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Scuola di Dottorato in Scienza e Alta Tecnologia - XXIV Ciclo

Dynamic Nuclear Polarization of heterogeneous systems for enhanced MRI

Anno Accademico 2009-2010-2011

Indirizzo in Fisica ed Astrofisica

Tutor: Prof. Cristiana Peroni

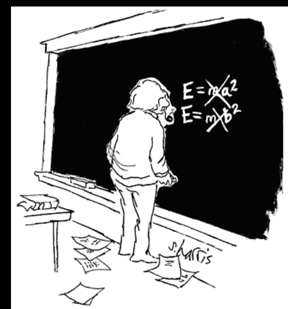
Azienda Ospitante: Bracco Imaging S.p.A

Tutor Aziendale: Dr. Fabio Tedoldi

Summary

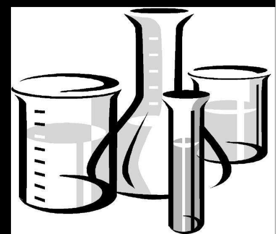
1. <u>Introduction</u>
   1.1 The challenge of Molecular Imaging by Magnetic Resonance
   1.2 Dynamic Nuclear Polarization

2. <u>Target definition</u>
   2.1 A comprehensive picture on relaxation mechanisms, spin diffusion, radical features and glassy structure role.



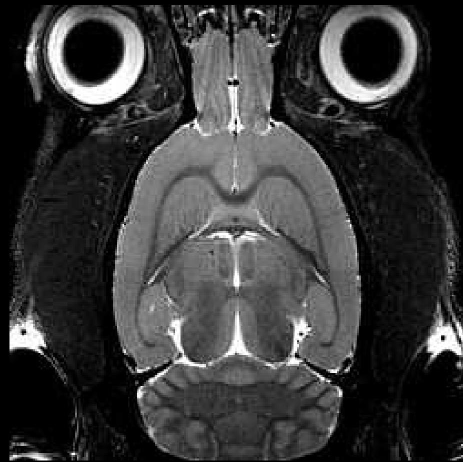
3. <u>Theoretical approach</u>
   3.1 Theoretical analysis of existing models
   3.2 Simulation tools
   3.3 DNP numerical investigation on prototype systems

4. <u>Experimental approach</u>
   4.1 Physical characterization of MR probes
   4.2 DNP experimental set-up
      4.2.1 *From low to high field*
      4.2.2 Start-up of a DNP polarizer prototype
      4.2.3 DNP analysis on specific samples



1.1 Magnetic Resonance Imaging

MRI definition: medical imaging technique used in radiology to visualize detailed internal structures.



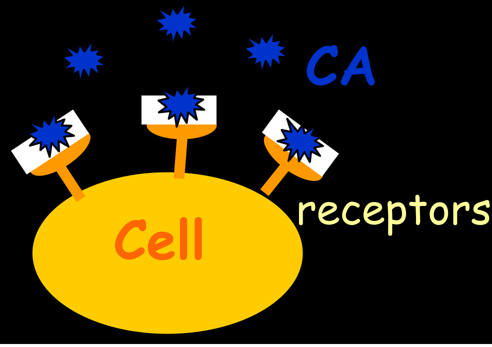
Resolution: 100 μm.

Sensitivity: about 1 nucleus every million adds up to signal.

Anatomy: high resolution allows high anatomical definition.

Physiology: Gd-based CA, fMRI and other techniques allow physiologic characterization.

Molecular: molecular targeting requires a huge amount of Gd ion (about 10^5 ion per cell) .

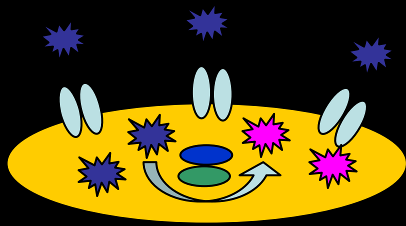


1.1 From Gd-CA to HP-CA

$$M \approx \Delta N = NP = N\tanh\left(\frac{\gamma\hbar B_0}{2k_BT}\right)$$

$$NMRsignal = f(M, T_1, T_2)$$

HP CA: a hyperpolarized agent shows a high polarization and can be detected with a high SNR compared to surrounding tissues.



APPLICATIONS:

→**Angiographic imaging**

→**Perfusion imaging**

→**Molecular and metabolic imaging**

Gd-Based CA: None increase of magnetization, only shortening of T_1, T_2 (by altering relaxation times of water proton in surrounding tissues and using proper MRI weighted sequences, CNR increases).

APPLICATIONS:

→**Angiographic imaging**

→**Perfusion imaging**

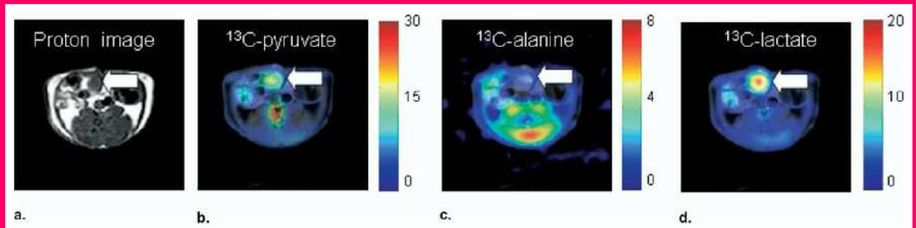
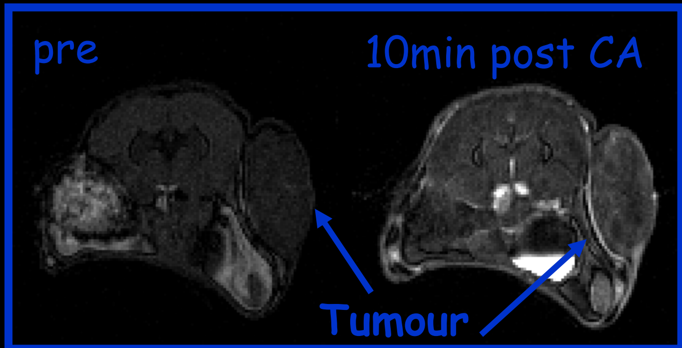


Figure 9. The metabolic pattern in a tumor model. Maps of the metabolites alanine (*c*) and lactate (*d*) obtained in a rat tumor model after injection of hyperpolarized ^{13}C-pyruvate (*b*). The first image (*a*) shows the corresponding proton slice. The ^{13}C maps have all been superimposed on the proton map. In all images, the position of the implanted tumor in indicated by a *white arrow*. All ^{13}C images have been individually scaled.



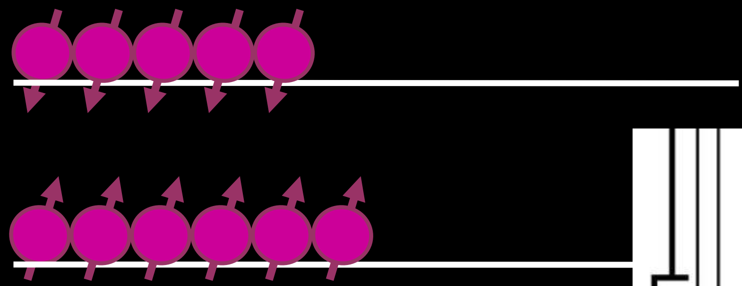
Golman et al, Cancer Res 2006, Vol. 66

1.2 Dynamic Nuclear Polarization

$$Thermal\ \ Polarization = Tanh\left(\frac{\gamma\hbar B_0}{2k_B T}\right)$$

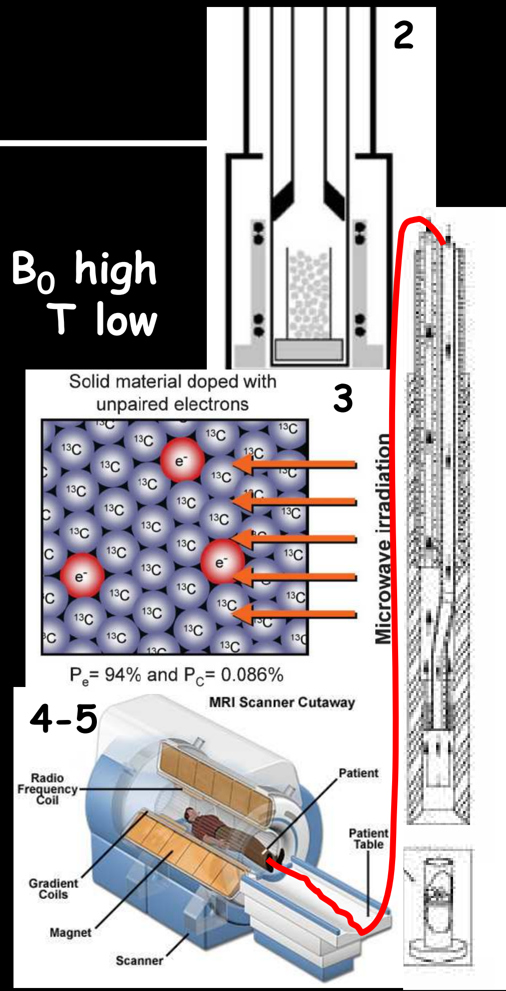
Hyperpolarization = establishment of an artificial non-equilibrium distribution of the nuclei.

