Interaction of Na ions with DNA G-quadruplex structures studied directly with Na beta-NMR spectroscopy

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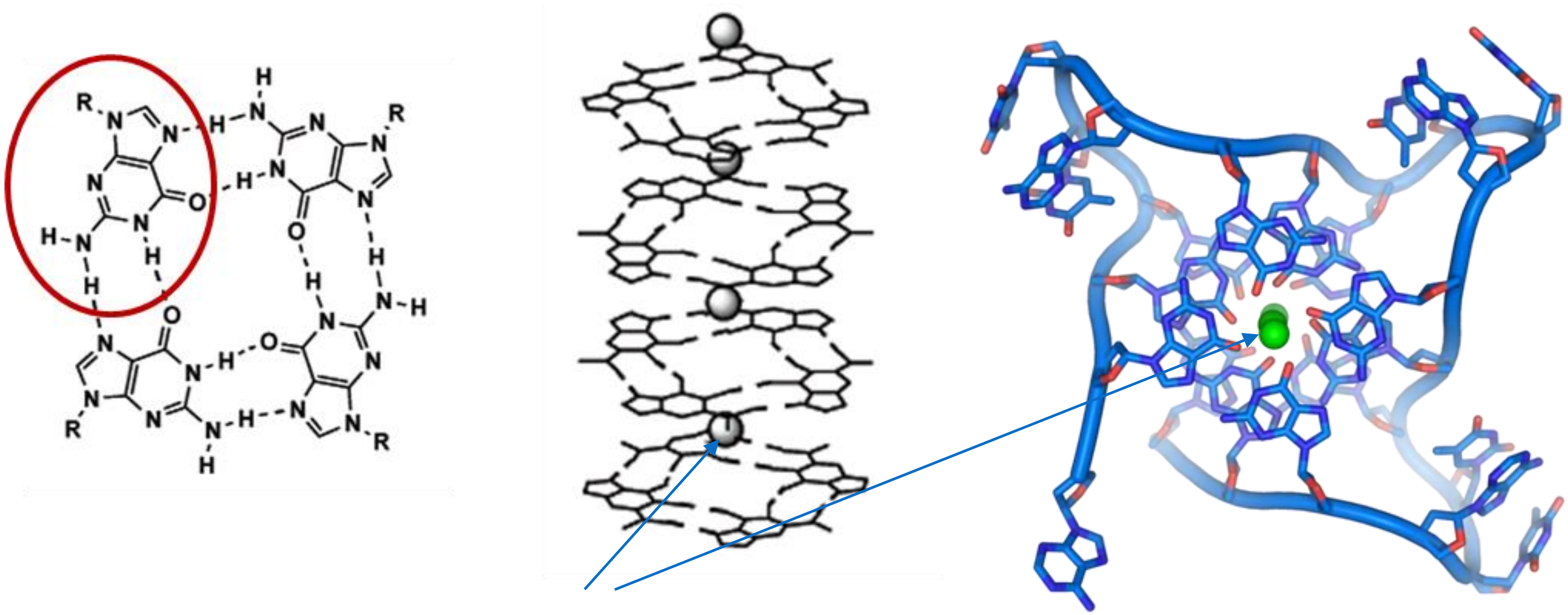
Motivation: DNA G-quadruplexes

- DNA G-quadruplexes:
  - ➢ Formed in guanine-rich DNA fragments
    - ✓ Present in telomeres (ends of chromosomes)
    - ✓ Present in promoter regions of many oncogenes
  - ➢ Synthesised for novel applications

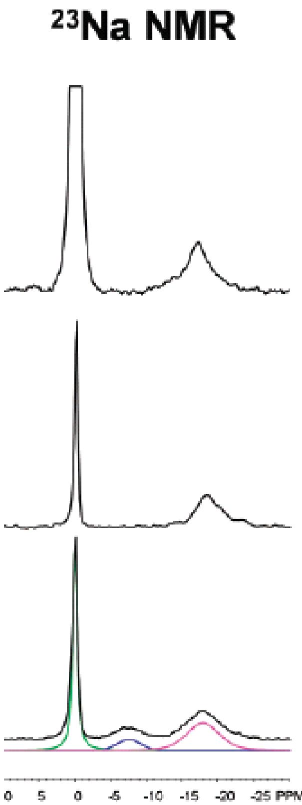


- Alkali metals in DNA G-quadruplexes
  - ➢ Important for their formation, stability and structural polymorphism
  - ➢ Until recently considered invisible in conventional Na+/K+ NMR

M. Trajkovski et al, J. Am. Chem. Soc. 134, 4132 (2012)

Alkali ions and G-quadruplexes

- Techniques used for their studies:
  - solid-state techniques: X-ray crystallography and solid-state NMR,
  - indirect solution NMR methods using spin-1/2 probes, 15NH4+ and 205Tl+
- Tightly bound ‘channel’ ions considered ‘invisible’ in solution-NMR
  - low signal intensity and unfavourable quadrupole spin relaxation properties
  - Only recently several direct solution NMR studies published [Won05]



=> still little known about alkali-metal cation dynamics in this important class of nucleic acid structures

Our aim:

Investigate Na interaction with G-quadruplexes using beta-detected 26-28Na NMR

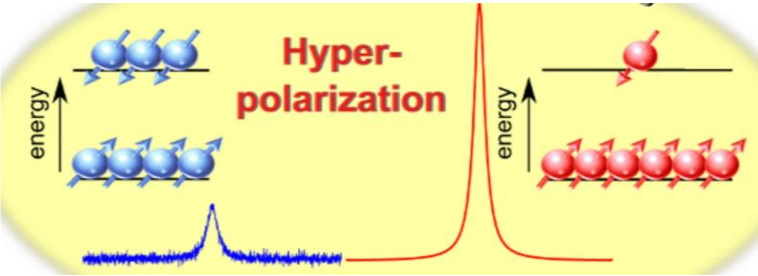
Primary gain: up to billion-times higher sensitivity than conventional 23Na NMR

[Won05] A. Wong, R. Ida, G. Wu, Biochem. Biophys. Res. Commun. 337, 363 (2005)

Ultra-sensitivity of beta-NMR

- From low sensitivity of NMR to 1e10 more sensitive beta-NMR:
  - Small degree of (thermal) polarization -> hyperpolarization; 1e5 gain
  - Inefficient resonance detection -> particle detection; 1e5 gain

