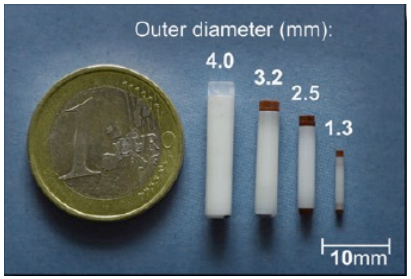
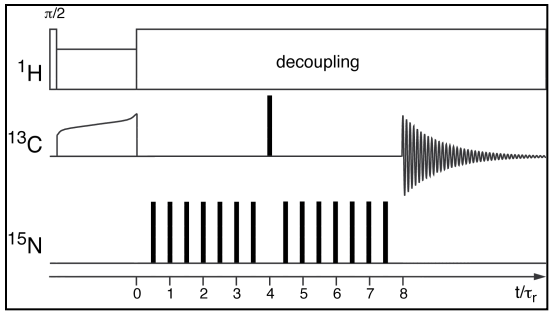
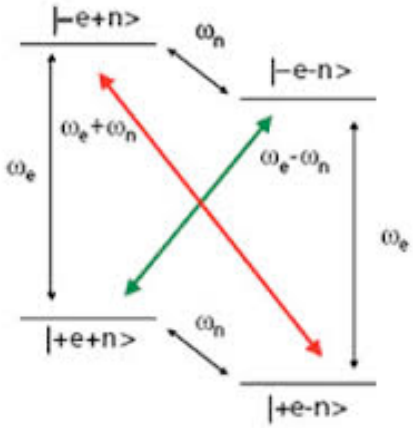
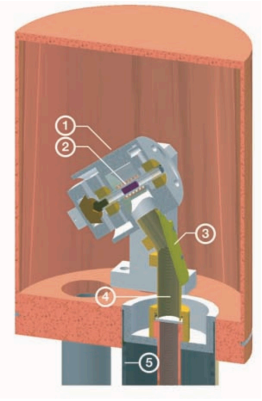
Instrumentation & developments in NMR, part 2: Solid-state NMR & dynamic nuclear polarization









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Oléron, June 2019

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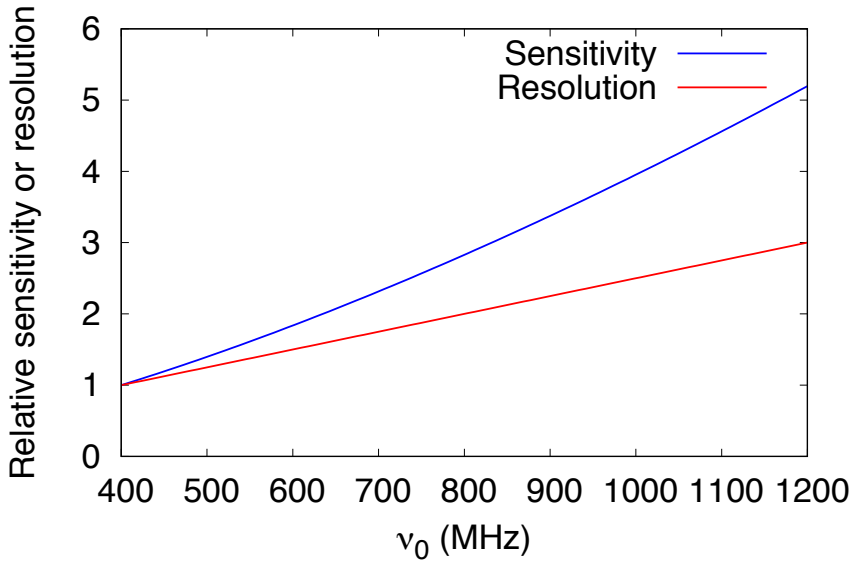
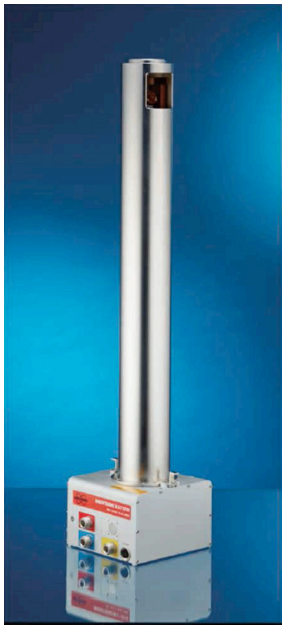
Higher B_0 – beneficial for solution and solid state!