Polarization from 10^{-6} to 10^{-1}



DNP STEP BY STEP

1. Sample preparation: molecules labelled with 13C (or other interesting nucleus), a glass-former (e.g. glycerol) and molecules carrying unpaired electrons (e.g. organic free radical).
2. Transfer of the sample at low temperature and field ~ T to obtain high electron polarization.
3. Microwave irradiation of the frozen sample to transfer polarization to the nuclear spin.
4. Rapid dissolution of the solid sample into an injectable liquid
5. Rapid injection and fast CS image acquisition



1.2 Physics of DNP

T~1K

B~1T

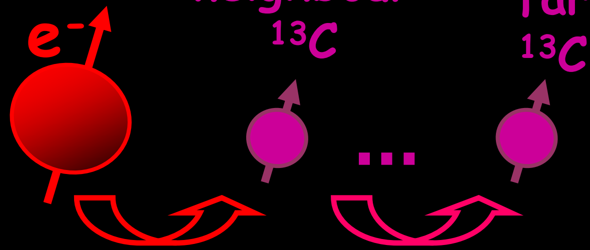
$$P = Tanh\left(\frac{\gamma\hbar B_0}{2k_B T}\right)$$

B_0= 3.35T, T=1.2K,

electron $\rightarrow$ P=0.95

$^{13}C \rightarrow P=7\cdot10^{-4}$

$\Rightarrow \gamma el \sim 2600\ \gamma^{13}C$



Overhauser-like effect (through MW)

Hyperfine interaction between nuclei and electrons

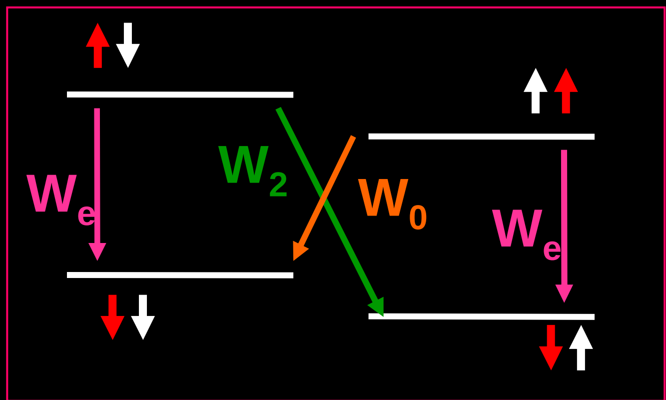
Spin diffusion

Dipolar interaction between nuclei

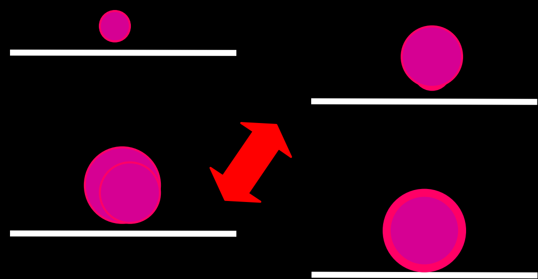
What kind of transfer mechanism are involved?

Solid effect
($\Delta\omega e < \omega n$)

Thermal mixing
($\Delta\omega e > \omega n$)



e-n system



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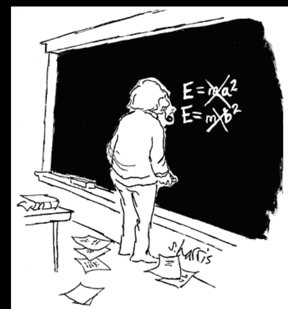

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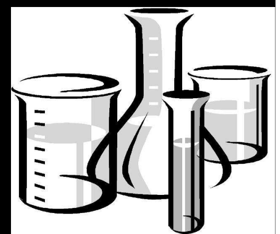

2.1 Known and unknown

Known	Unknown
Sample preparation	
Solution recipe: •Labelled molecule of interest •Radical •Glass former (if the neat compound does not vitrify)	•How should the ideal radical be? •Which features of that radical are crucial? •Are vitrifying and not vitrifying compounds characterized by different relaxation time behaviour as function of temperature (i.e. different molecular motions)?
Sample transfer at low temperature	
The solution must form a glass at the solid state to avoid radical confinement outside crystalline domains.	•How does the glass former influence the system? •How are relaxation times and recovery laws affected?
Polarization transfer from electronic to nuclear spins	
Microwave irradiation, opportunely tuned, can transfer polarization from electrons to nuclei.	•Which is the mechanism involved in our system: Solid Effect, Cross Effect, Thermal Mixing? •How do these processes work? •How are they influenced by Spin Diffusion?

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2.1 Target definition

ISSUE	EXPERIMENTAL APPROACH	THEORETICAL APPROACH
Final polarization dependence on radical concentration/distribution	Hyperpolarization experiment on samples containing different amount/kind of radical. Hyperpolarization of non glassy solution.	Hyperpolarization simulated experiment on samples containing different amount of radical.
The role of Spin Diffusion	Hyperpolarization experiment on samples containing different amount of 13C nuclei.	Dynamic behaviour of a simulated system with different SD conditions.
Hydrogen polarization	Hyperpolarization of 1H nuclei → pure SE mechanism	Hyperpolarization simulated experiment on 1H nuclei system →pure SE mechanism
Role of Gd	Comparison between sample with and without Gadolinium.	

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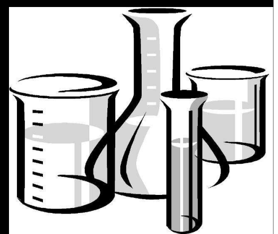


3.1 Existing model

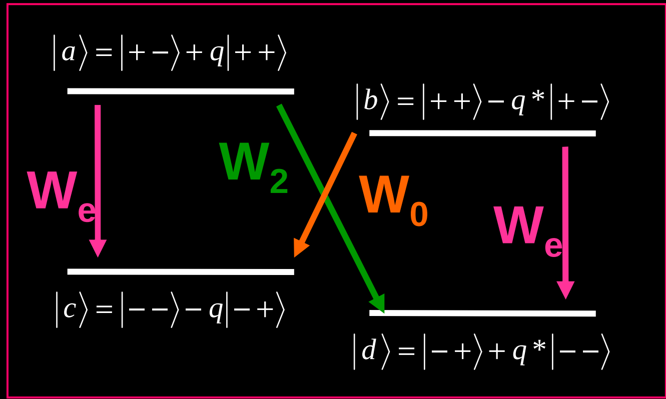
Khutsishvili, G. R., *Spin Diffusion*
Soviet Physics Uspekhi, 1966, 87, 211 - 25

Abragam, A. & Goldman, M. "Principles of dynamic nuclear polarization"
Rep. Prog. Phys., 1978, 41, 395-467

J. H. Ardenkjaer-Larsen, S. M. & Johannesson, H.
"Dynamic Nuclear Polarization with Tritylsat 1.2 K"
Applied Magnetic Resonance, 2008, 34, 509-522

SOLID EFFECT

$$\frac{\partial M}{\partial t}=\frac{M_0-M}{T_d}+D\Delta M-C\sum_n\frac{M-M_0}{|r-r_n|^6}-2AM-\Gamma_\pm\sum_n\frac{M\mp\frac{\gamma_e}{\gamma_n}M_0}{|r-r_n|^6}$$

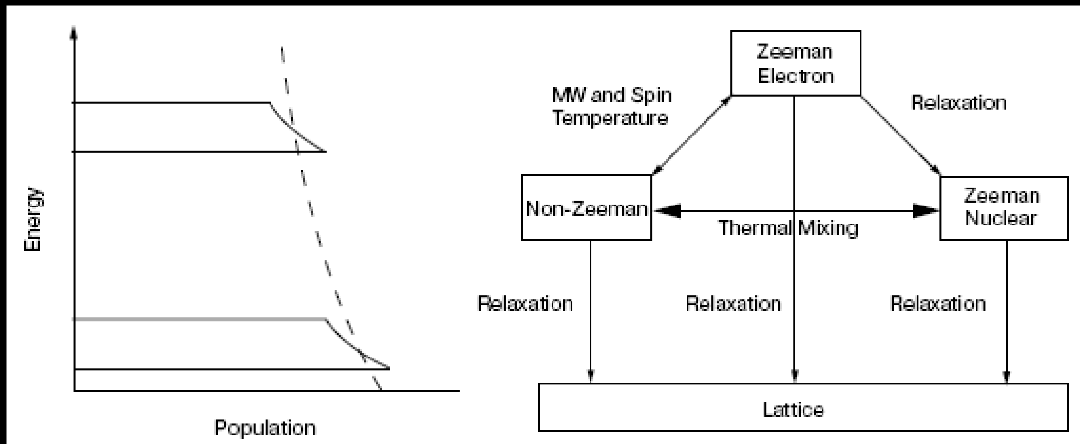


THERMAL MIXING (Provotorov)

$$\frac{\partial\alpha}{\partial t}=-W(\alpha-\beta)-\frac{1}{T_{1e}}(\alpha-\alpha_L)$$

$$\frac{\partial\beta}{\partial t}=-W\frac{\Delta^2}{D^2}(\alpha-\beta)-\frac{1}{T_{1n}}(\beta-\beta_L)$$

$$con\;\; W=\pi\omega_1^2 g(\Delta)$$



THERMAL MIXING (Borghini)

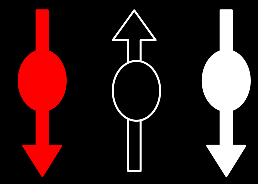
$$-\Delta_0 P_0+\frac{N_I}{N_s}\frac{T_{1S}}{T_{1I}}2I\omega_I P_I=\int_{-\infty}^{\infty}(\Delta_0-\Delta)g(\Delta)Tanh\left[\frac{1}{2}\beta(\Delta_0-\Delta)\right]d\Delta$$

Goldman, M.
"Overview of Spin Temperature, Thermal Mixing and Dynamic Nuclear Polarization"
Applied Magnetic Resonance, 2008, 34, 219-226

3.2 Simulation tools: description

Poster at **GRC In vivo Magnetic Resonance 2010**
Development of a Mathematica-based tool for simulation of Dynamic Nuclear Polarization via Solid Effect on a 3D lattice.
S. Colombo Serra, A. Rosso, F. Tedoldi

System: 3D cube lattice of nuclear spins doped with electrons.



