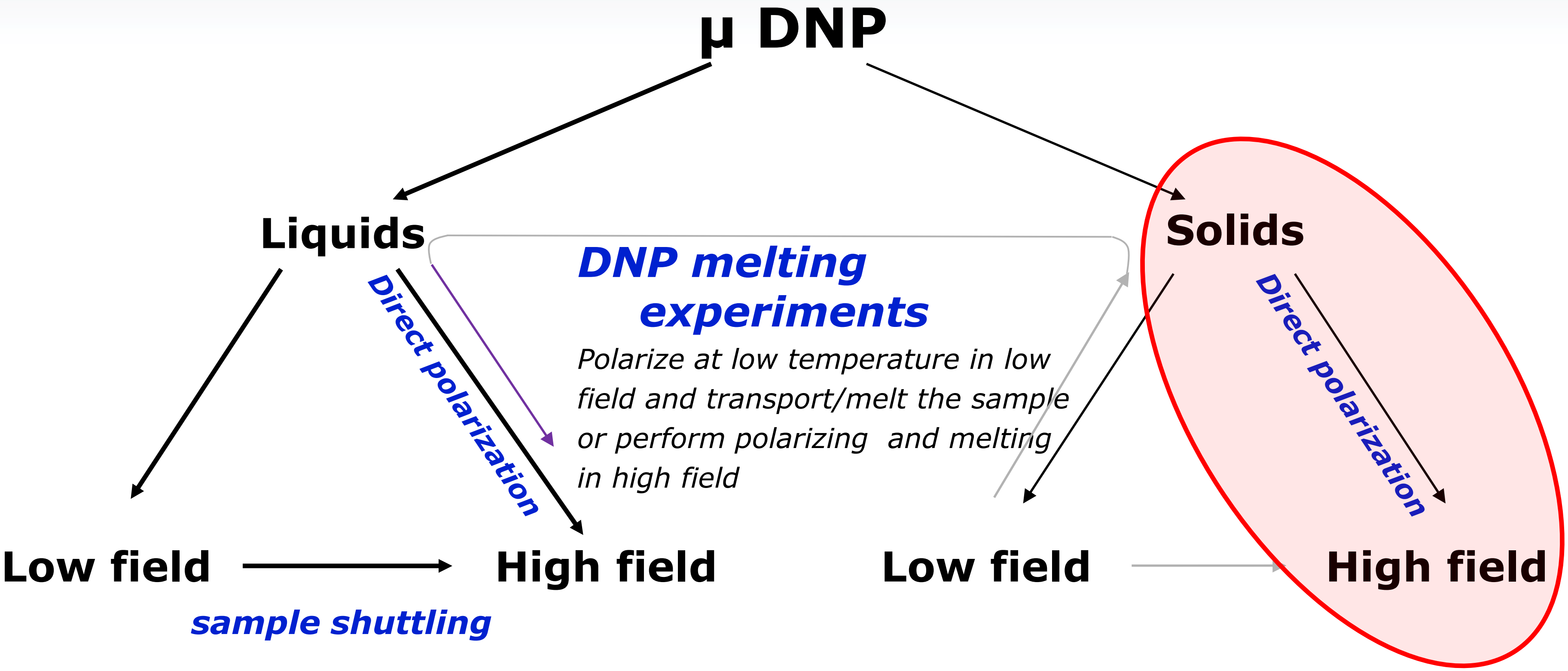
- Ingredients:
  - Unpaired electron spin
  - µ wave excitation



4% e^- polarization @ 700 MHz / 90 K

0.006% ^1H polarization @ 700 MHz / 90 K

Solid-state DNP NMR



μ DNP

Liquids

Solids

Low field

High field

Low field

High field

Direct polarization

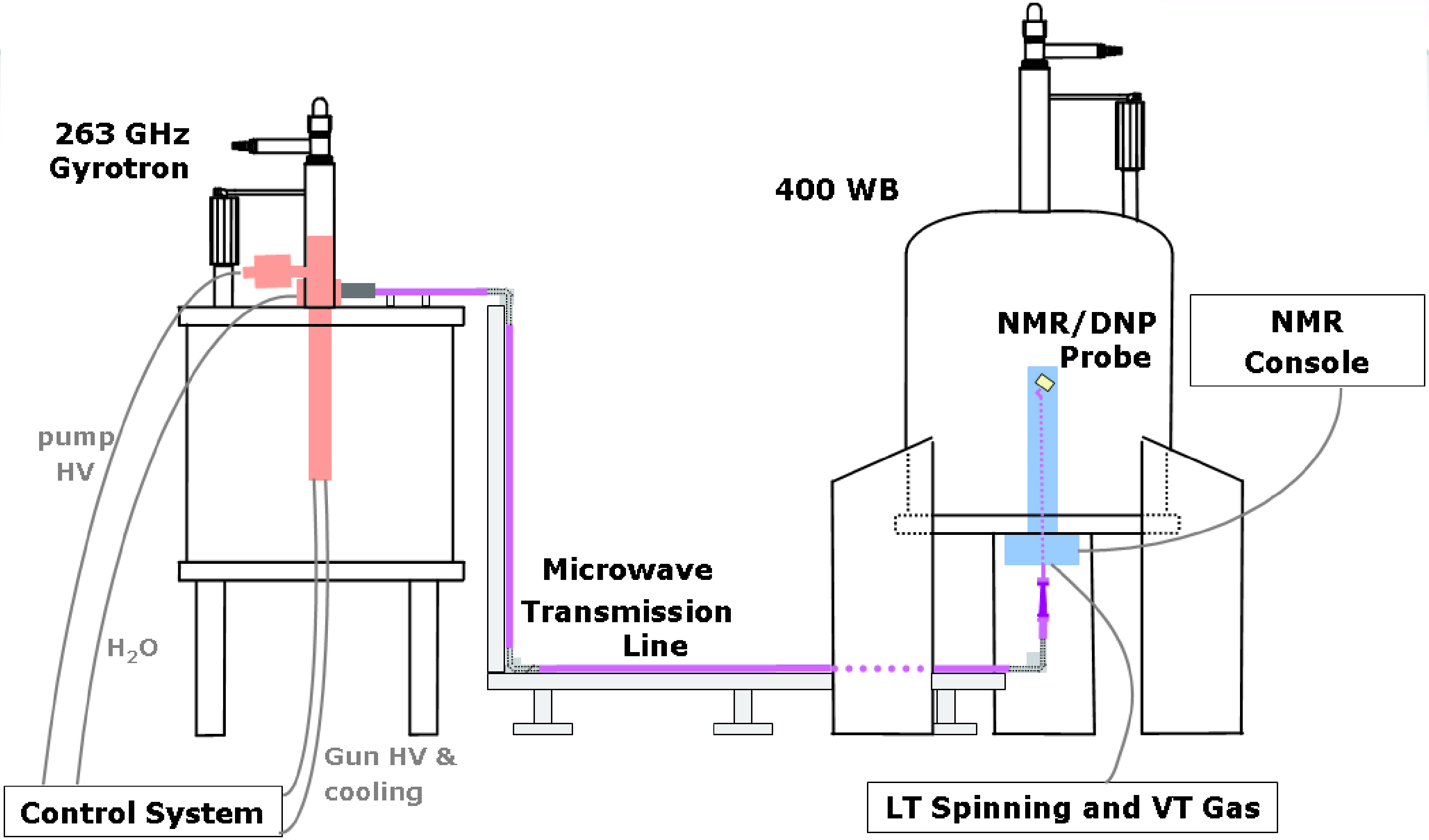
Direct polarization

sample shuttling

DNP melting experiments

Polarize at low temperature in low field and transport/melt the sample or perform polarizing and melting in high field