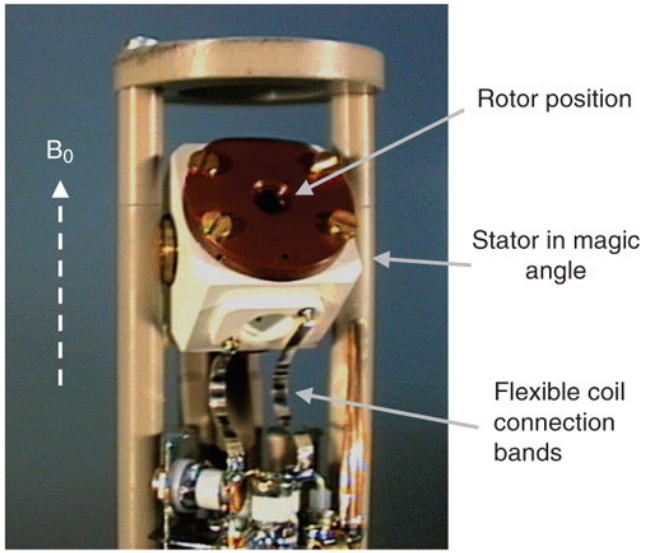
- Much progress in NMR concerns improvements in **resolution** and **sensitivity**
- Resolution ~ B_0, sensitivity ~ $B_0^{3/2}$
- Stronger magnets are thus always beneficial!
- but especially for solid-state NMR:
  - larger linewidths, lower sensitivity to begin with
  - thus, increased T_2 relaxation due to larger CSA or a potential TROSY optimum at higher B_0 are not a concern
  - strong RF fields available on solid-state probes can much more easily cover the increased frequency range at higher B_0

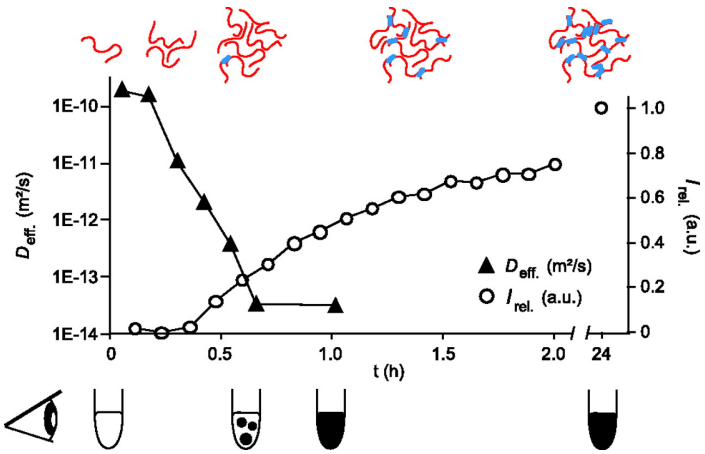


Equipment progress: solid vs. solution

- As in solution, solid-state NMR greatly profited from **higher B_0 fields** and **improved probehead** and **spectrometer** equipment
- However, **cryoprobes** (with samples at ambient temperatures) only just became available
- **No gradients** available either (except for prototypes)





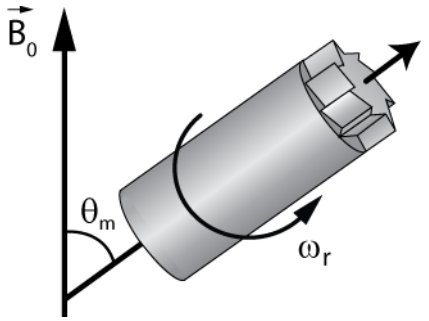


Beckonert et al., Nat Protoc 5, 1019, 2010
Ader et al., PNAS 107, 6281, 2010

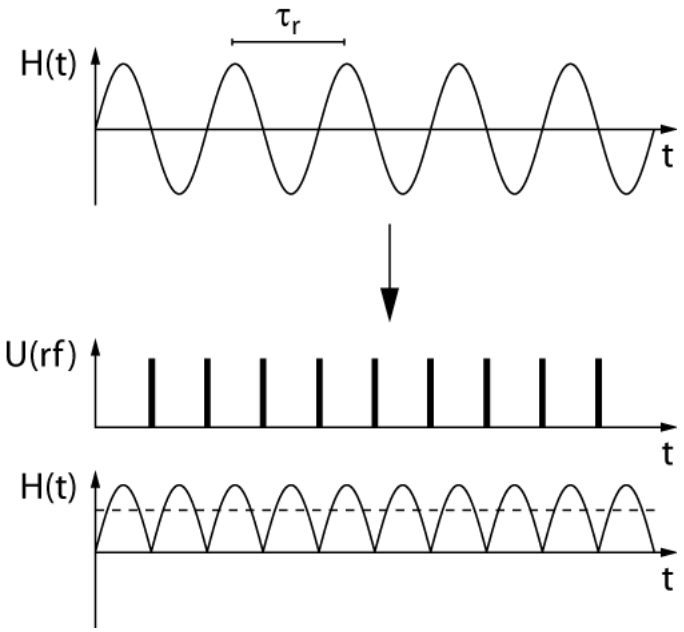
Progress in methodology: Recoupling pulse sequences

$$\hat{H} = \sum_{l=0}^{2} \hat{\boldsymbol{A}}_l \cdot \hat{\boldsymbol{T}}_l$$

space spin



- General idea:
  - Using tailored RF irradiation,
  - (re)activate / **recouple** the interaction of interest
  - by creating **interference** between **sample** (space) and **spin** (RF) **rotation**