MONTECARLO: The simulation generates an initial state of the system and makes it evolving step by step, establishing which of the possible transitions will happen, according to the values of their rates .

Random choice → event selection

0 (e.g. flip-flop spins (i,j)) 1

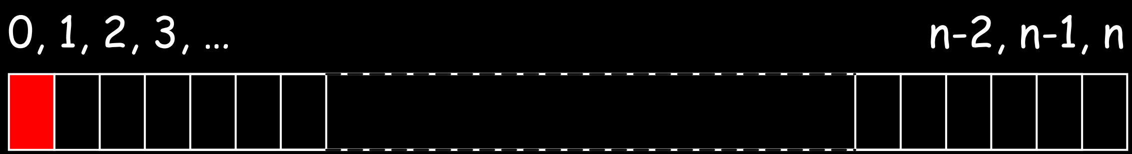
N_{coppie} x $W_{flip-flop}$	N_{spins} x W_{MW}	N_{spins} x W_{relax}

DISCRETE DIFFERENTIAL EQUATION: A discrete equation describing the system evolution can be written with a matrix formalism $\tau(t+1) = W \cdot \tau(t) + B$, where W and B contain rates of all possible transition events.

In very simple cases the equation can be solved analytically, otherwise a computational method has to be applied.

$$\tau_i(t+1) = (1 - 2p_D - p_{\mathrm{dec}} - p_{\mathrm{inj.}}\delta_{i,1})\tau_i(t) + p_D\tau_{i+1}(t) + p_D\tau_{i-1}(t) + p_{\mathrm{inj.}}\delta_{i,1}$$

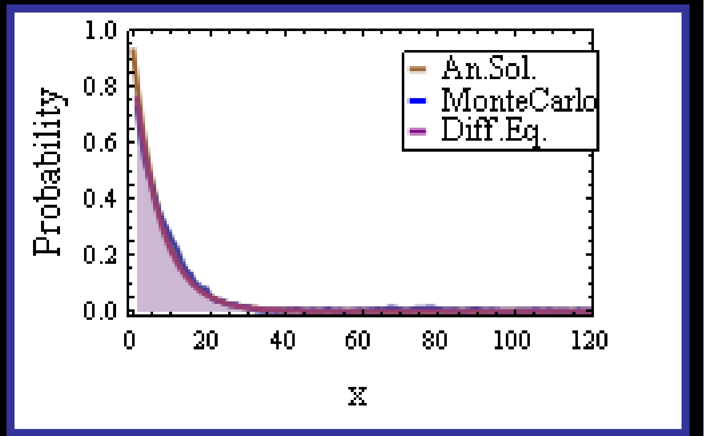
3.3 Simulation tools: test and comparison



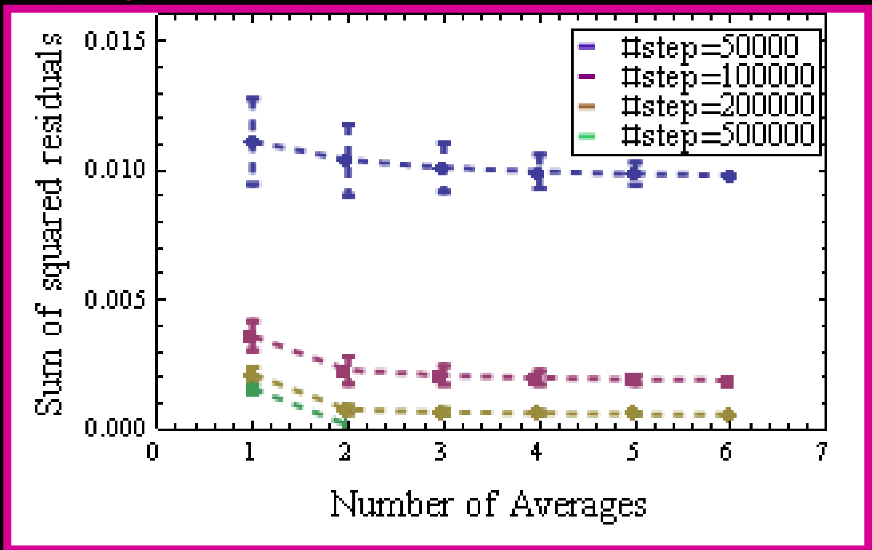
Simple model: One dimensional chain of n spins, state 0 or 1

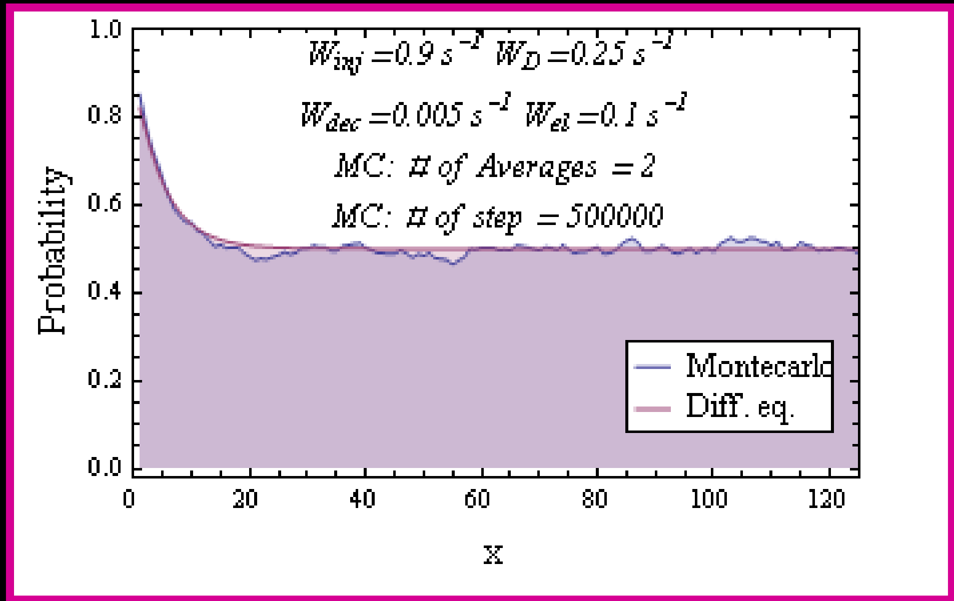
events: exchange between nearest neighbours (i±1) → pD
decay (from state 1 to 0) → pdec
injection (from state 0 to 1) only for i=1 → pinj

Analytic solution compared to Montecarlo and differential equation simulation tools.