Solid-state DNP NMR



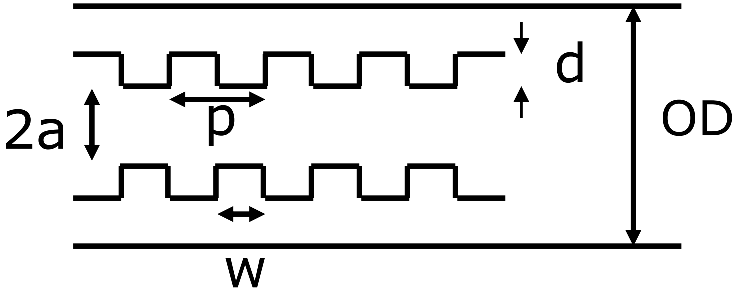
Principle components of an DNP-NMR spectrometer for low-temperature MAS solid-state NMR

Gyrotrons for DNP applications:
Basic specifications of 263 GHz gyrotron

Microwave frequency	263 GHz
Microwave output power	1-50 W
Power stability	± 1%
Frequency stability	± 10 ppm
Frequency tuning	± 50 MHz
Output microwave beam	Gaussian beam
Operation modes	Continuous on operation 10 days or longer
	Easy and safe operation

Microwave transmission to the NMR sample

- Internal mode converter:
  - Transform gyrotron cavity TE03 cavity mode to a Gaussian beam
  - Step-cut launcher (axial cut in wall)
  - Five-mirror transmission system to steer and shape RF beam
- Corrugated waveguide:
  - Very small ohmic loss for Gaussian beam
  - Loss possible due to mode conversion in case of tilt or offset
  - Some broadband capability



$p = \lambda/3$
$d = \lambda/4$
$w < 0.5p$
Gaussian beam waist = 0.64a

Transmission line components

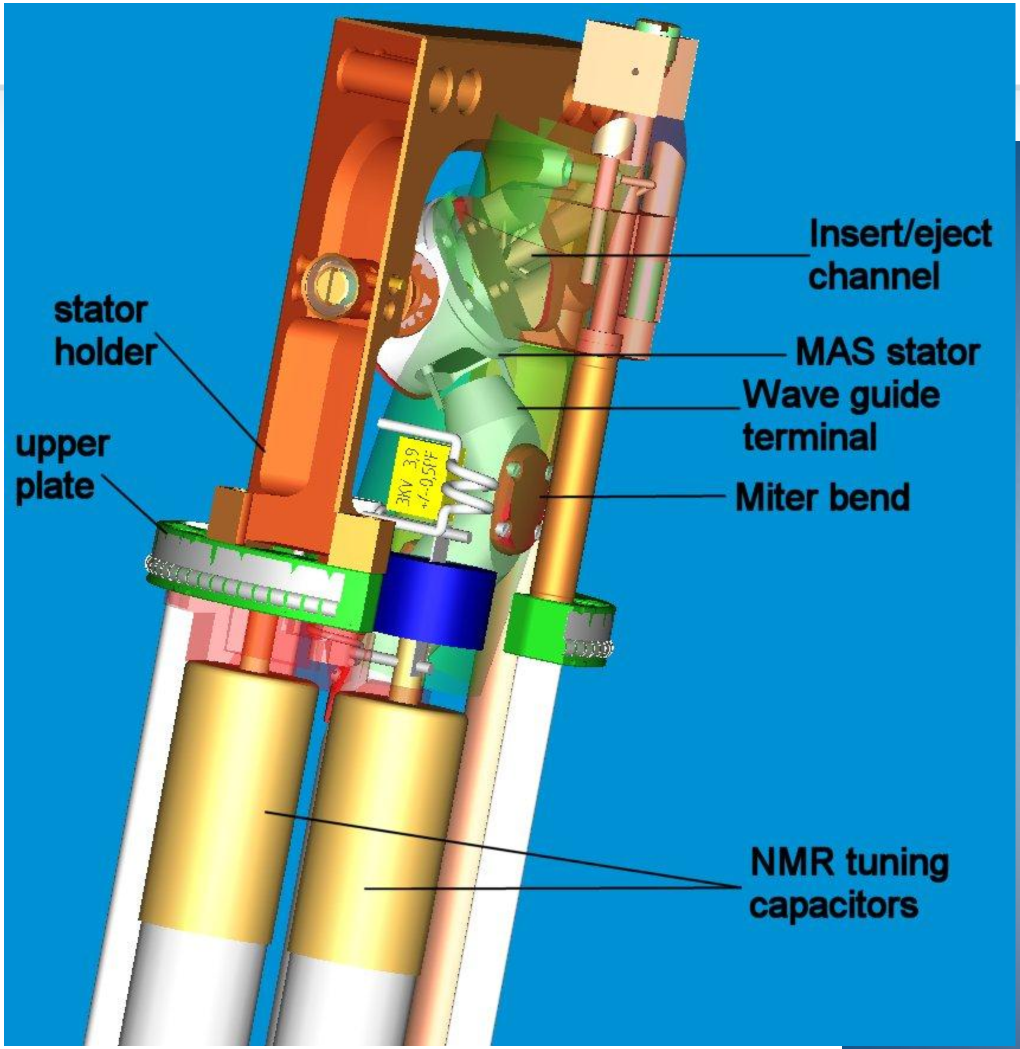
Inside NMR probe:

8 mm waveguide

Main (19 mm) transmission line

- Microwave transmission line from gyrotron window to NMR sample
- Corrugated waveguide:
  - 0.28 x 0.28 mm groove every 0.38 mm (1/3 wavelength at 263 GHz) in 19 mm ID waveguide
  - Waveguide sections joined end to end and held on support structure
  - Helical tap for 8 mm probe waveguide
- Directional coupler for power and frequency measurements