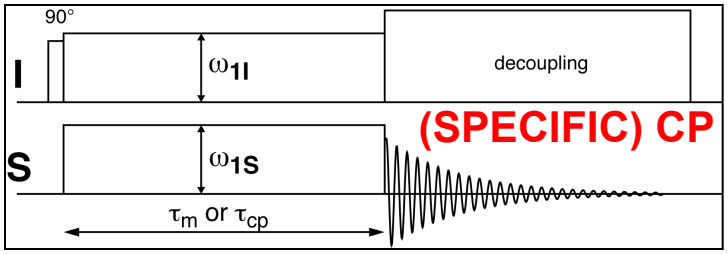


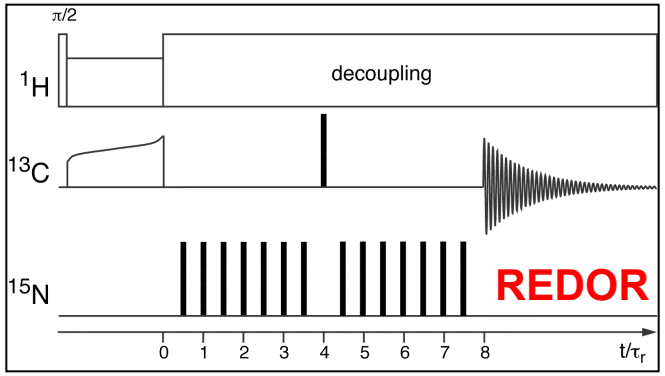
Progress in methodology: Recoupling pulse sequences

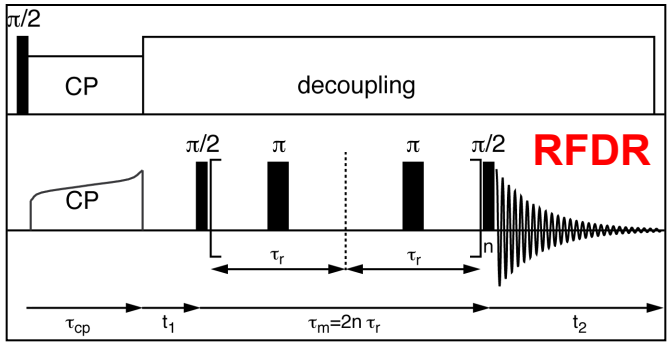
- Develpoment of a wide range of recoupling pulse sequences

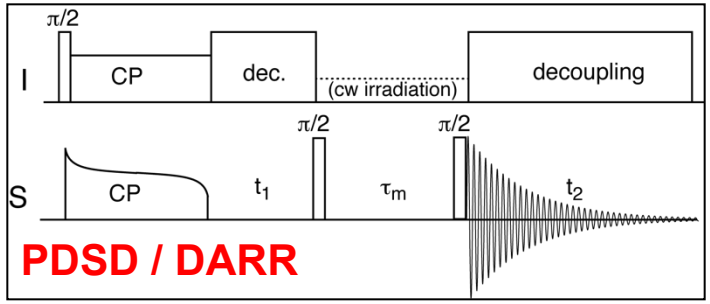
Choose your preference:

- dipolar coupling (→ distances) or chemical shift anisotropy (→ conformation)
- heteronuclear or homonuclear
- for shorter (filtering; dynamics) or longer distances (structure)
- broad-band or chemical-shift selective