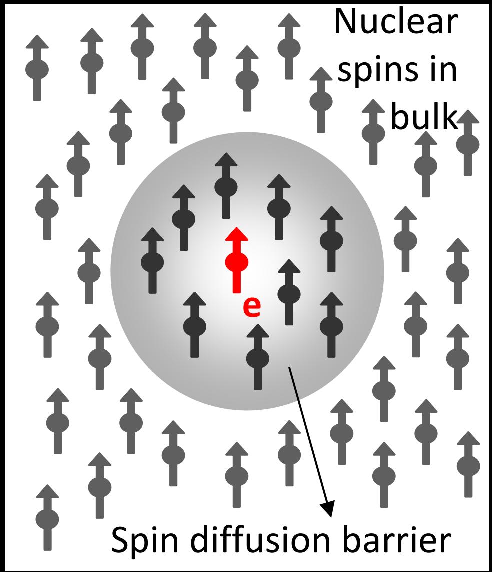


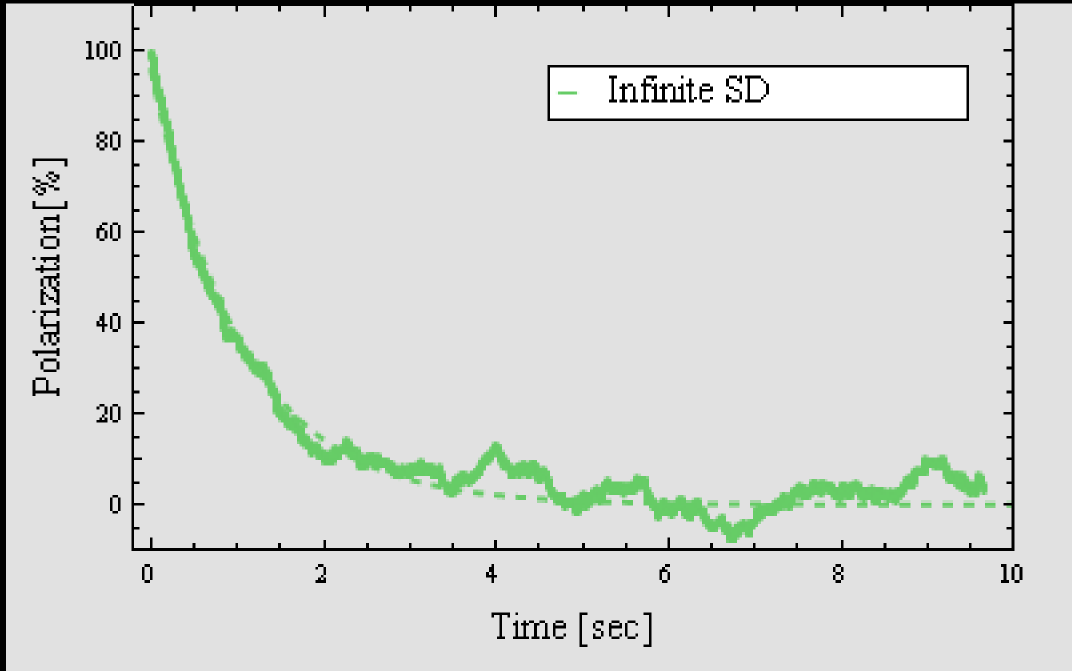
Comparison between Montecarlo and differential equation simulation tools (pinj(r) and pdec(r) .





3.3 Simulation tools: first results on Spin Diffusion





Simulation parameter:
Initial state = 100% of polarization
Observed physical phenomenon = relaxation by electron impurities
T_{1e}= 1µs
$\Delta\omega_n$= 40 KHz
Nspins = 512
A = 3 Å
SD condition: INFINITE

$$\frac{1}{T_{1,th}} = \frac{\sum_{i=1}^{NSpin} 2W_i}{\sum_{i=1}^{NSpin} i}$$

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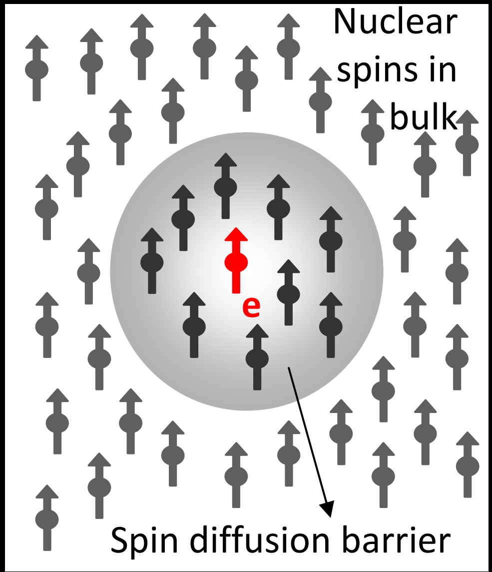

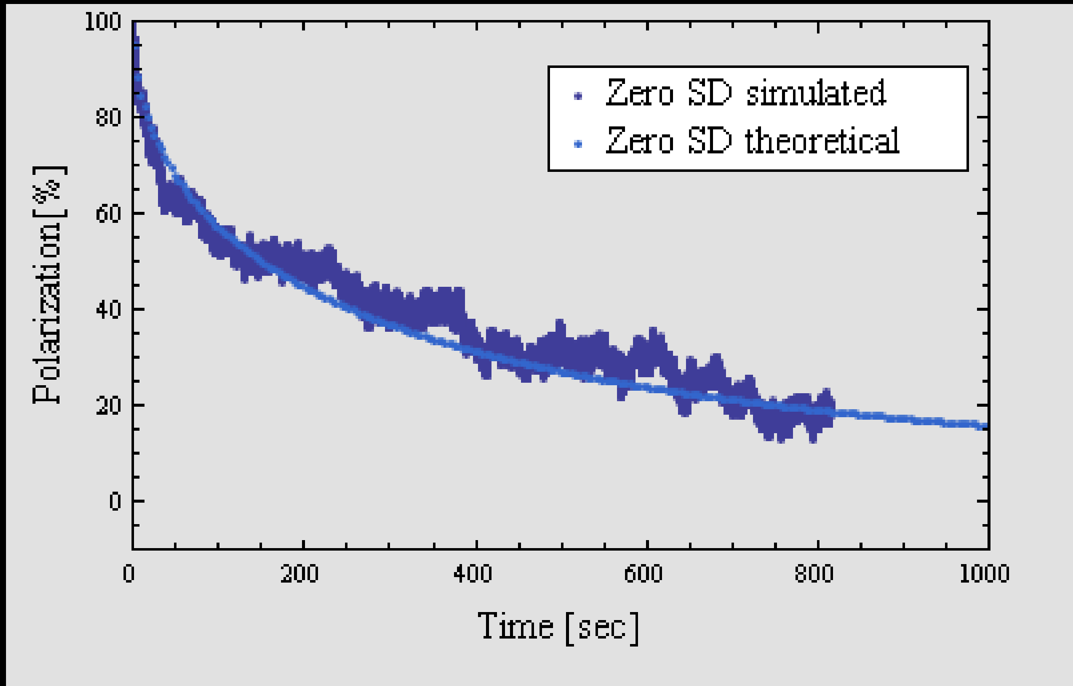

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Initial state = 100% of polarization

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T_{1e}= 1µs

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A = 3 Å

SD condition: ZERO

$$\text{Recovery Law} = \int 4\pi r^2 e^{-t/T_1} dr$$

$$\approx \sum_{i=1}^{Nspin} \frac{1}{Nspin} e^{-t/T_1(i)}$$

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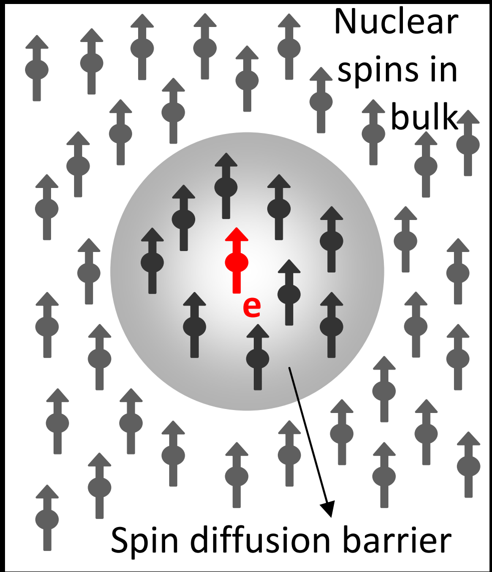

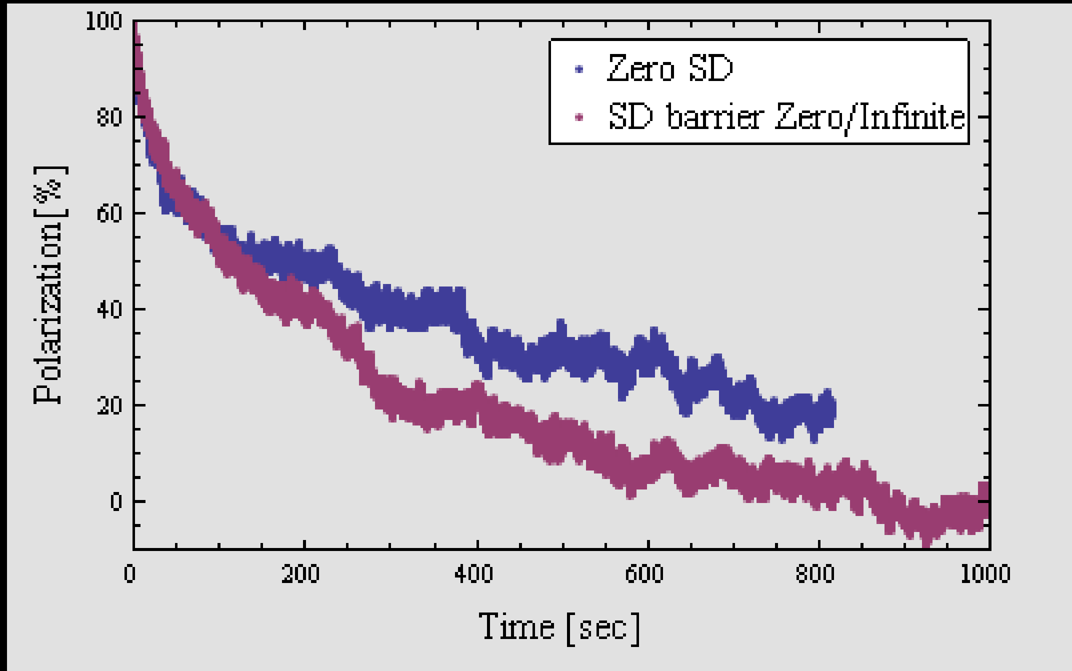

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T_{1e}= 1µs
$\Delta\omega_n$= 40 KHz
Nspins = 512
A = 3 Å
SD condition: Barrier → ZERO/INFINITE

$$\frac{1}{T_1} = \left(\frac{4\pi}{3} C\, Nel\, b_{SD}{}^3 \right)^{-1}$$

$$T_{1,th} = 400s,\ T_{1,ex} = 250s$$

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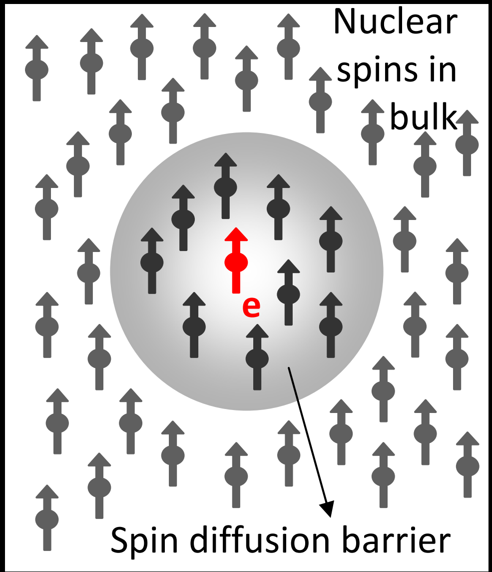