- Hyperpolarization – using optical pumping with lasers



- Anisotropic emission of beta particles from decay of polarized nuclei

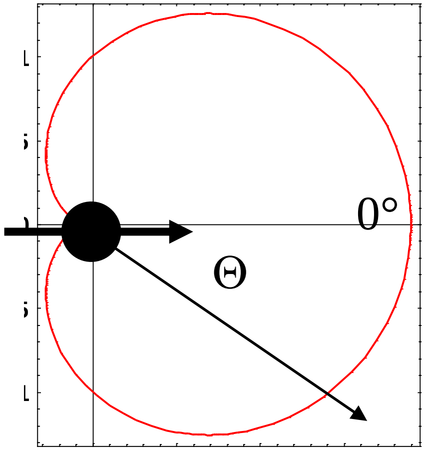
Velocity of beta-particle (v/c close to 1)

Angle between beta-particle emission and direction of spin polarization

$$D(\Theta) = 1 + a\frac{v}{c}\frac{\langle I_z \rangle}{I}\cos(\Theta)$$

Asymmetry factor (-1,1), depends on details of beta decay

PI (0-100%): degree of spin polarization

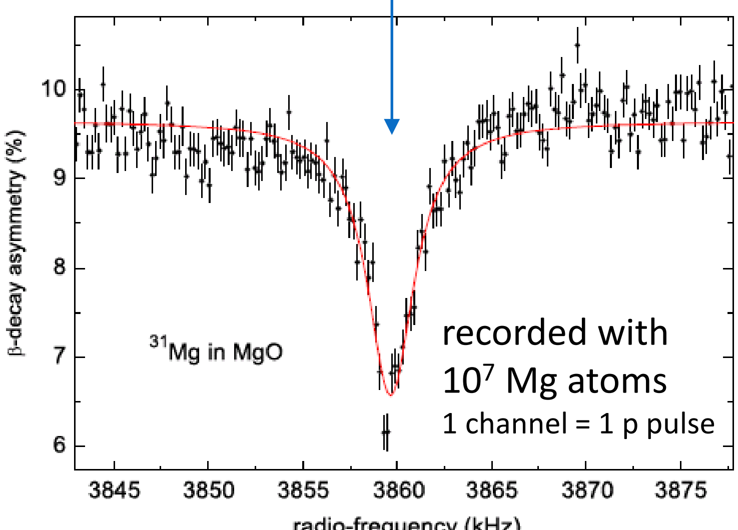


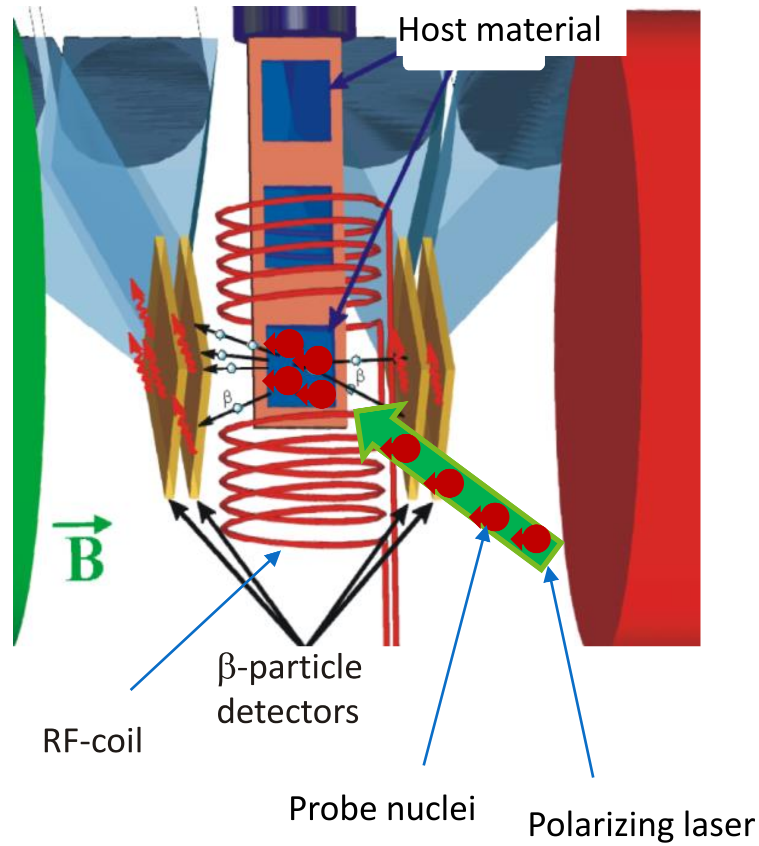
Beta-NMR measurement

- Same principles as conventional NMR
- Detection of resonance:
  - **Baseline: asymmetry in beta decay in space**
  - **In resonance with rf: decrease in asymmetry**