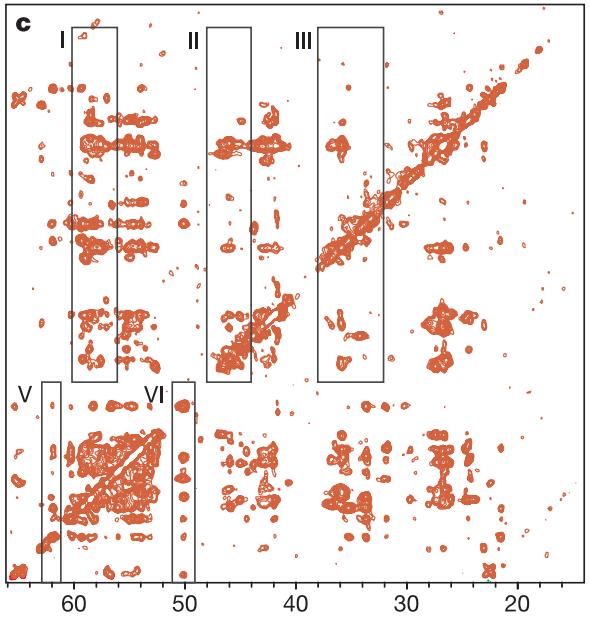


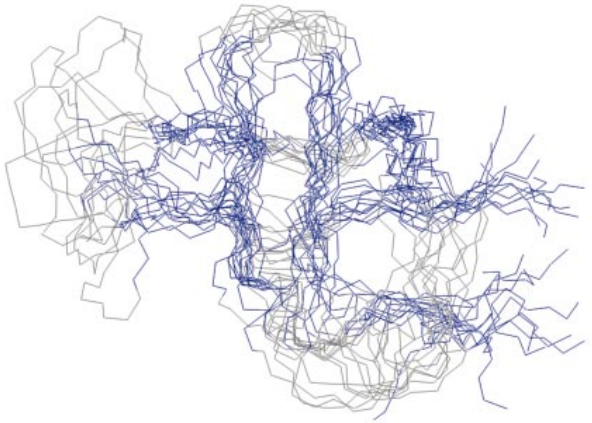
HORROR / DREAM, selective recoupling, R and C sequences, PAR / PAIN, ...

ssNMR pulse sequences and structural biology

Notably:

- Progress in **structural biology applications** was often made via rather **simple** experiment types (SPECIFIC-CP, spin diffusion)!
- For structural information:
  - **prefer many imprecise** over **a few precise** distance restraints
  - for long(er) distance restraints, require recoupling methods **less affected** by **dipolar truncation**
  - For structure calculation purposes, **complex relationship** between **interspin distance** and **signal intensity** in solid-state NMR is problematic!

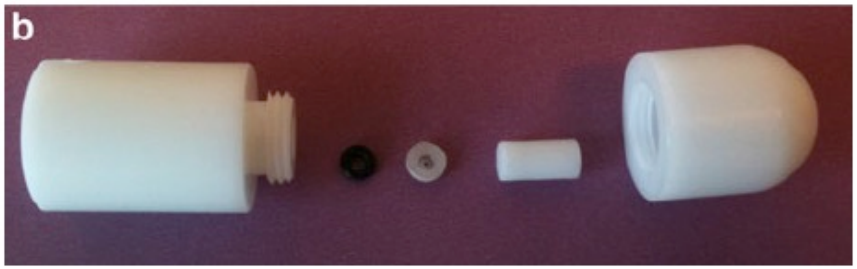


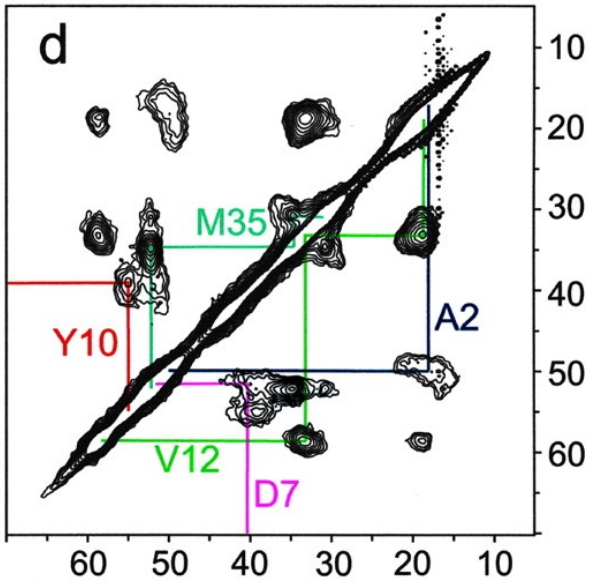


Castellani et al., Nature 420, 98, 2002

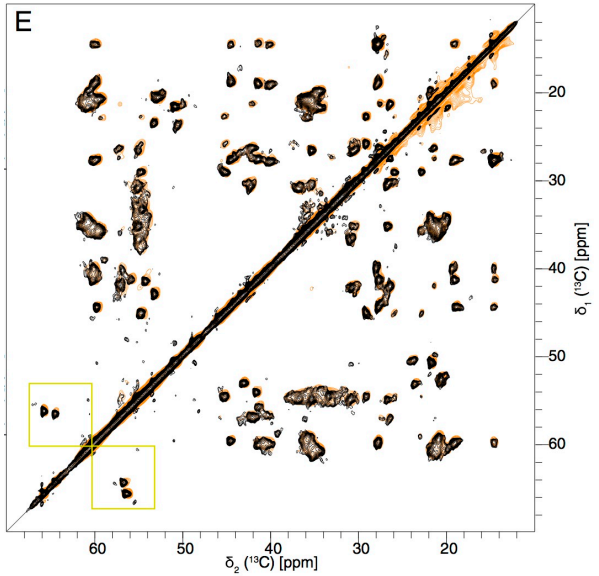
The importance of sample preparation

- **Avoid lyophilization** or (random) **precipitation** for sample preparation!
  - sufficient **water content** and
  - **local, short-range order** are important!
- Soluble proteins: **microcrystals**
- Fibrils, membrane proteins: **pelleting by ultracentrifugation** (directly into NMR rotor)
- **Screen** for optimal conditions giving best spectra! (e.g. avoiding fibril polymorphism)