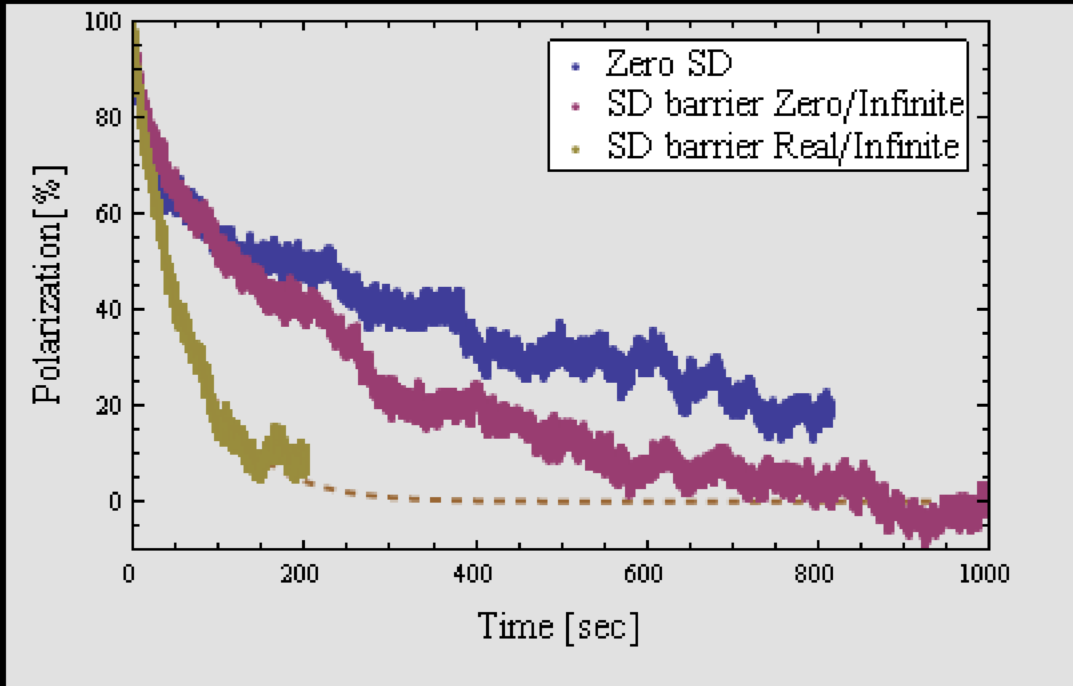

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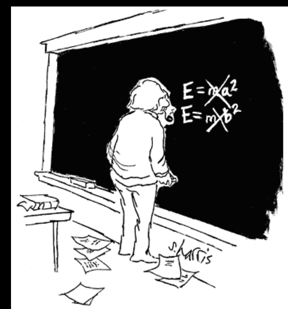

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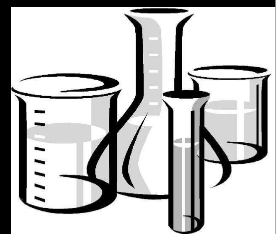
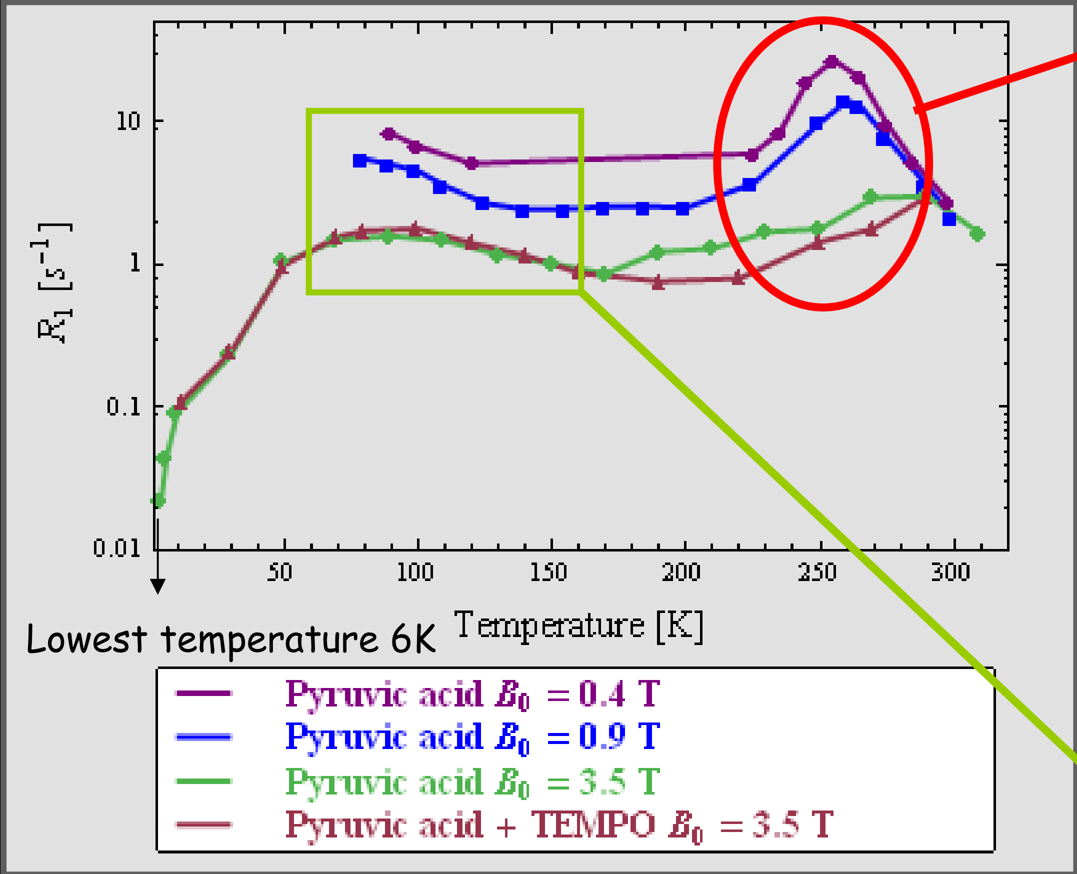
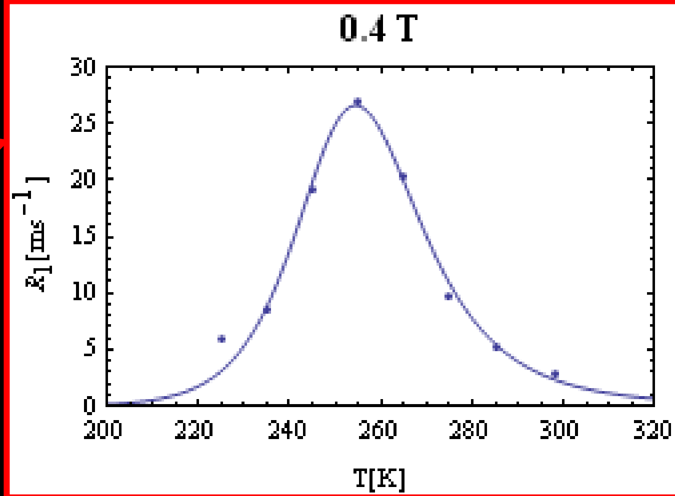


4.1 Physical characterization of NMR probes

A quite wide characterization of NMR features of examined samples has been performed.

Ex: PYRUVIC ACID ($CH_3COCOOH$)





Activation Energy		
Compound	EA[K]	σEA[K]
Pir 4kG	5670	346
Pir 9kG	5770	296
Pir 34.6kG	3390	477

Activation Energy		
Compound	EA[K]	σEA[K]
Pir 4kG	170	5
Pir 9kG	160	29
Pir 34.6kG	120	18

Urea and Butyric acid (pure and in glycerol solution) were NMR characterized. Butyric acid pure and in glycerol solution was further characterized (EPR, SQUID, NMR with different freezing methodology)

4.1 Physical characterization of NMR probes: summary of results

NMR RESULTS

- Evidence of relaxation by paramagnetic impurities in liquid state.
- Analysis of molecular motion allowing relaxation.
- Limited relaxation effect by paramagnetic impurities at SS and temperature above 6K (at least on 1H nuclei): some molecular motion (related to the glassy structure) still exists and allows nuclei relaxation.
- Strong dependence on cooling process.
- Efficient relaxation mechanism connected to the presence of glycerol (i.e. to a glassy solvent).