DNP NMR MAS probes



Solid-State NMR/DNP system: 400 MHz / 263 GHz

FMP Berlin, February 2009

Solid-state NMR/DNP system: 600 MHz / 395 GHz

BBIO, Billerica, July 2012

Solid-state NMR/DNP system: 800 MHz / 527 GHz

University of Utrecht, November 2012

Requirements for Solid State DNP-NMR

Thermal mixing/cross effect (TM/CE) mechanism demonstrated at high field

- Follows ***general trend***:

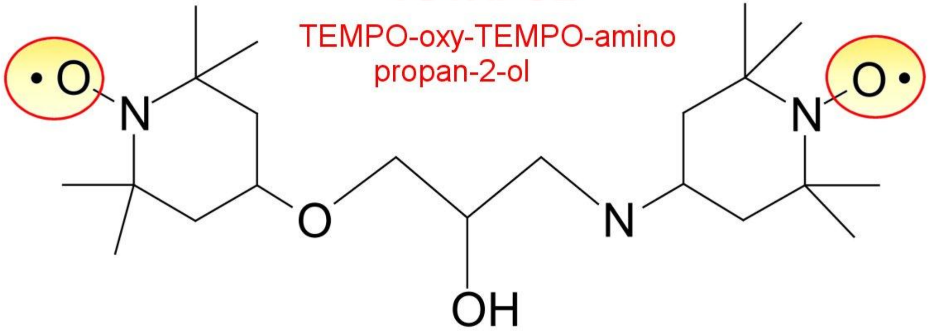
DNP signal enhancement $$\sim \left(\frac{B_1^2}{B_0} \right) T_{1e}\, T_{1n}$$

- B_1 = microwave field amplitude
- B_0 = static magnetic field
- T_{1e} = electron spin lattice relaxation time
- T_{1n} = nuclear spin lattice relaxation time

Biradicals:
two unpaired electron spins in one molecule

TOTAPOL

TEMPO-oxy-TEMPO-amino propan-2-ol

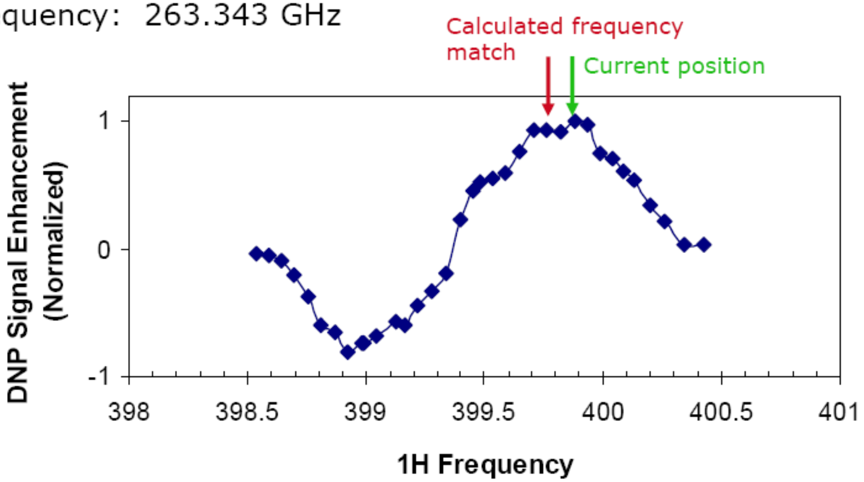


T_{1e} (100K):
on the order of 100 μs

Data courtesy of M. Rosay, et al., 2009

- Intermolecular e-e dipolar coupling small (at 10…20mM conc.)
- Intramolecular e-e dipole coupling: 22 MHz

- High sample temperature (117 K) for stable temperature during 2-day acquisition of field dependence curve
- Gyrotron frequency: 263.343 GHz



Song, et al. JACS 128, 2006
Maly, et al. JCP 128, 2008

Solid-state DNP NMR: applications

Examples

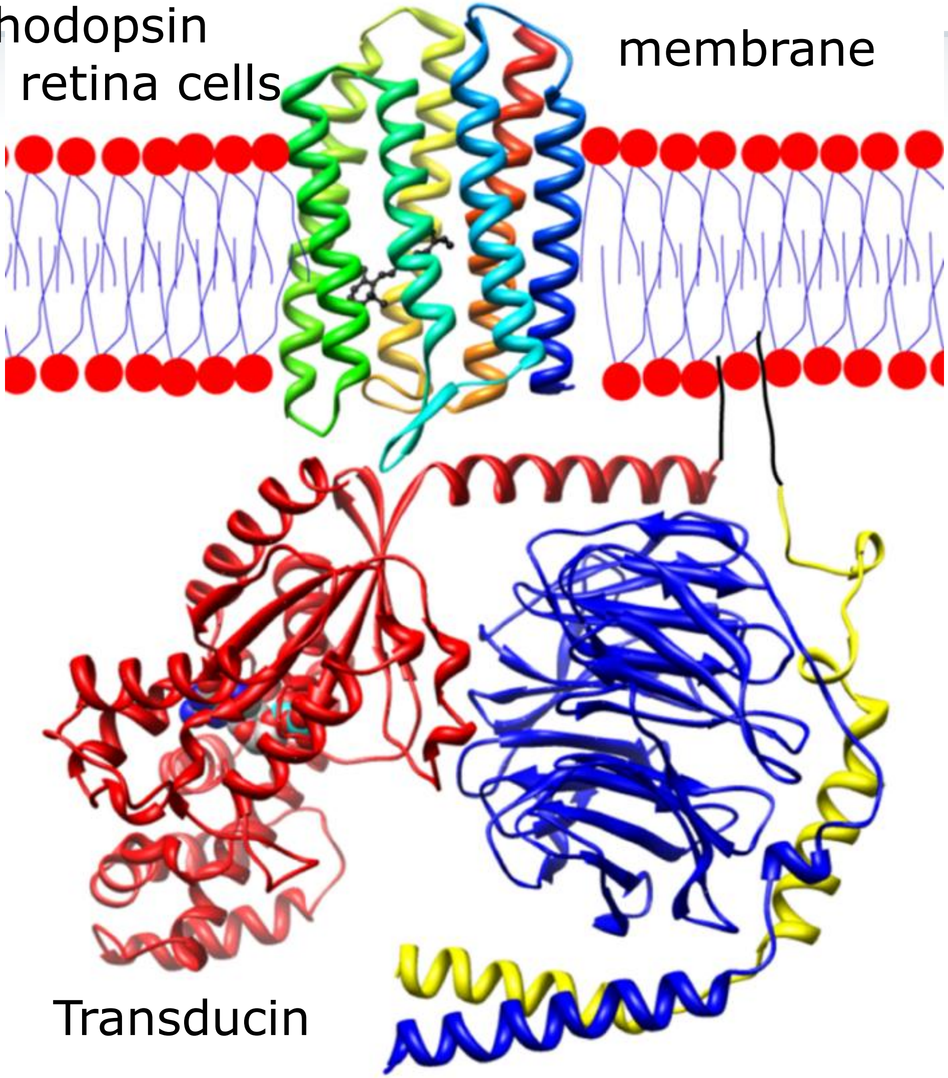
- **Membrane proteins (proteo and bacteriorhodopsin) retinal conformations, role of 1H spin diffusion**