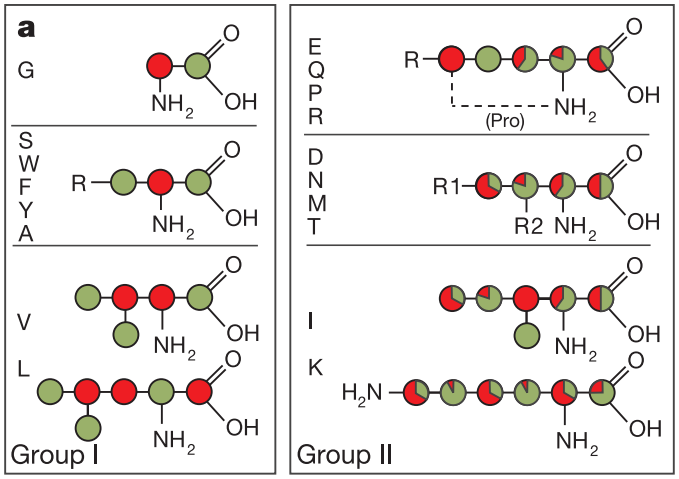


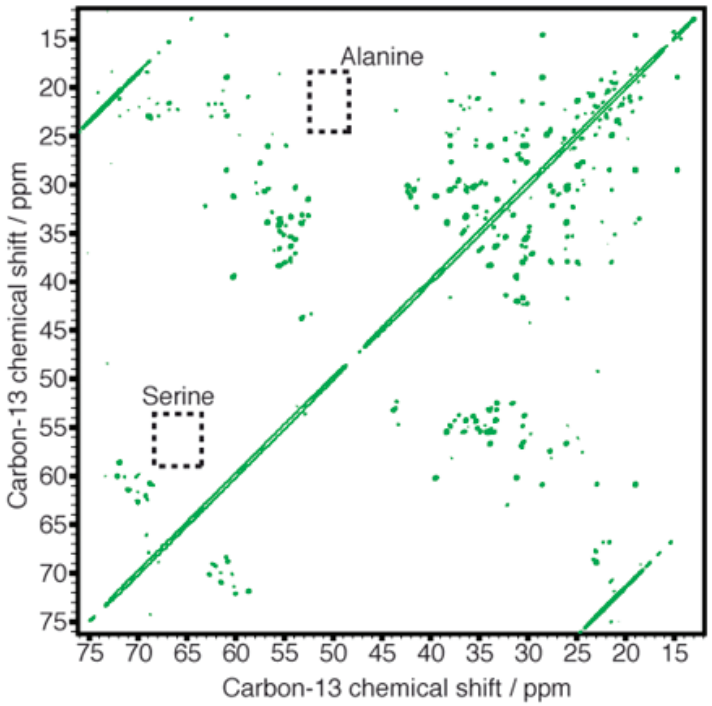
vs.



Petkova et al., PNAS 99, 16742, 2002
Aulikki Wälti et al, PNAS 2016
Bertini et al., J Biomol NMR 54, 123, 2012