EPR RESULTS

- In presence of glassy structure: EPR line is narrower, T1 is shorter, T2 does not change.
- Different line width between nitroxides and trytil radicals ($\Delta\omega_e \sim \omega_n$).

SQUID RESULTS

- All samples show paramagnetic behaviour. No magnetic ordering of the radical can be revealed

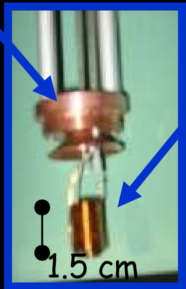
4.2 DNP experimental setup: 0.7 T (UNIPV)

Microwave horn

NMR coil

Cryostat and pumping system

zoom



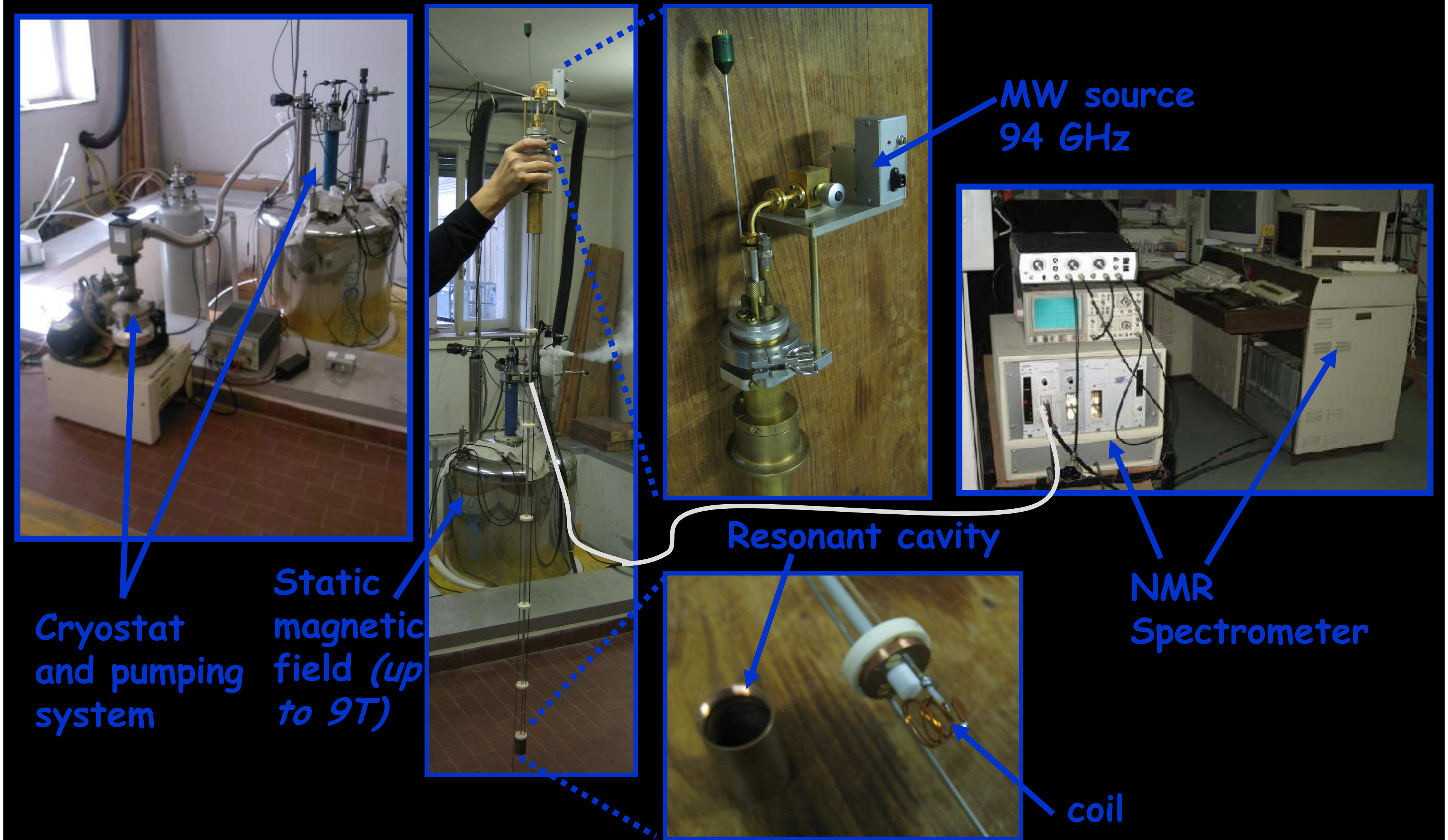
Microwave source

20 GHz

Magnet

NMR Spectrometer

4.2 DNP experimental setup: 3.35 T (UNIPV)



4.2 DNP results: from 0.7 T to 3.35 T

Polarization level

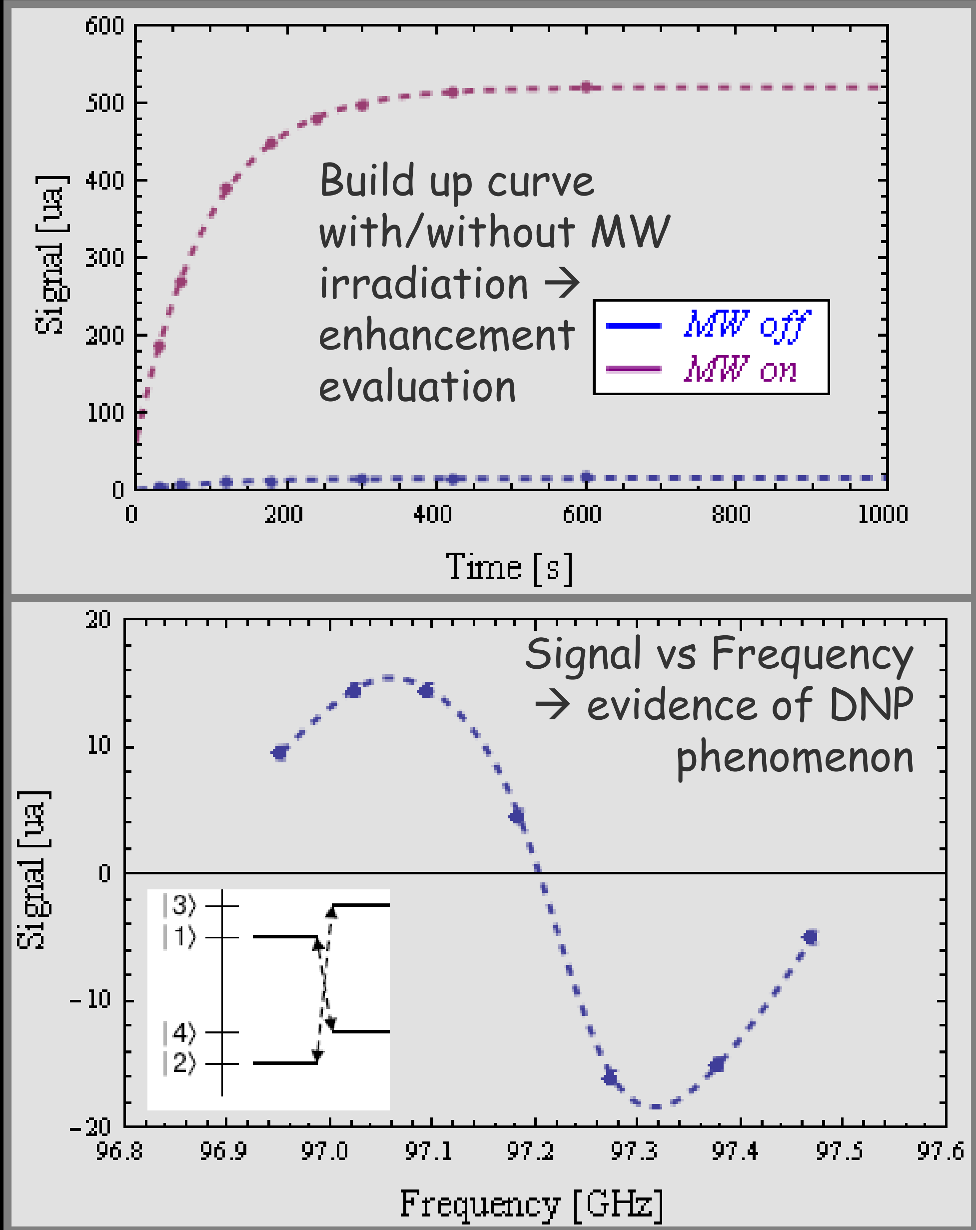
B_0 = 0.7T, 4.2 K → 0.06%

B_0 = 3.35T, 4.2 K → 0.7%

B_0 = 3.35T, 1.8 K → 3%

Results:

- Literature results reproduction
- Development of know-how and expertise in DNP technique (completely unexplored till 2009)

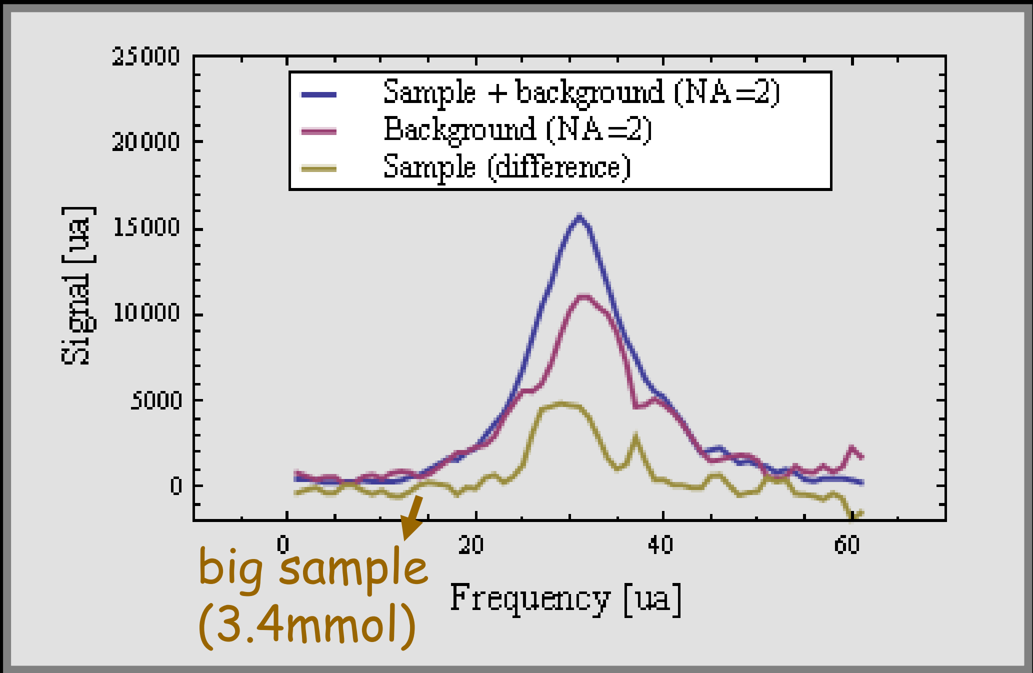


4.2 DN Polarizer start up: thermal signal measurement.

How can we estimate **absolute polarization**? By comparing the hyperpolarized signal to the thermal signal.

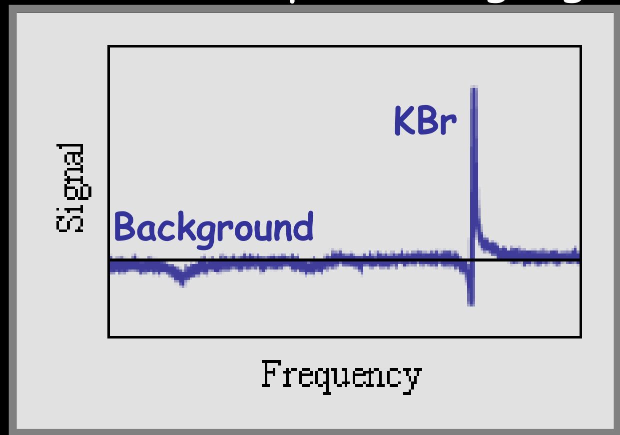
Thermal signal evaluation is very troublesome:

- A huge background hides the sample signal.
- T_1 of 13-Carbon nuclei at low temperature is really long.
- Coil sensitivity is low.



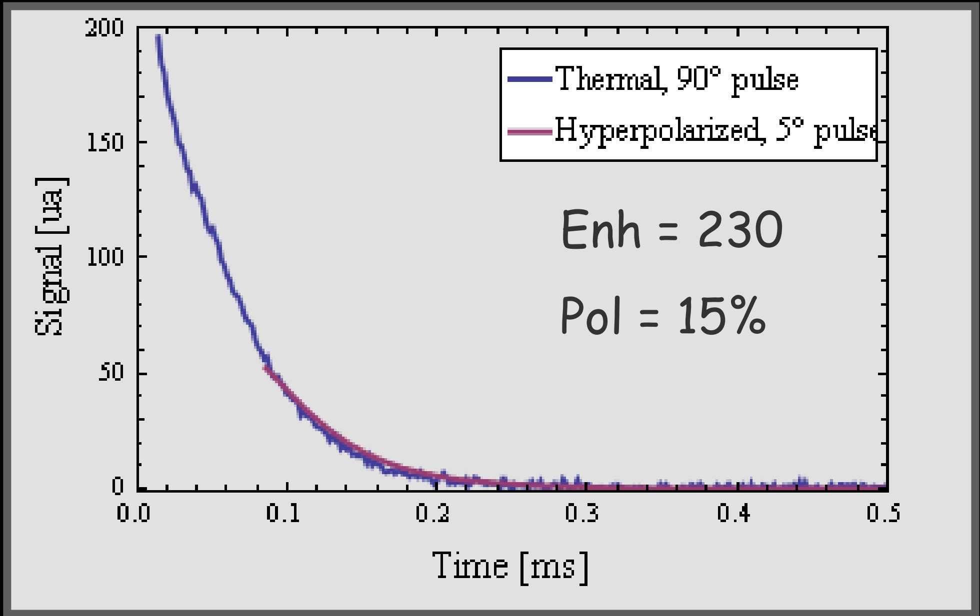
1° attempt: evaluation of the ratio between hp signal and thermal signal of a big sample (failed, probably bg and sample signals have different phases)

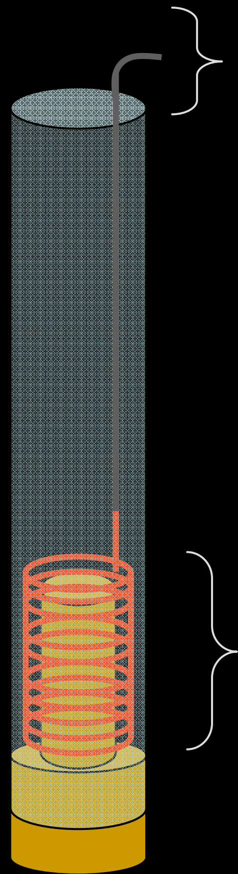
2° attempt: avoid overlapping between sample and bg signal

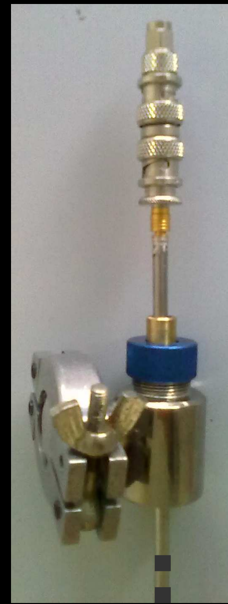


4.2 Thermal signal measurement: 3° attempt

The 1° attempt is the most direct, but the coil sensitivity is too low and too much affected by the background. By making a dedicated coil this problem can be solved.



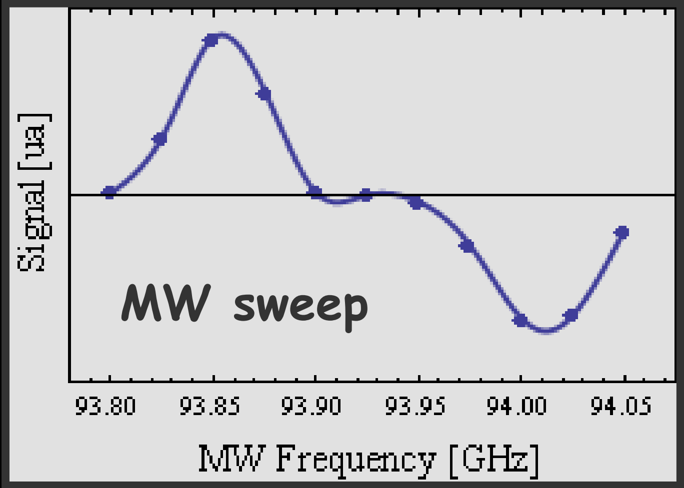




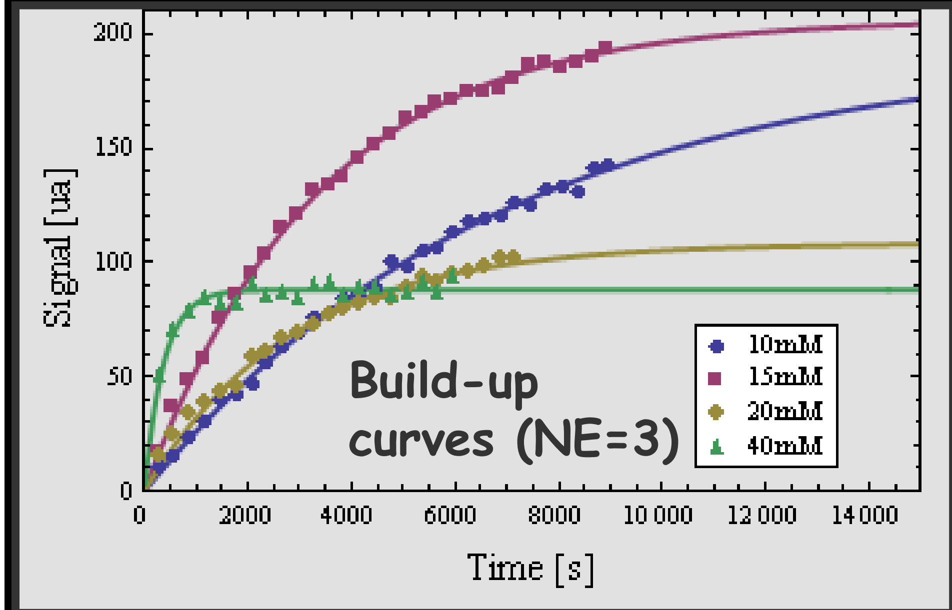
The thermal signal was measured successfully. The hyperpolarized signal saturated the receiver. The experiment requires a further optimization (i.e. decreasing of the signal).