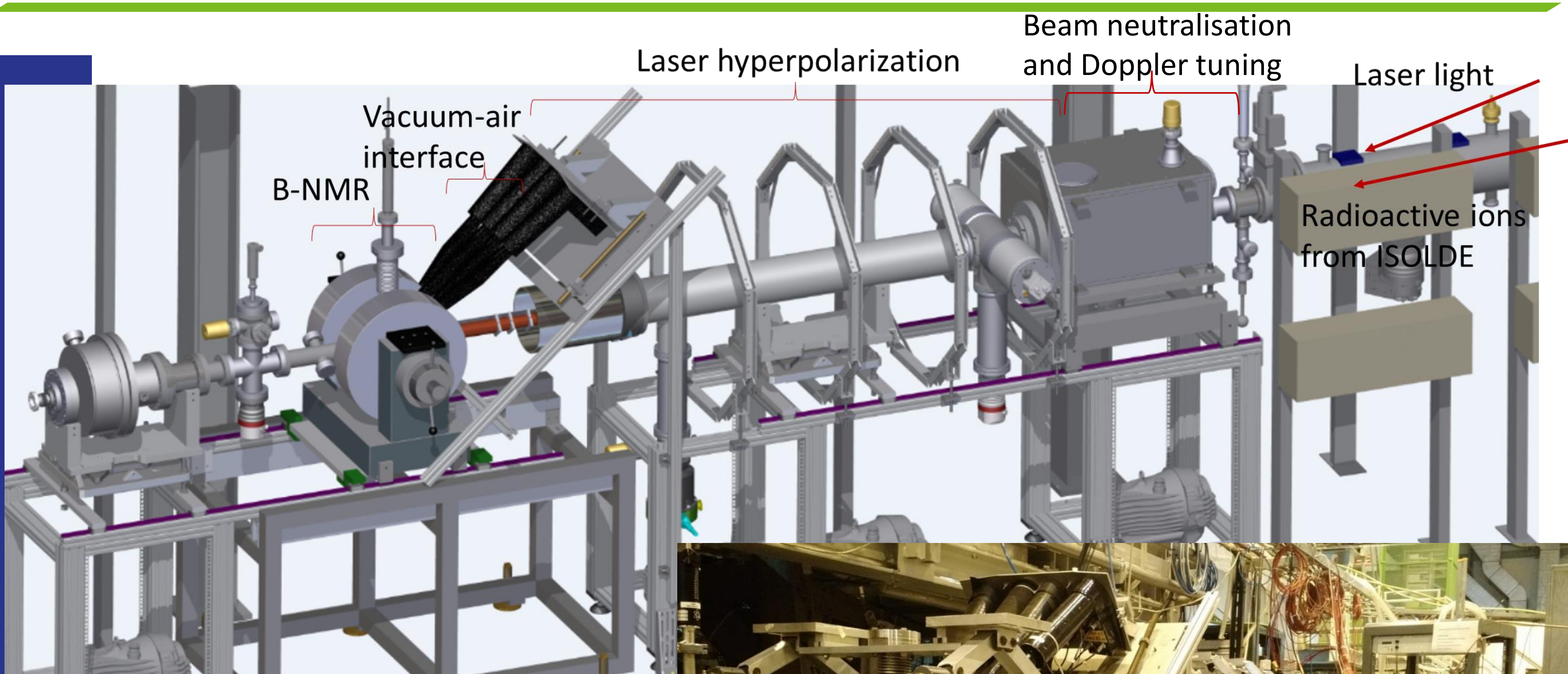
- When combined with hyperpolarization

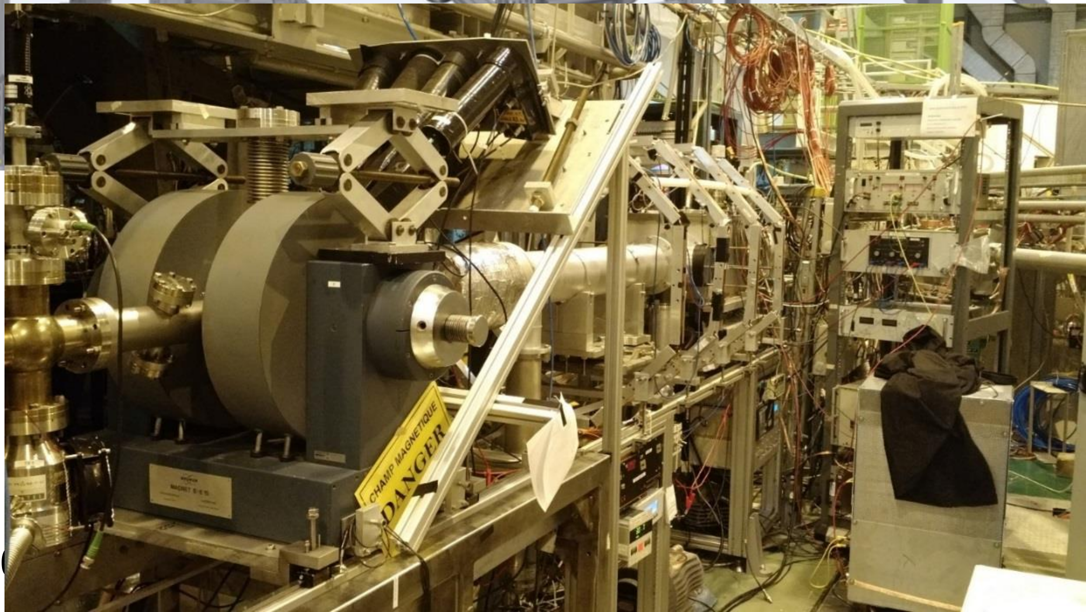
=> Beta-NMR can be up to 10^{10} more sensitive than conventional NMR