Isotope labeling: Selective ^{13}C incorporation

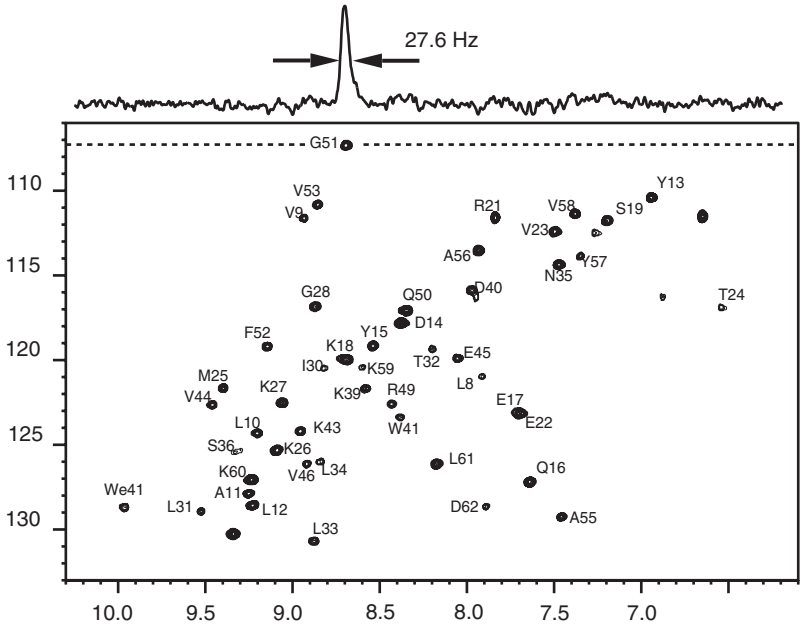




- e.g. using [1,3-^{13}C]- / [2-^{13}C]-glycerol, [1-^{13}C]- / [2-^{13}C]-glucose as carbon sources
- **Reduce** cross-peak **ambiguity**
- **Increase resolution** (remove broadening by J coupling)
- Can mix molecules with **complementary labeling** patterns to obtain **inter-molecular** correlations (e.g. in fibrils)!

Castellani et al., Nature 420, 98, 2002
Loquet et al., JACS 132, 15164, 2010

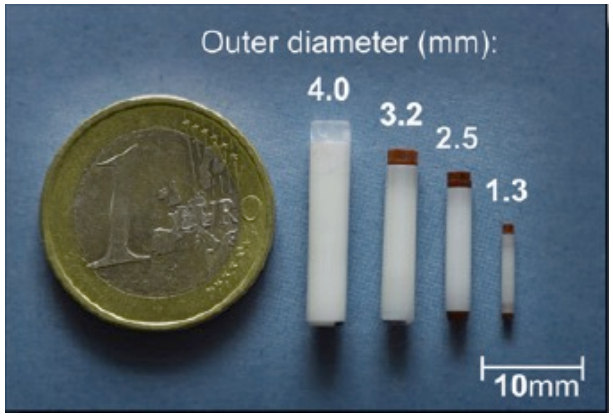
Isotope labeling: Deuteration

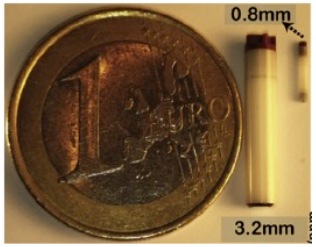


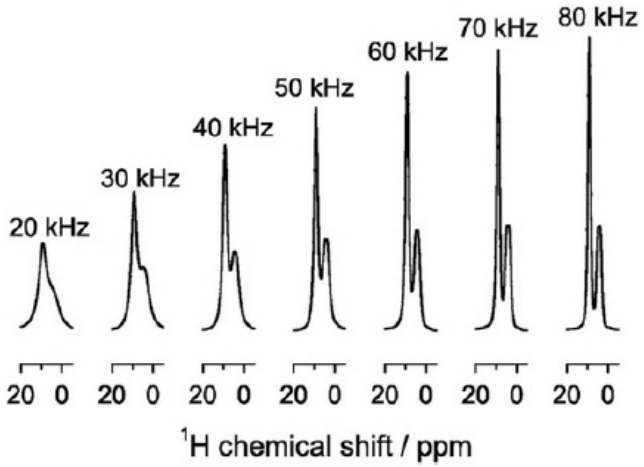
Chevelkov et al., Angew Chem Int Ed 45, 3878, 2006

- **(Per)Deuteration** (possibly also with limited ^{1}H back-exchange) yields “solution-like” spectra for solid proteins even at low MAS speeds!
- Dilution of strongly coupled ^{1}H network essential
- **^{1}H detection** (sensitivity increase!) becomes possible