- **TIM: binding ligands to enzymes**

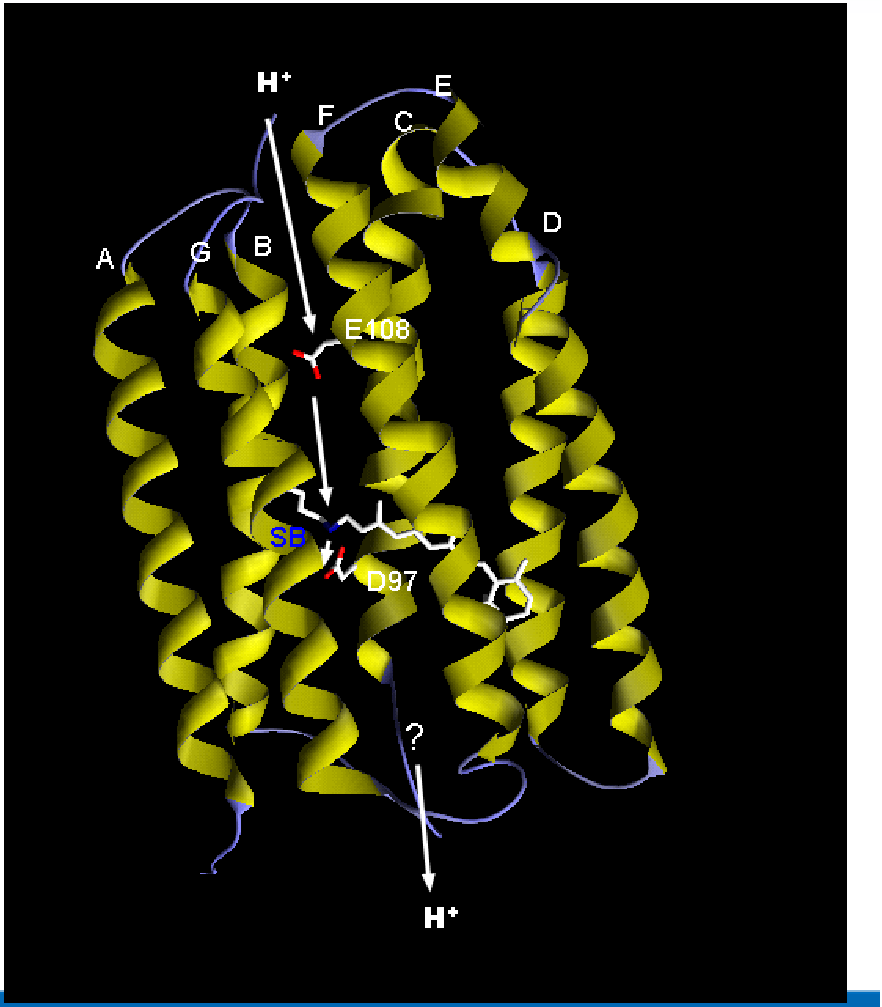
- **Molecules adsorbed on surfaces**

Rhodopsin

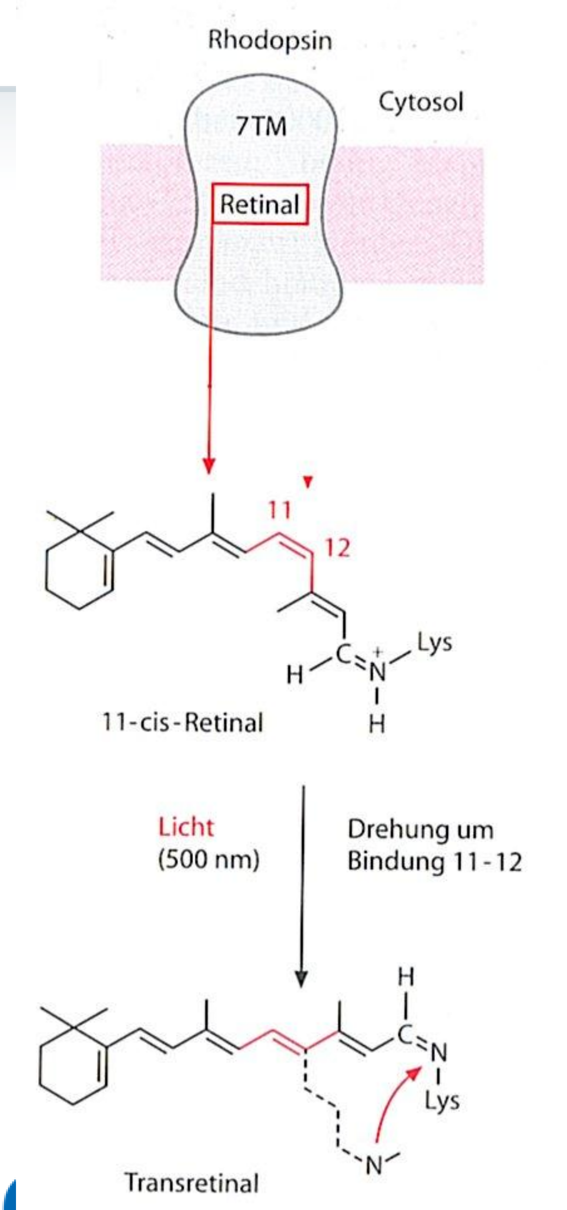
Rhodopsin
in retina cells



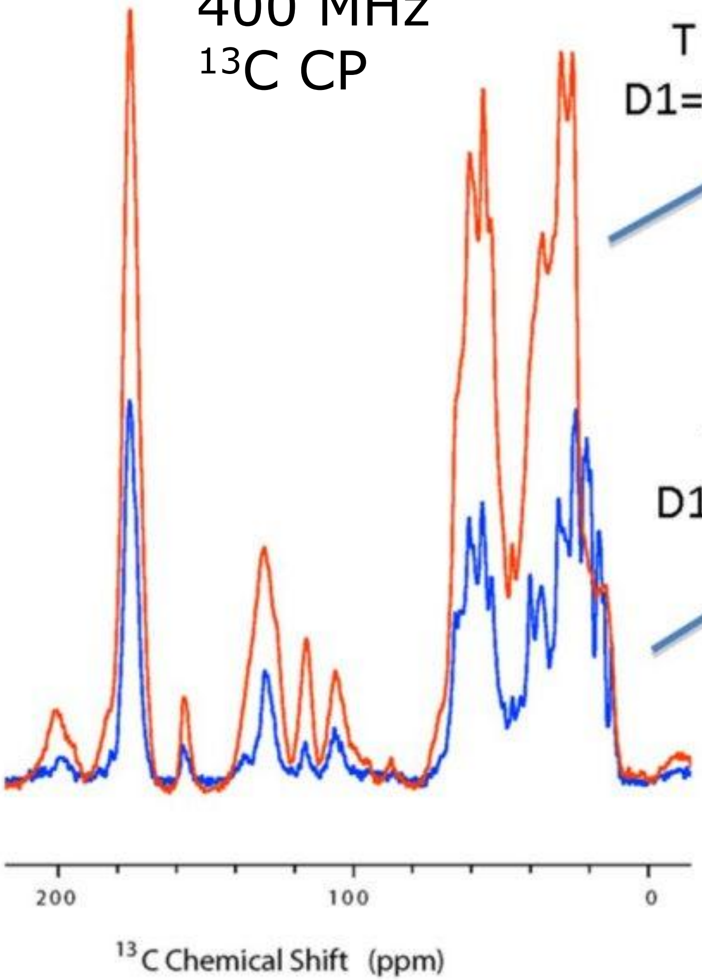
Proteorhodopsin for photosynthesis:
27 kDa membrane protein



Proteorhodopsin – LT.MAS



400 MHz
^{13}C CP



T = 100K,
D1=2sec, 8kHz spinning

T = RT,
D1=2sec, 8kHz spinning

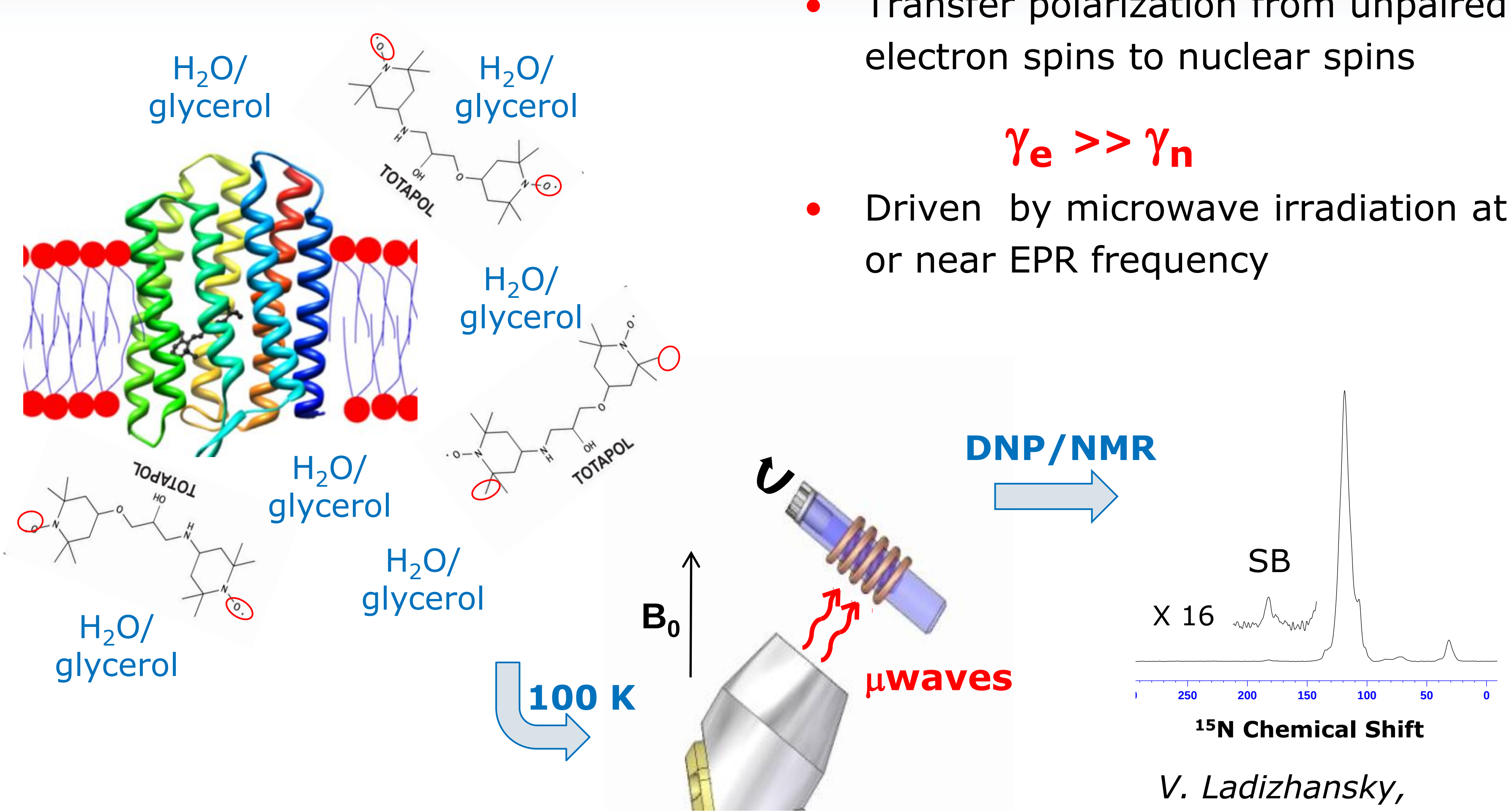
Data courtesy of C. Glaubitz, Univ. Frankfurt.

Solid-State Dynamic Nuclear Polarization (DNP)