Experimental setup

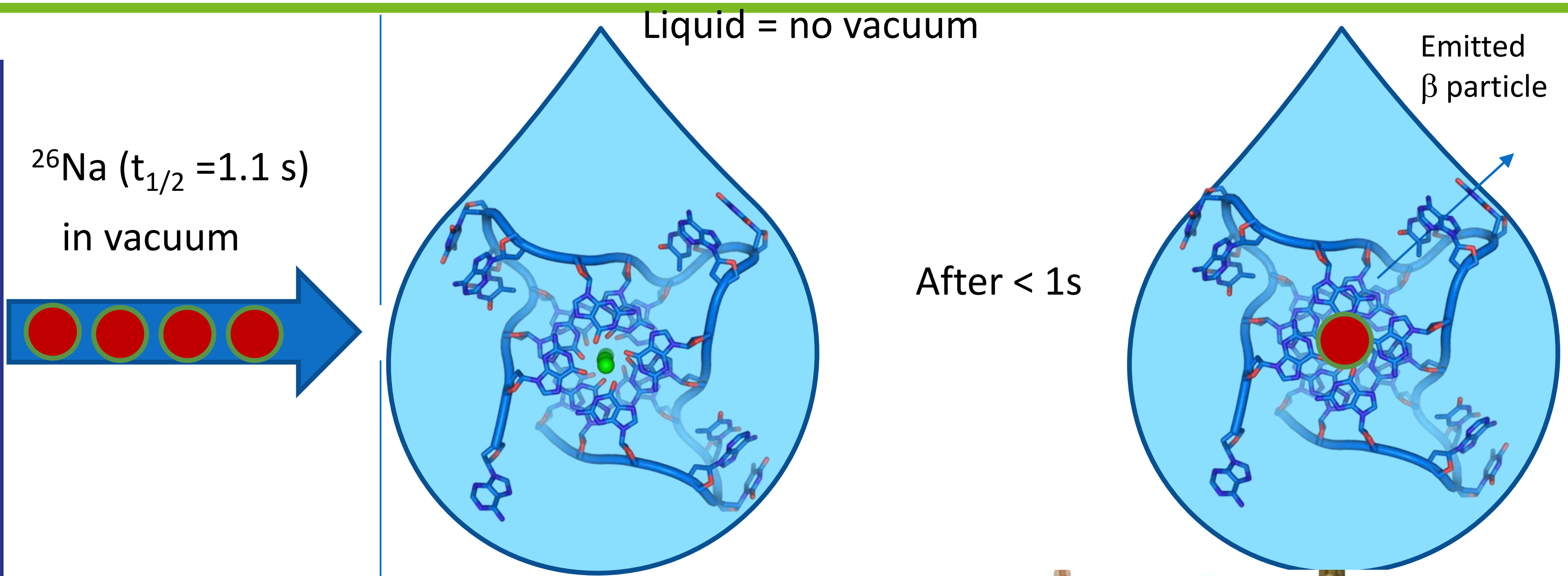




Commissioned with 26,28Na in autumn 2016

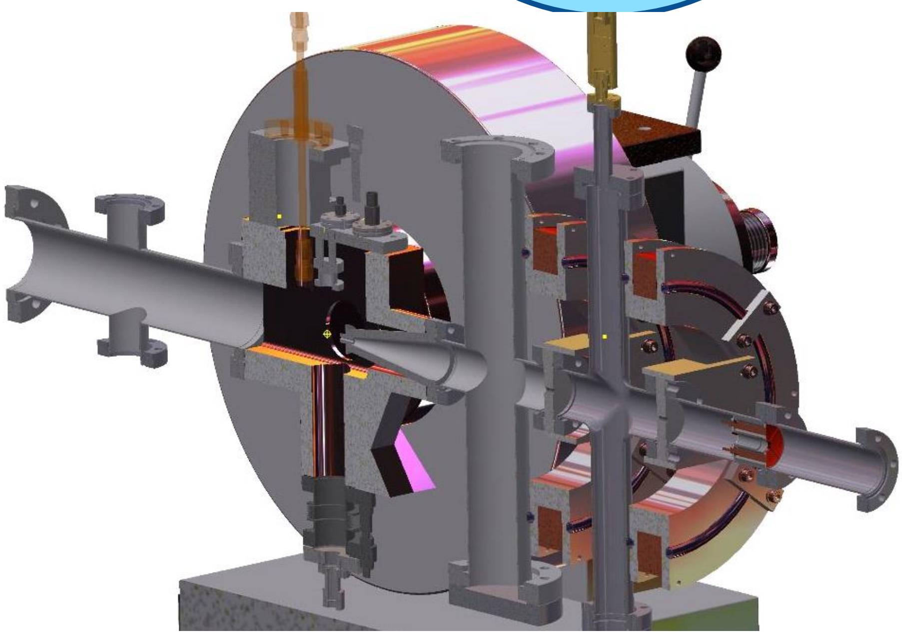
M. Kowalska et al., Journal of Physics G, in print (2017)

Getting probe nuclei into liquid samples



Challenges and constraints:

- Vacuum/liquid interface with little loss in atom beam and polarization
  - at present, differential pumping system with strong pumps, under tests now (4 orders of magnitude pressure difference with 1 pinhole)
- Binding to biomolecule before decaying -> choose suitable systems to study



Probe nuclei: 26,27,28Na

Nucleus	Radioactive half-life	Nuclear spin I	Magnetic moment (μ_N)	Quadrupole moment (mb)	Observed β–asymmetry
26Na	1.1 s	3	2.86	-5	30 %
27Na	0.3 s	5/2	3.89	-7	30 %
28Na	30 ms	1	2.43	40	40 %

- Favourable atomic transition (NaI D2 line at 589 nm) => P_I =30-60%, pumping both ground state HFS levels => 30% higher P_I
- a_β factors close to 1

⇒ beta-NMR resonances easy to observe, even if small fraction of original P_I maintained after implantation in liquid sample

- Very well produced from several targets

- Q < than Q(stable 23Na) = +100 mbarn

⇒ weaker quadrupole interaction => longer relaxation time and smaller peak broadening

- Different t1/2

⇒ probing different timescales, e.g. t1/2(28Na) comparable with relaxation time observed G-quadruplex liquid 23Na NMR studies

- Compatible with present stage of setup: VIS, IR polarization of atoms

Feasibility and goal of studies

- G-quadruplex interaction with Na^+ very suitable for beta-NMR studies:
  - Very strong binding with alkali metals [Ske10]
  - Reaction extremely fast – expected < $t_{1/2}$ of ^{26}Na, even ^{28}Na, [Ske10]
  - Long enough NMR relaxation times: tens ms reported [Won05, Ida08]
  - NMR chemical shifts of 23Na-NMR in 20 ppm range => can be resolved with our B field homogeneity

- Goal: determine how and at what time scale Na^+ binds to model G-quadruplexes
  - observations at different Na+ and G-quadruplex concentrations => determine dissociation constant of reactions
  - measure relaxation times of observed signals => identify different positions occupied by Na+

- Planned liquid hosts:
  - Ionic liquid – verifications at good vacuum, chemical shift calibration
  - Water and NaCl solution – chemical shift references
  - (crown ethers)
  - DNA model G-quadruplex solutions at different concentrations and temperatures
    - ✓ E.g. d[TAG3CG3AG3AG3A2] synthesized and well studied by Ljubljana coauthors
    - ✓ Chemical shifts (signal at -18 ppm expected, [Won05]) and relaxation times

[Ske10] P. Sket and J. Plavec, J. Am. Chem. Soc. 132, 12724 (2010)
[Ida08] R. Ida and G. Wu, J. Am. Chem. Soc. 130, 3590 (2008)

Beamtime request

Uc, Ta or Ti target, surface ionization, GPS or HRS

beam	Yield from UCx	Yield from Ta	Yield from Ti
26Na	3.0e7 [Kei00]	4e6	1.5e6
27Na	8.5e6	1.2e4	1.7e5
28Na	9.6e5	7e3 (interpolated)	n.a.

15 shifts, (split into 1-2 runs over 1 year):

- 3 shifts (24 h) to perform preparatory NMR measurements with ionic liquids, with one and two liquids, and at several pressures: 1 shift to see 1st NMR spectrum, 2 shifts to record some 20 spectra
- 3 shifts (24 h) to record NMR resonances in water and crown ethers, with min 1 spectrum per hour
- 9 shifts to investigate the G-quadruplex interaction with 26-28Na, again with min 1 spectrum/hour
- General assumptions
  - 1 shift: establish change-exchange cell and doppler tuning settings => 1^{st} polarization signal and b-NMR resonance
  - 1 h: several NMR resonances in good vacuum, 1 resonance in bad vacuum
  - 1h between different hosts (and conditions)
  - Time for contingency

Thank you

Funding:

ENSAR2

Beamtime request

Beamtime request

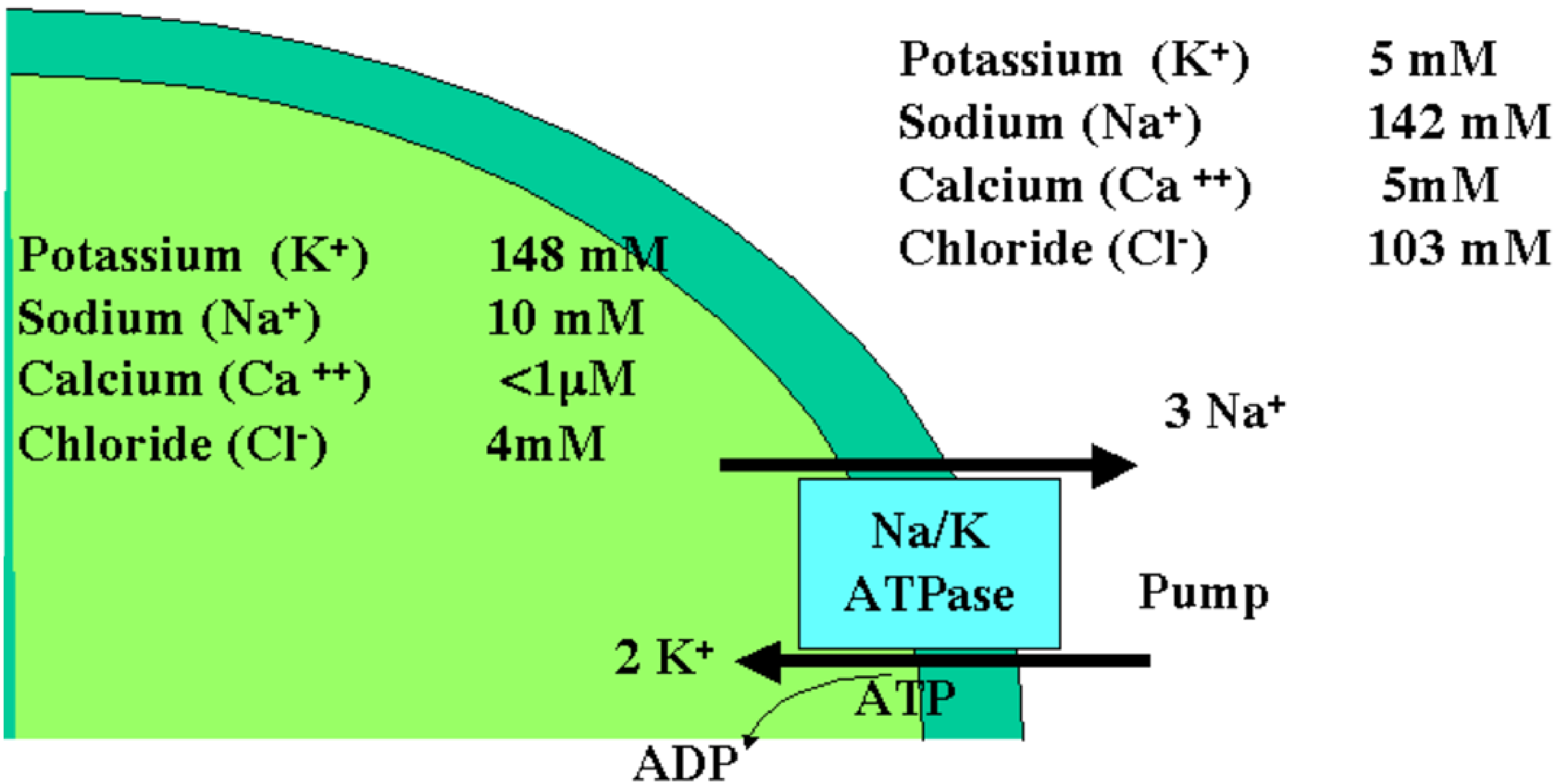
The shift request takes into account the $^{26-28}Na$ yields (Table 2) and is based on the times required previously to record good quality NMR spectra using the COLLAPS setup, which was 5-10 min for β asymmetries above 5% and some 20-30 min for asymmetries of below 5%, rather independent of the ISOLDE production yields.

Thus, we assume that we will need: 1 shift to establish the optimal settings of the charge exchange cell and post-acceleration electrode for the 1st polarization signal and subsequent scanning of a β-NMR resonance on the best-produced ^{28}Na (based on commissioning beamtime in 2016). We also know that in good vacuum 1 h should be enough to record several NMR resonances, whereas for worse vacuum required for aqueous samples 1 h might be needed for one scan (due to very low expected asymmetry). In addition, between measurements in different hosts, we assume that 1 h will be needed to remove the previous liquid, titrate the new one, as well as achieve the right pressure and temperature. We also need some time for contingency.