MAS: fast, very fast, ultrafast, warp speed



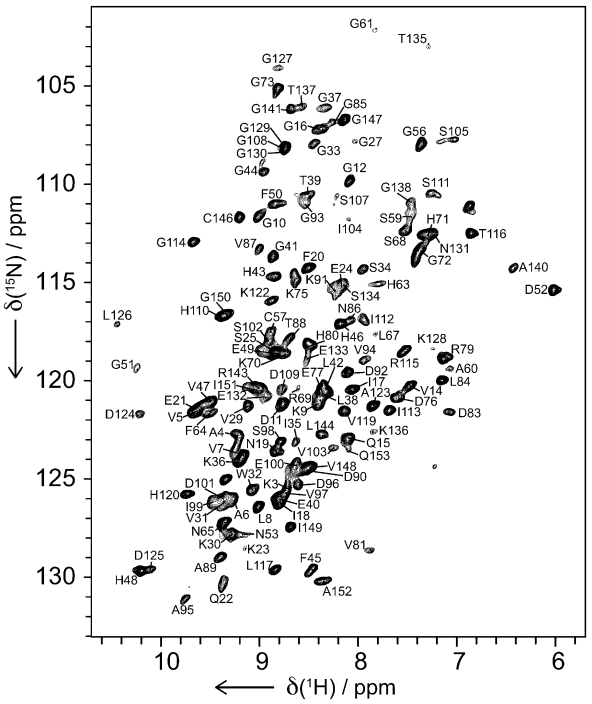




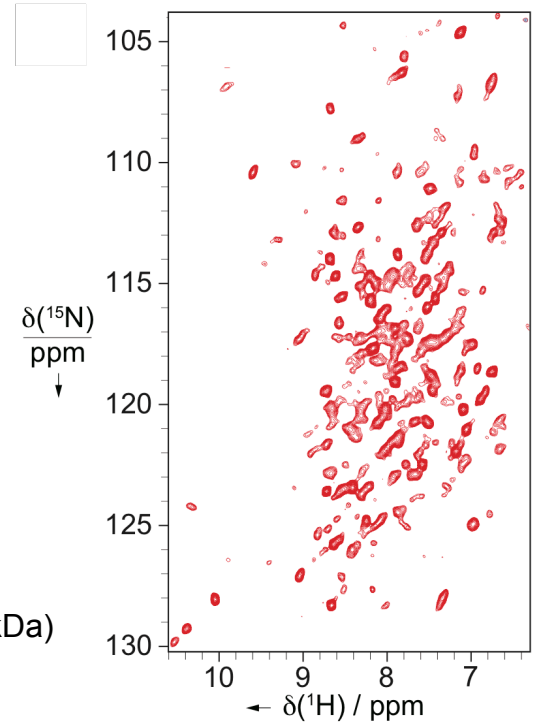
- Smaller rotors allow for faster MAS speeds, currently up to ~ 110 kHz
- **New "regime"** for solid-state NMR: different recoupling methods, new low-power pulse sequences also permitting **faster repetition**
- **Smaller sample amounts** required!
- Longer coherence lifetimes → **higher-dimensional spectra**, J transfers
- Above 100 kHz, deuteration no longer required for high-resolution ^{1}H-detected spectra!

Demers et al., Solid State Nucl Magn Reson 40, 101, 2011
Böckmann et al., J Magn Reson 253, 71, 2015

Deuteration and fast MAS combined



SOD
(2 x 16 kDa)



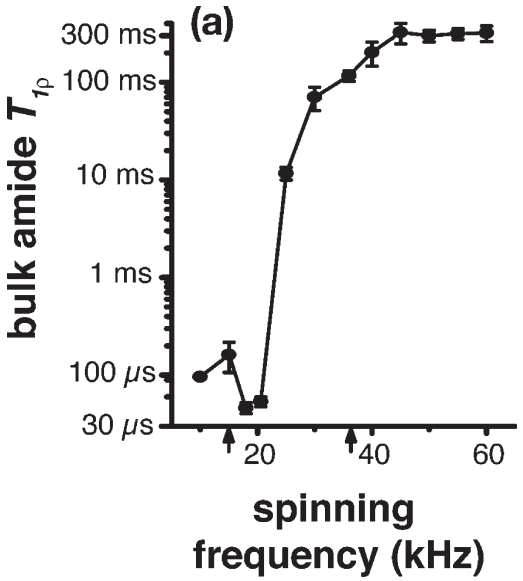
MeV capsids
(n x 43.5 kDa; MDa-size assembly!)

- Combine (per)deuteration and high MAS speeds for best results
- As opposed to solution-state NMR, **linewidth** does **not depend** on **molecular size**, i.e. can in principle access arbitrarily large molecules!

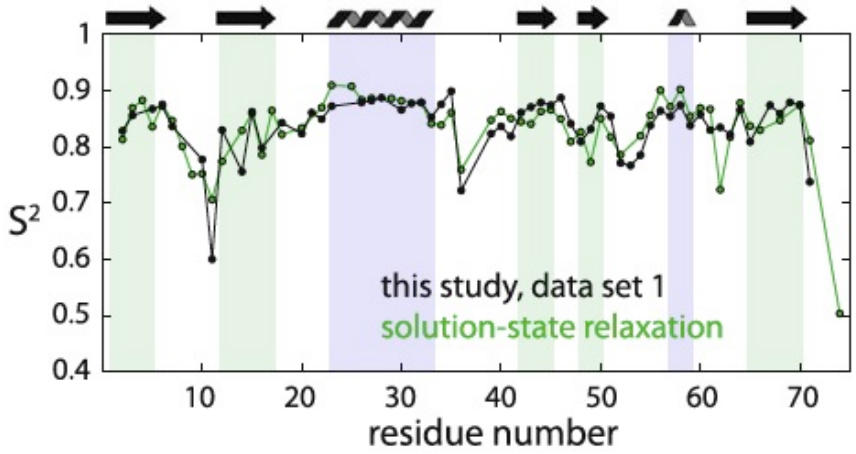
Knight et al., Angew Chem Int Ed 50, 11697, 2011; Schneider et al., unpublished

Access to dynamics via fast MAS

- Site-specific **relaxation** measurements for **dynamics** studies, previously inaccessible to solid-state NMR, become possible
  → measure motion on **ns-to-ms time scales**, which are difficult to access for solution-state NMR due to overall molecular tumbling!