- Transfer polarization from unpaired electron spins to nuclear spins

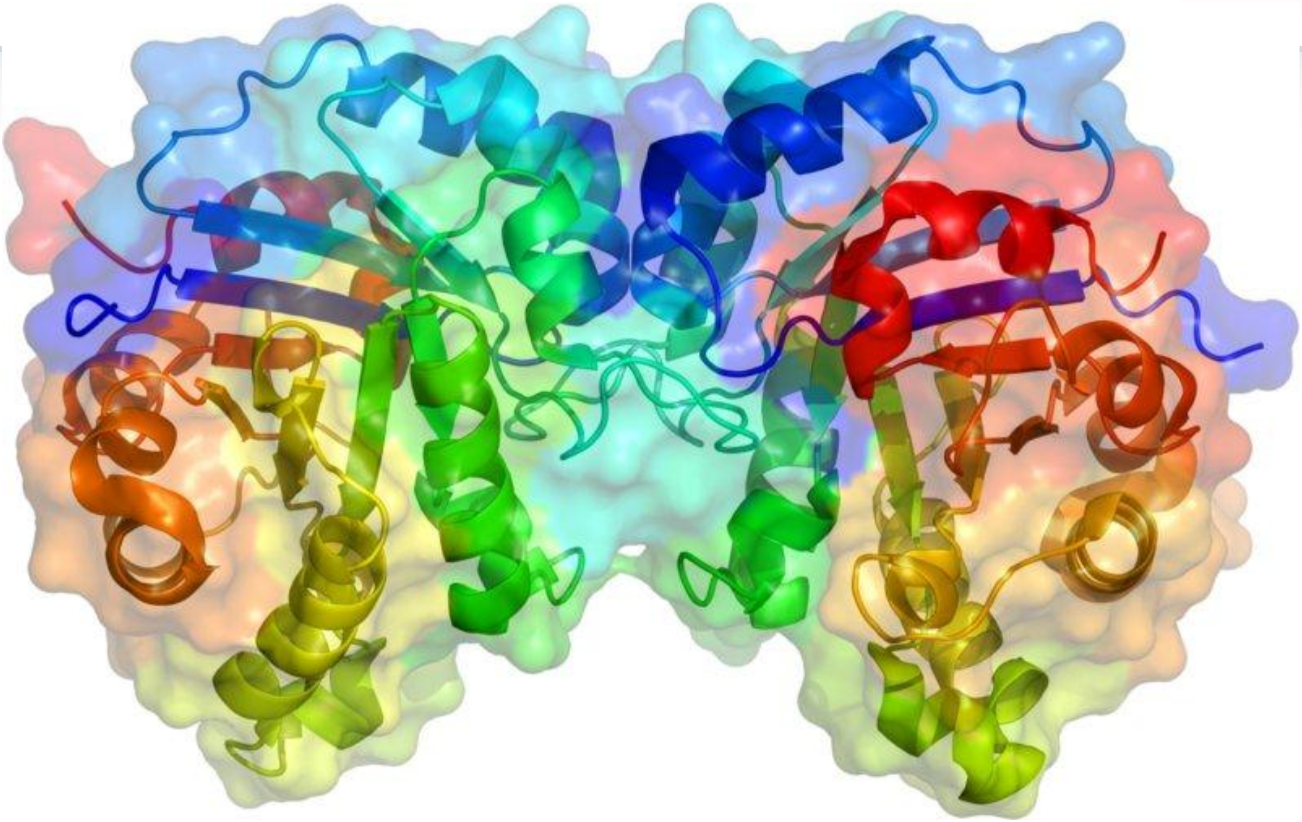
$$\gamma_e >> \gamma_n$$

- Driven by microwave irradiation at or near EPR frequency



V. Ladizhansky, University of Guelph

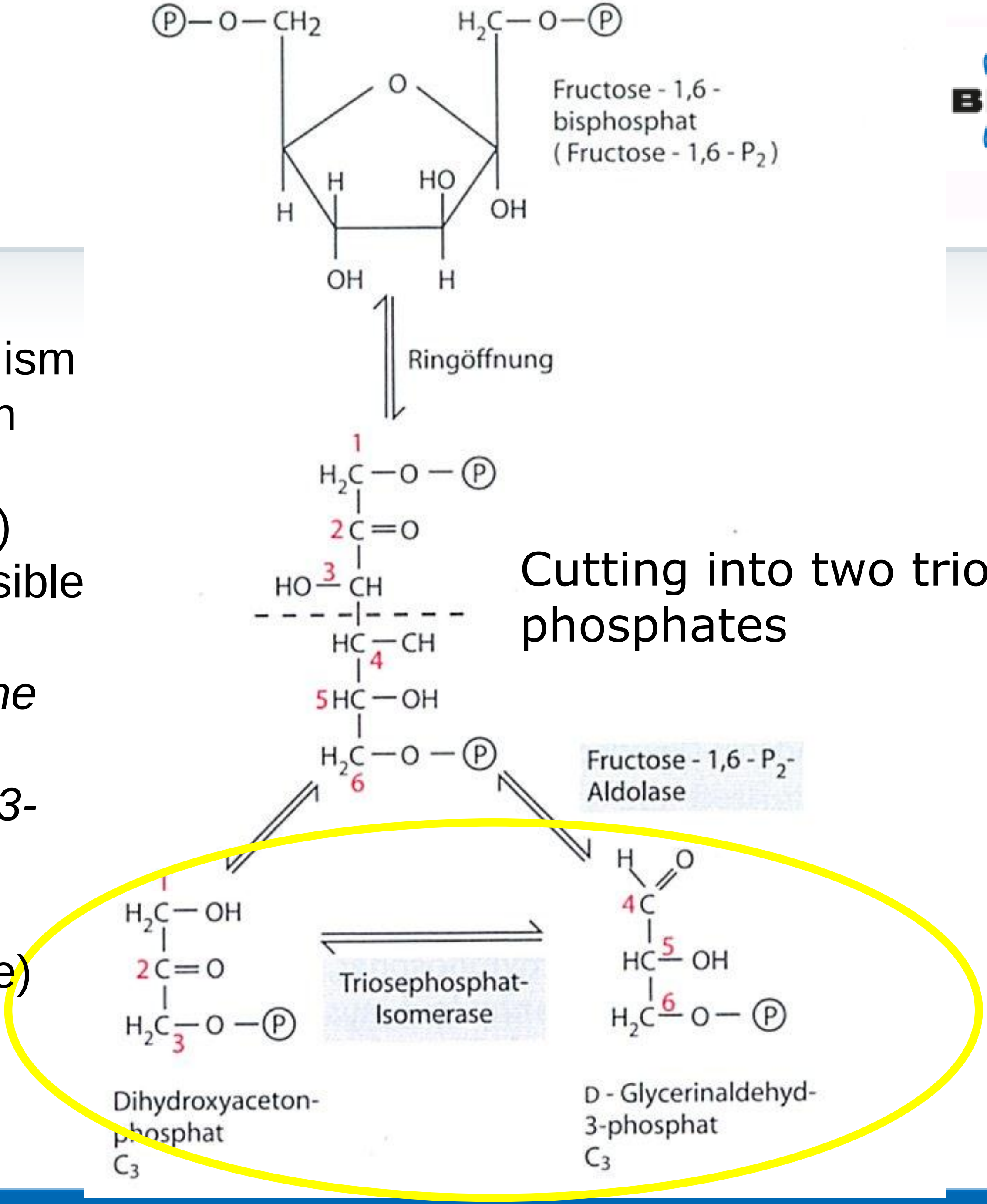
DNP experiments on TIM (TPI)



Triose phosphate isomerase dimer.
Each subunit 250 aa with 8 α-helices outside and 8 parallel β-strands inside.
α/β protein folds including alternating pattern of α-helices and β-strands form a solenoid that closes on itself in a toroidal shape (TIM barrel)