ISOLDE

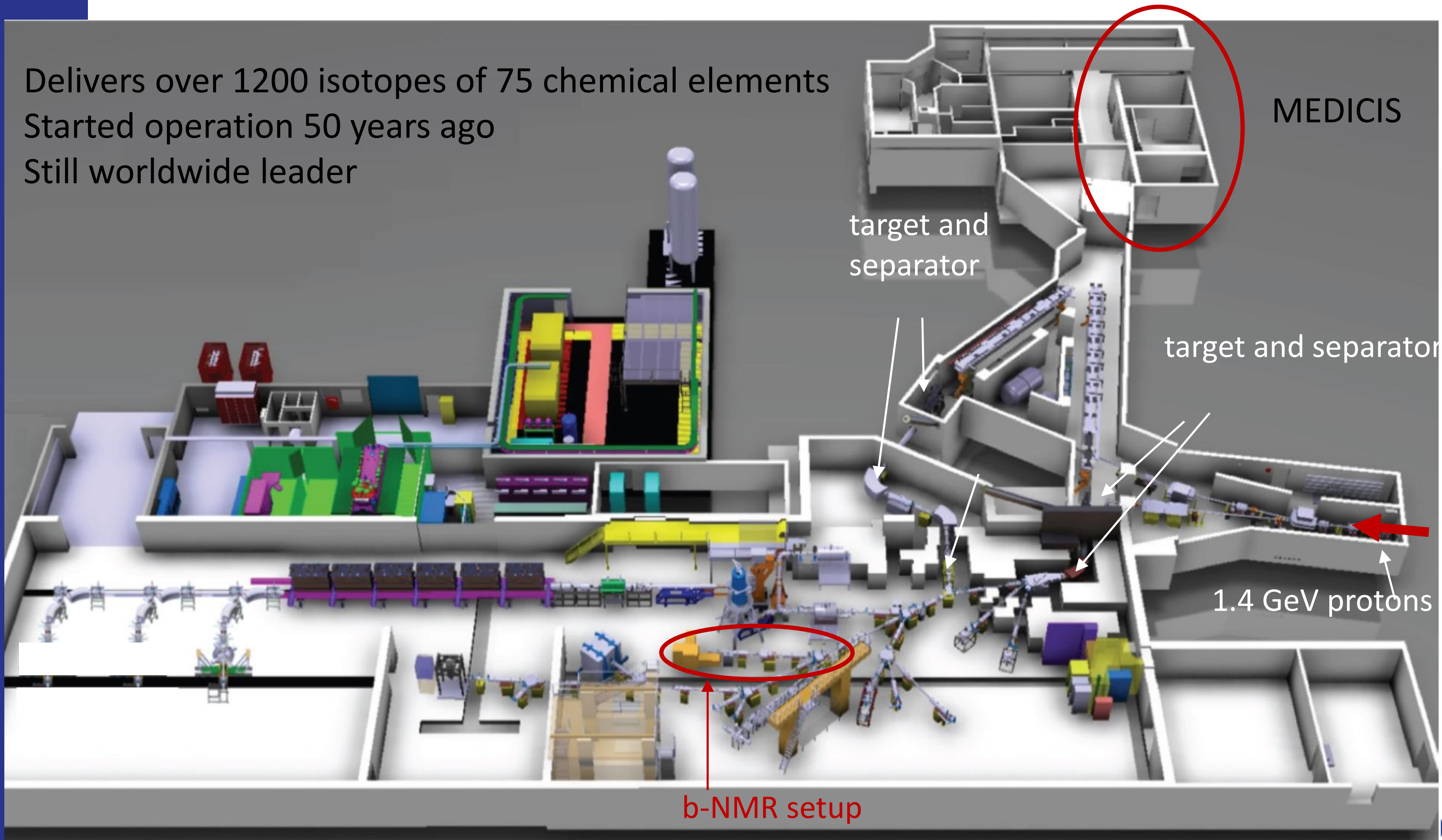
Na, K concentrations

- 2 mm drop = 4 mm3 volume = 4 uL
- Na+ inside cell: 10 mM in 4uL = 10e-3*4e-6 = 4e-9 moles = 40nmoles of Na+
- = 40e-9 *6*1e23 ions = 24e15 ions = 2.4e16 ions

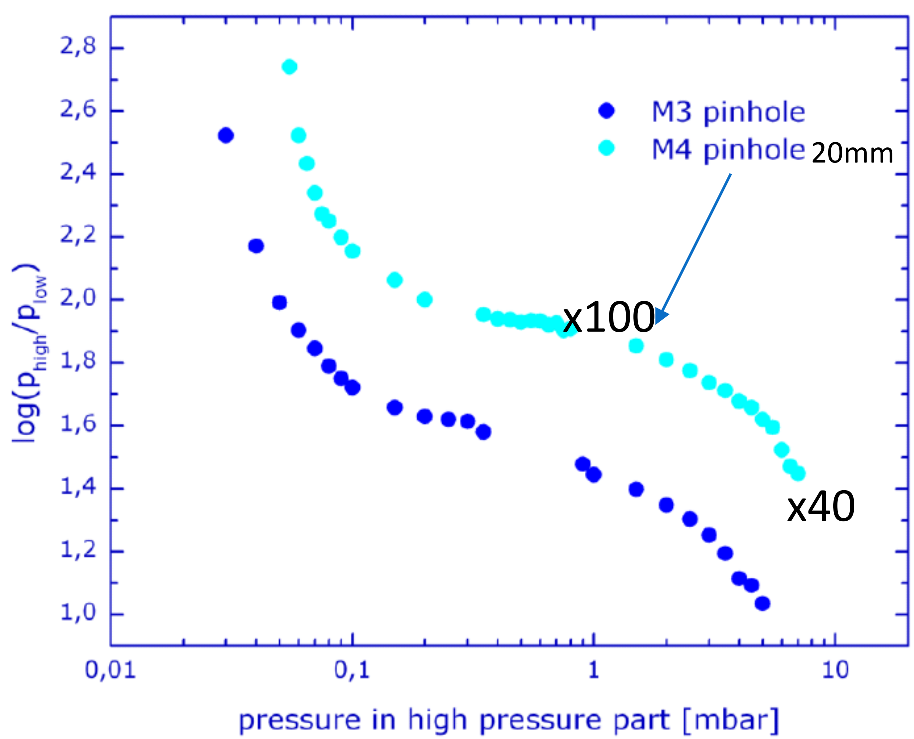


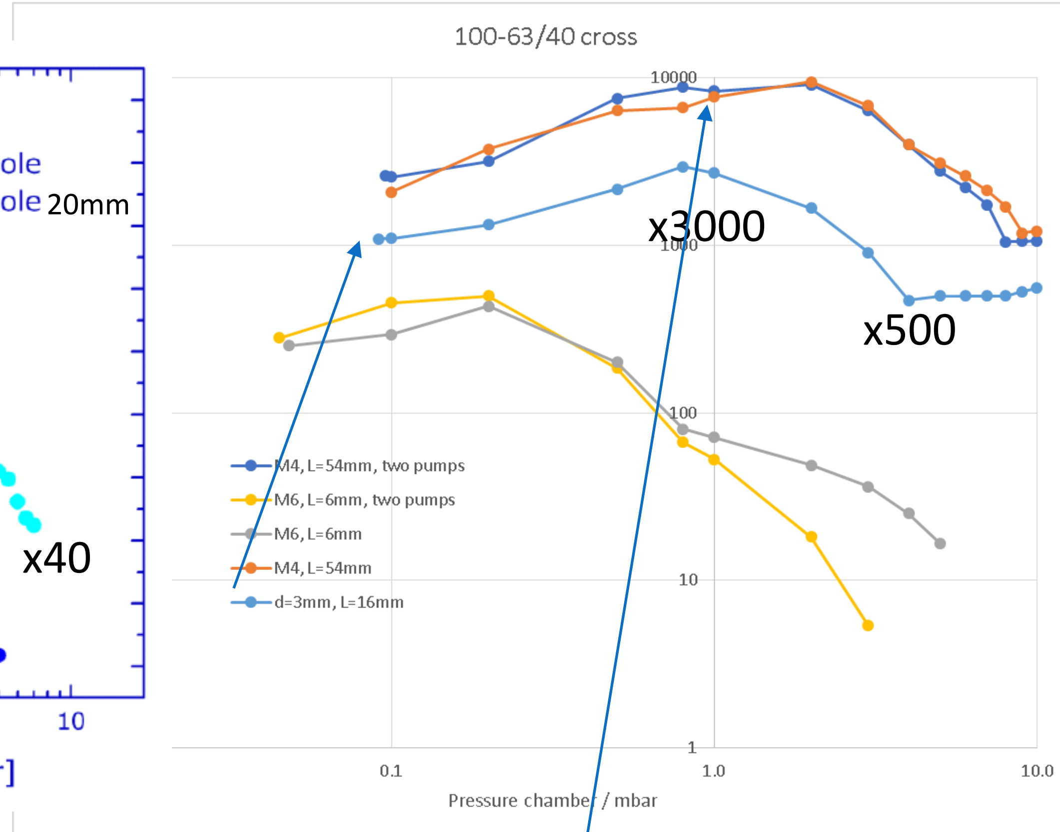
ISOLDE

CERN's facility for production and research with radioactive nuclei



Differential pumping





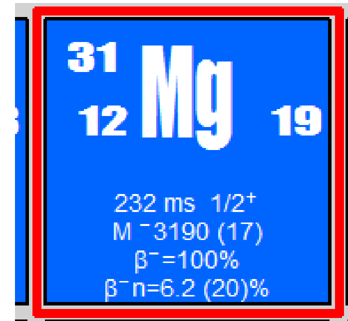
- Before: up to 2 orders of magnitude in vacuum with 1 pinhole
- Now: up to 4 orders of magnitude