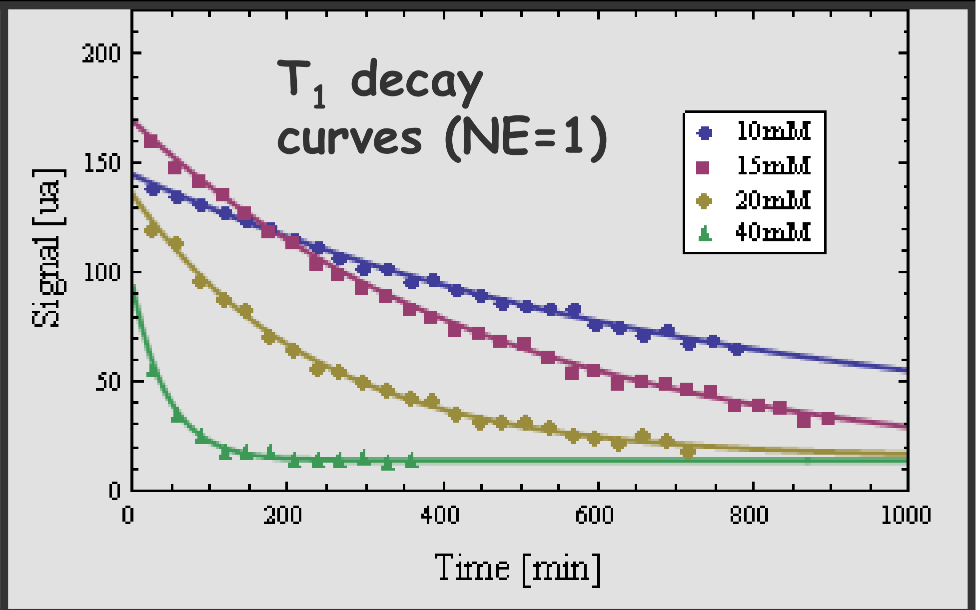
4.2 DNP results: UREA + Trytil @ variable [el]

SAMPLE: Glycerol/Water (1:1), ^{13}C-labelled Urea, Trytil radical (10, 15, 20, 40 mM)



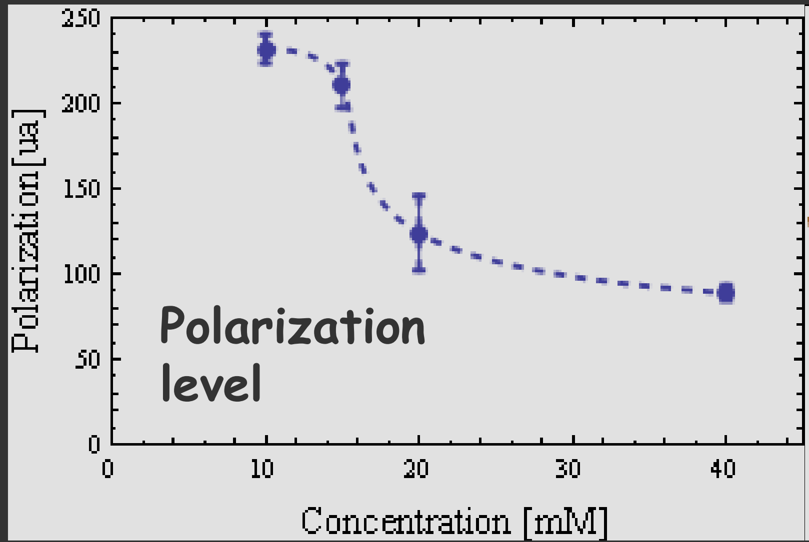
el [mM]	T1 [s]	τpol [s]	pol [ua]
10	53 340	8412 ± 460	232 ± 8
15	31 800	3600 ± 62	210 ± 13
20	14 940	2687 ± 1100	124 ± 22
40	2700	328 ± 46	89 ± 4

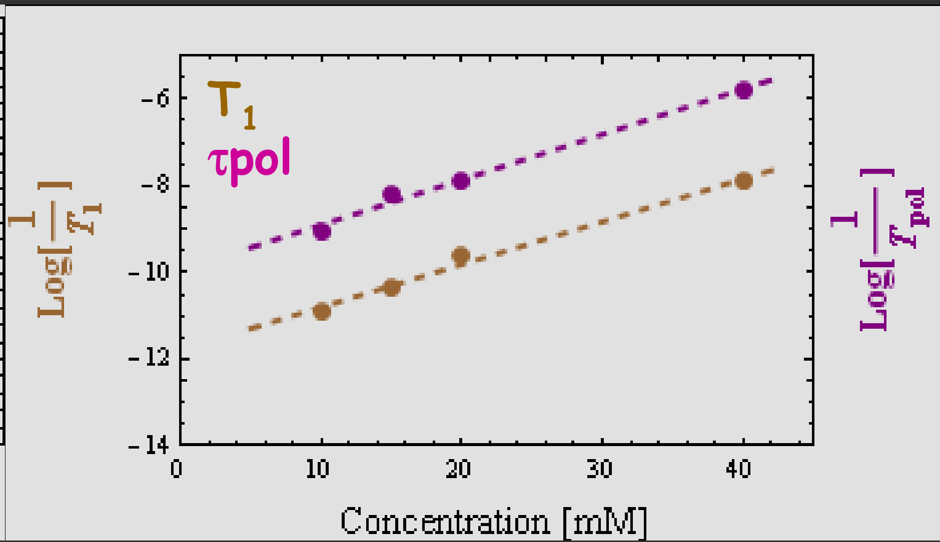


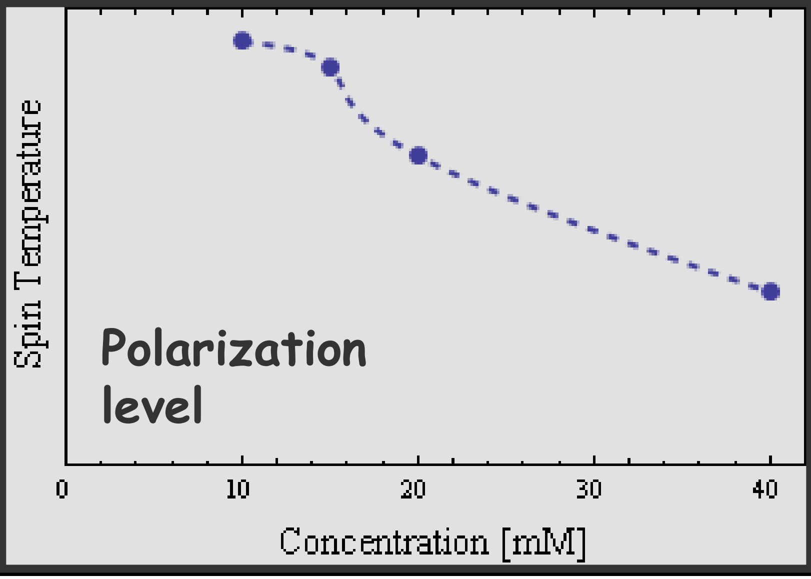


4.2 DNP results: UREA + Trytil @ variable [el]

EXPERIMENTAL RESULTS







THEORETICAL PREDICTION
(Provotorov model)

- -qualitative prediction of experimental results
- - not widely applicable
- - doesn't allow % polarization evaluation

Conclusions

Theoretical activities:

- ✓ Two simulation tools developed and compared
- ✓ Further optimization and final tests on going *(hopefully one publication)*

Experimental activities:

- ✓ Development of two polarizer prototypes
- ✓Development of new MCAs (hopefully one publication)
- ✓ NMR characterization of standard MCA: experimental activity on going
- ✓ DNP investigation of standard MCA: experimental activity on going *(hopefully one publication)*

Publication on Gd-based contrast agents (Cancer Res. 2011 Jan 6):

E-3810 is a potent dual inhibitor of VEGFR and FGFR that exerts antitumor activity in multiple preclinical models - Bello E, Colella G, Scarlato V, Oliva P, Berndt A, Valbusa G, Colombo S, D'Incalci M, Cavalletti E, Giavazzi R, Damia G, Camboni G. -