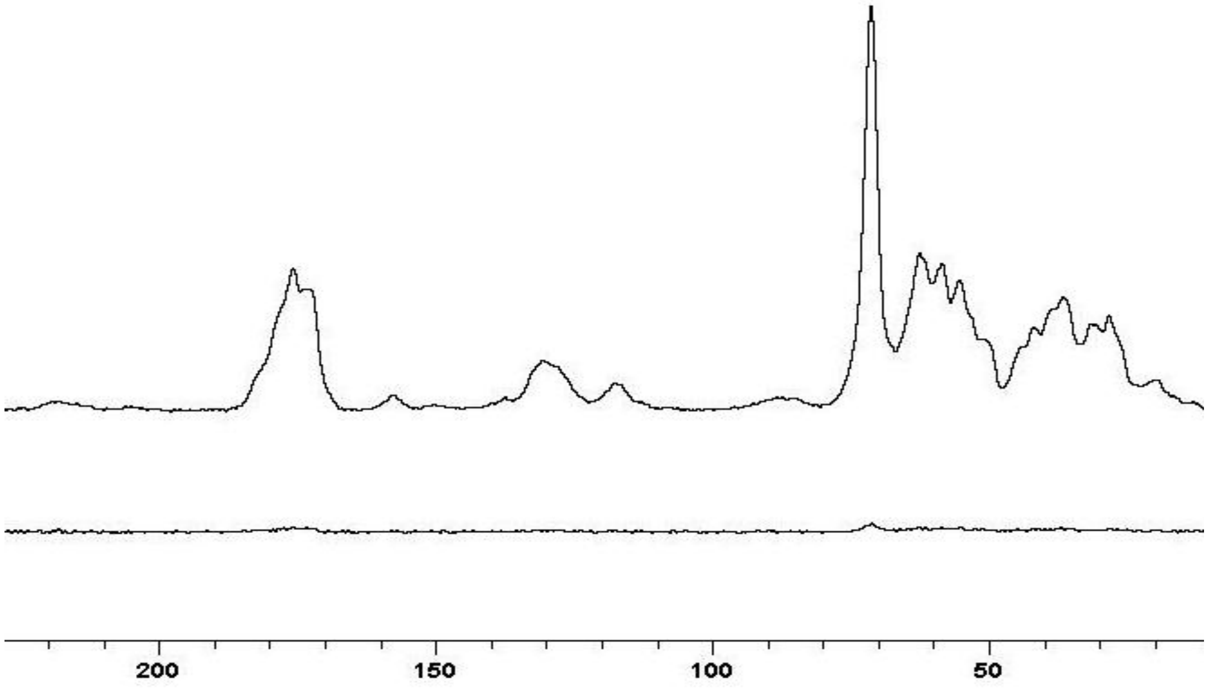
TPI

- to be found in almost every organism
- important enzyme in glycolysis (energy production)
- catalyzes the reversible interconversion of *dihydroxy acetone phosphate* and *D-glyceraldehyde 3-phosphate* (both are isomers of triose phosphate)



Cutting into two triose phosphates

DNP experiments on TIM (TPI)



^{13}C Chemical Shift (ppm)

- Frozen solution of microcrystalline yeast triose phophate isomerase (TIM) with 20 mM TOTAPOL in glycerol/water
- 32 scans, 2 second recycle delay
- 9 kHz MAS
- 105 K sample temperature

Acknowledgment:

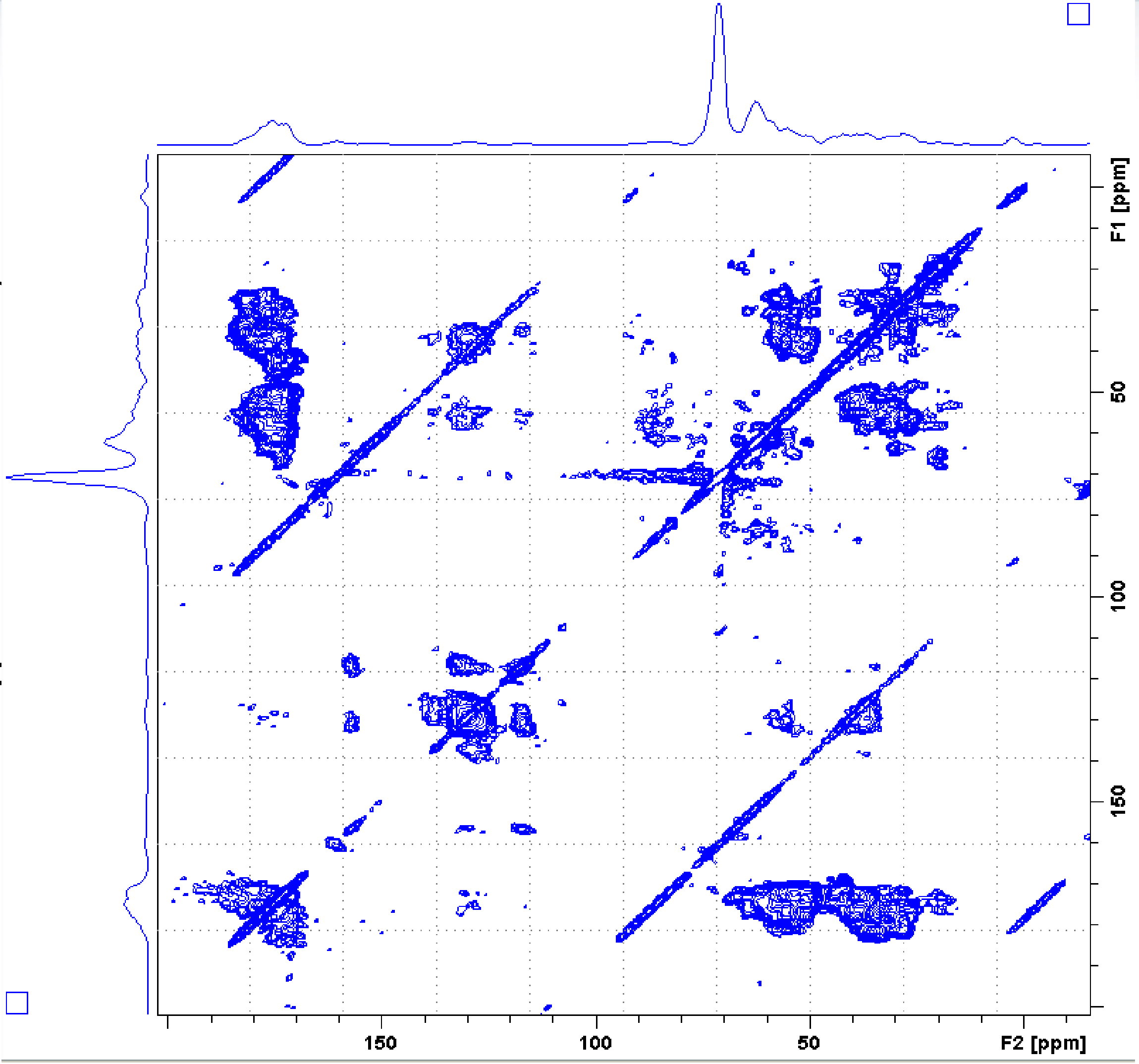
Ann McDermott, Yimin Xu, Ansgar Siemer, NYSBC