=> confident that studies with aqueous samples feasible

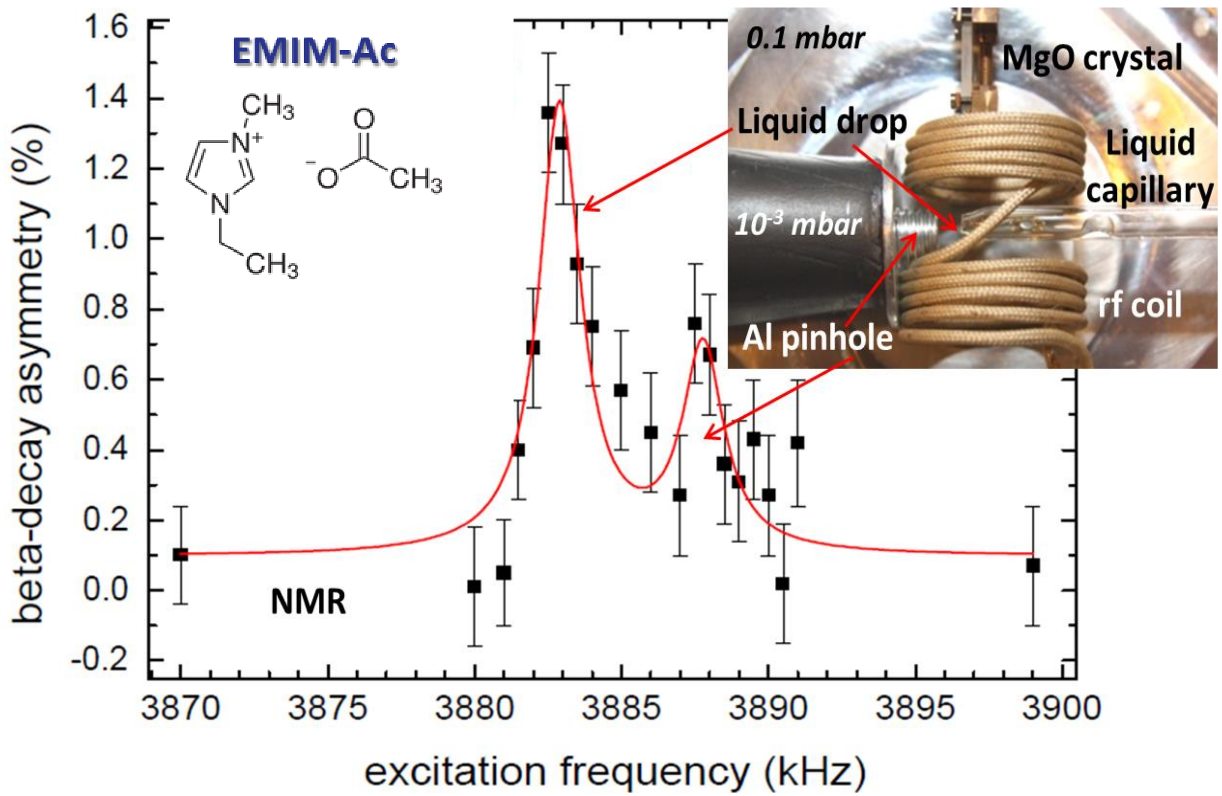
First liquid beta-NMR signal

- 31Mg
- 1e5 ions/s
- **1e7 ions in resonance**

B-static = 0.3 T
B-rf= 0.4 G
Width = 5 kHz



A. Gottberg, M. Stachura, M. Kowalska, et. al., ChemPhysChem 15, 3929 (2014)



NMR in (chemistry and) biology

Most versatile method to study structure and dynamics of molecules in solution

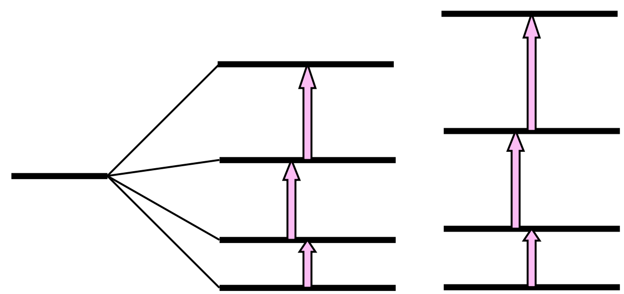
- **Observables**: chemical shift (Larmor frequency) and relaxation times in different hosts
- **Determined properties**
  - local electronic environment (i.e. **number and type of coordinating groups**)

Depends on environment

$$\Delta E_{mag} = |g_I| \cdot \mu_N \cdot B + \frac{1}{2} Q \cdot V_{zz}$$

known

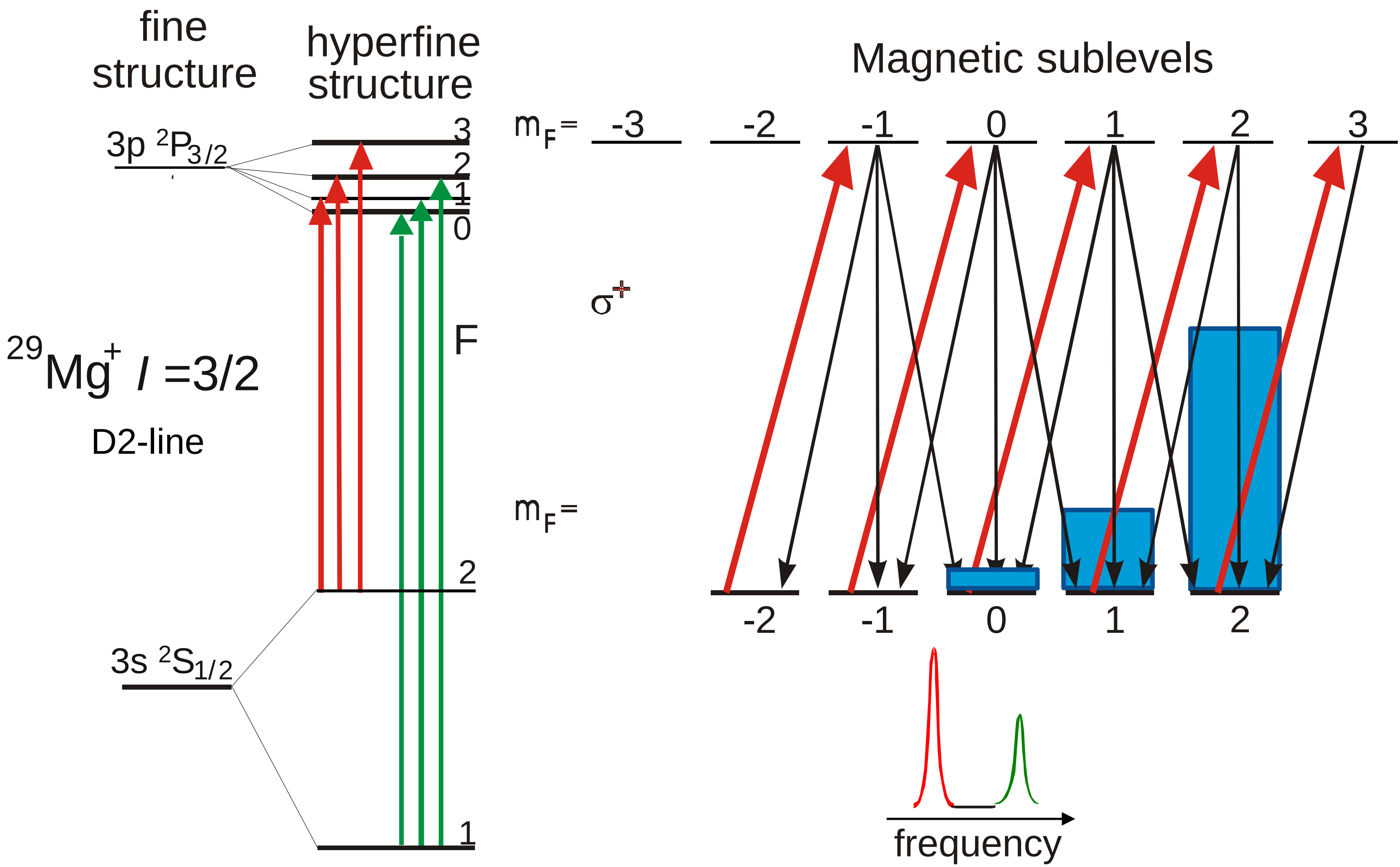
Same B, different shielding by host



$B = 0$ $B_0 + B'$ $B_0 + B''$

- **Derived information**: comparison to quantum-chemical models (e.g DFT)
  - kinetics and dynamics and ligand binding of the **metal ions and biomolecules**
  - 3D structure of proteins and **protein-metal complexes**

Optical pumping and atomic spin polarization

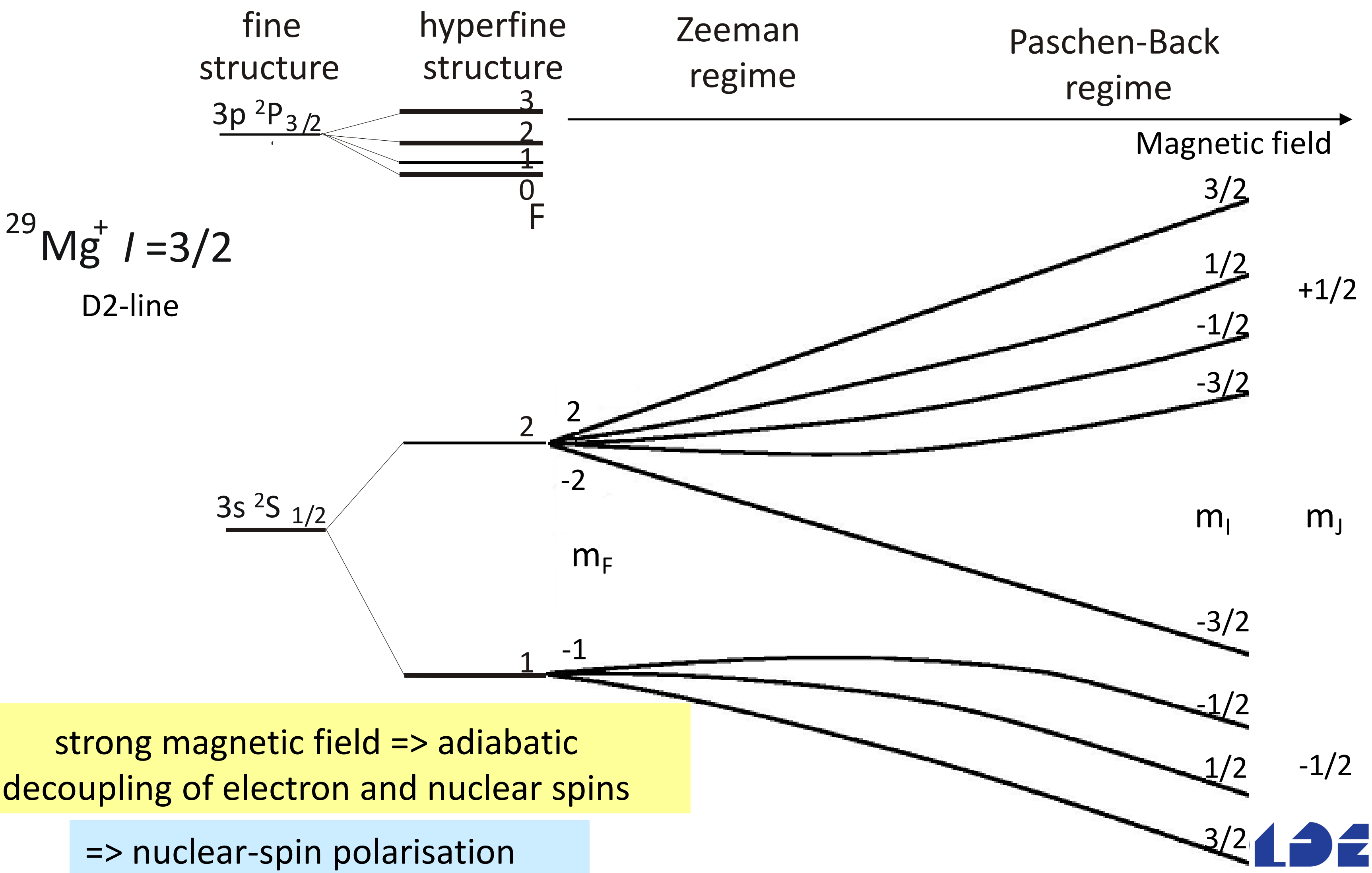


σ+ circularly polarized light => Δm_F = +1
(or -1 for σ-) => atomic spin polarization

Mg^+: 280nm, UV

Nuclear spin polarization

- Nuclear spin polarization via hyperfine interaction; spin decoupling in strong B field



strong magnetic field => adiabatic decoupling of electron and nuclear spins

=> nuclear-spin polarisation