DNP experiments on TIM (TPI)

- DNP-enhanced ^{13}C -^{13}C - correlation experiment
- Protron-driven spin diffusion with 22 ms mixing time
- 9 kHz MAS, 105 K sample temperature
- 100 kHz decoupling
- 4 scans, 2.5s recycle delay, 768 t1 points. Total experiment time: 2h 10min

Sample courtesy of Ann McDermott, Yimin Xu,

Ansgar Siemer, NYSBC



DNP on molecules adsorbed on surfaces

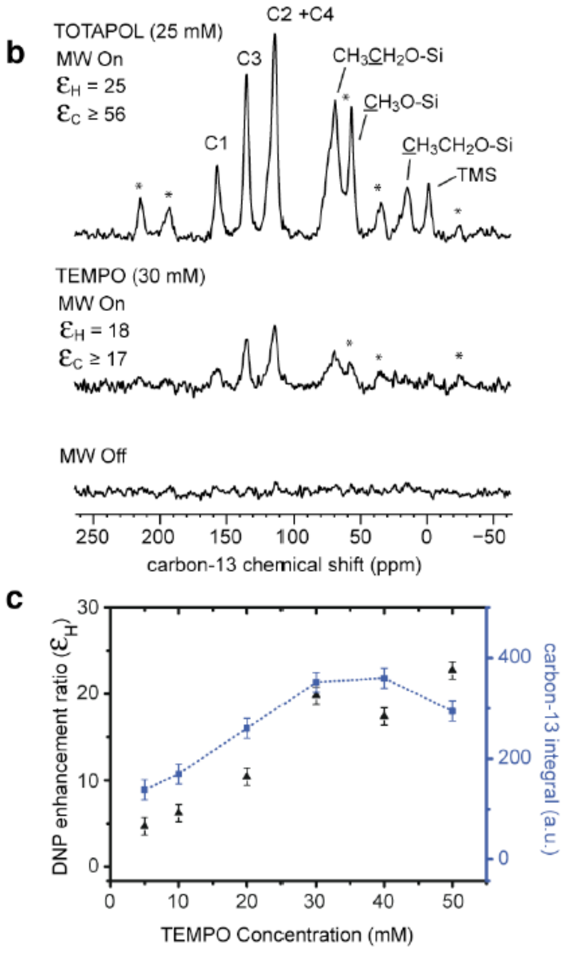
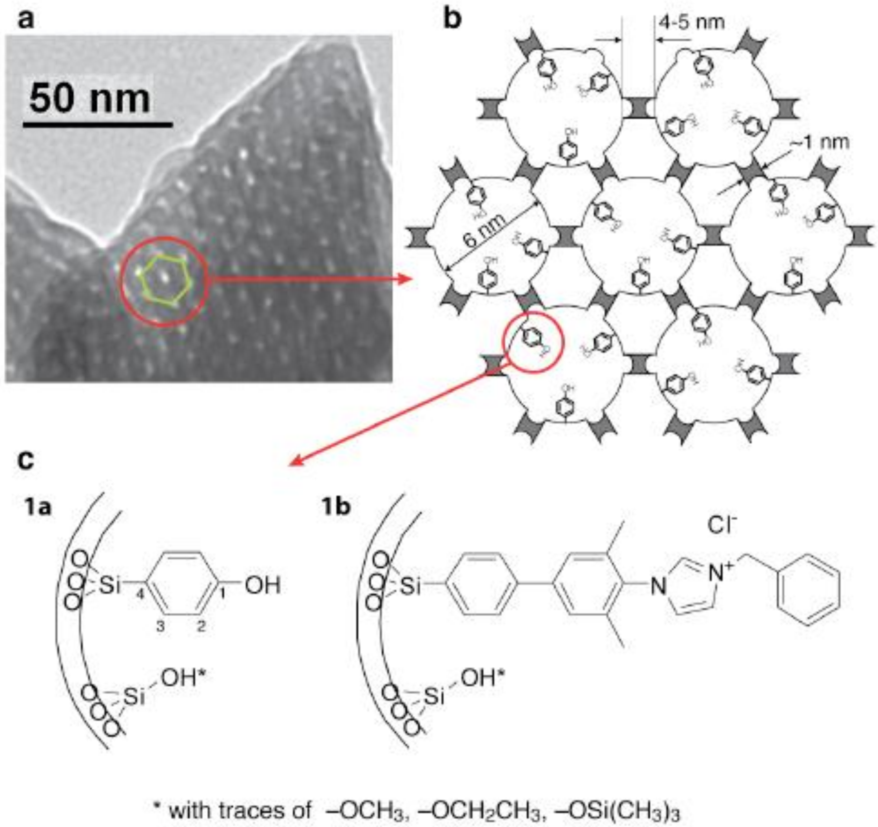


Figure 2. (a). Pulse sequence used for 1D CP MAS. The microwave (MW) irradiation was switched on or off continuously. (b) Carbon-13 CP MAS spectra of **1a** with (top and middle spectra) and without (bottom spectra) MW irradiation at 263 GHz to induce DNP. All spectra were recorded with 2048 scans, with an interval between scans of 1 s. The figures compare the best enhancements observed using TEMPO and TOTAPOL radicals ($T \approx 105$ K, $B_0 = 9.4$ T, $\omega_H/(2\pi) = 400$ MHz, $\omega_C/(2\pi) = 100$ MHz, $\omega_{rot}/(2\pi) = 8.0$ kHz). (c) Experimental ^1H DNP enhancement (ε_H, black triangles) and integrals of the ^{13}C peak at 115 ppm (blue squares) as a function of the TEMPO concentration.



* with traces of $-OCH_3$, $-OCH_2CH_3$, $-OSi(CH_3)_3$

Figure 1. (a) Transmission Electronic Microscopy (TEM) image of the nanoporous silica material. (b) Schematic diagram of the pore and channel network with phenolic derivatization. (c) Different covalently incorporated aromatic substrates.

A. Lesage, M. Lelli, D. Gajan, M.A.Caporini, V. Vitzthum, P. Mieville, J. Alauzun, A. Roussey, C. Thieuleux, A. Medhi, G. Bodenhausen, C. Coperet, L. Emsley in: Euromar, Florence, 2010.