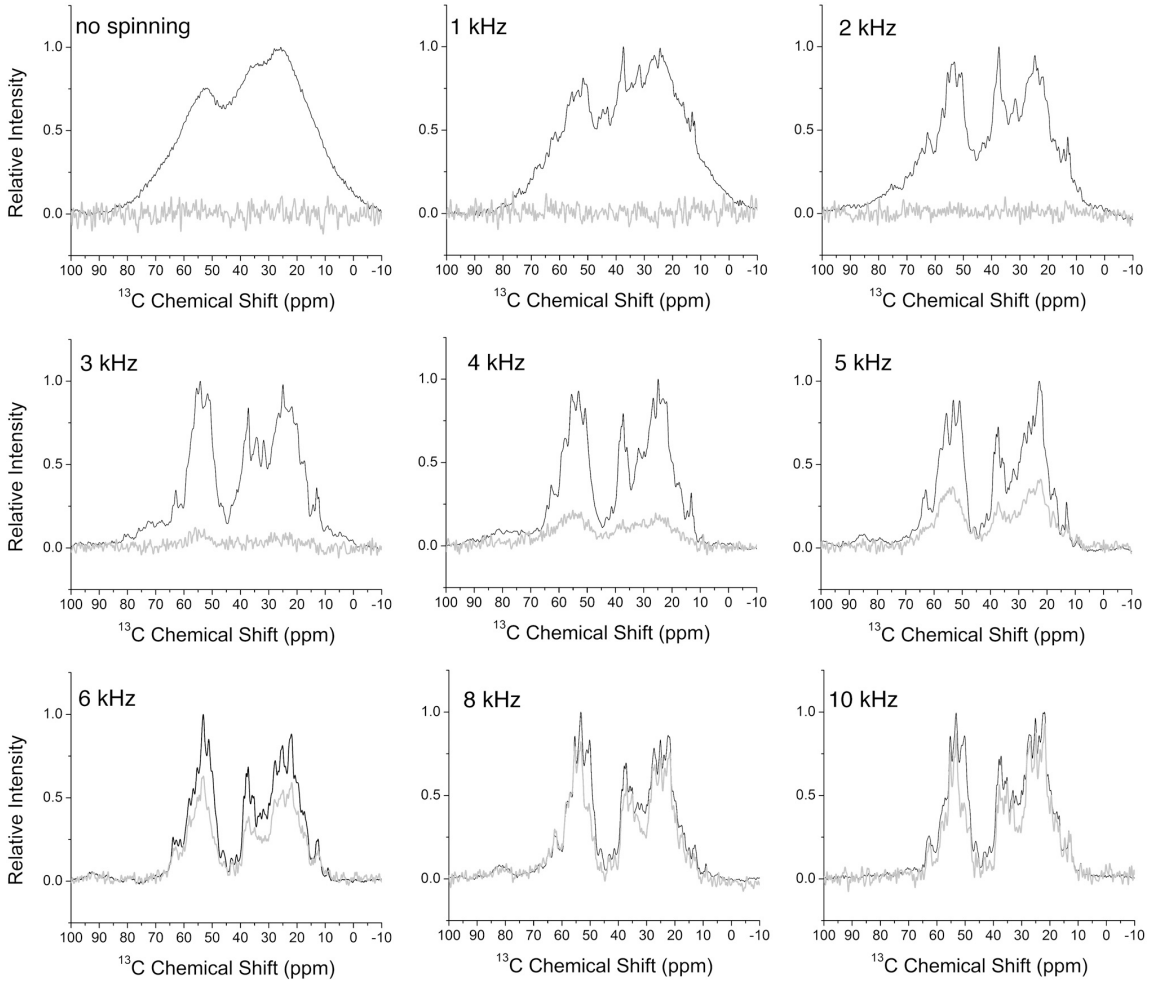


Lewandowski et al., JACS 133, 16762, 2011



Haller & Schanda, J Biomol NMR 57, 263, 2013

Sedimentation from solution at fast MAS



- Centrifugal forces during MAS can be an order of magnitude larger than in ultracentrifuges

⇒ Depending on molecular size and spinning speed, proteins can be **reversibly sedimented from solution in the MAS rotor** for solid-state NMR experiments!

Bertini et al., PNAS 108, 10396, 2011

Sensitivity

- Defined as **signal to noise** ratio per **unit acquisition time**:

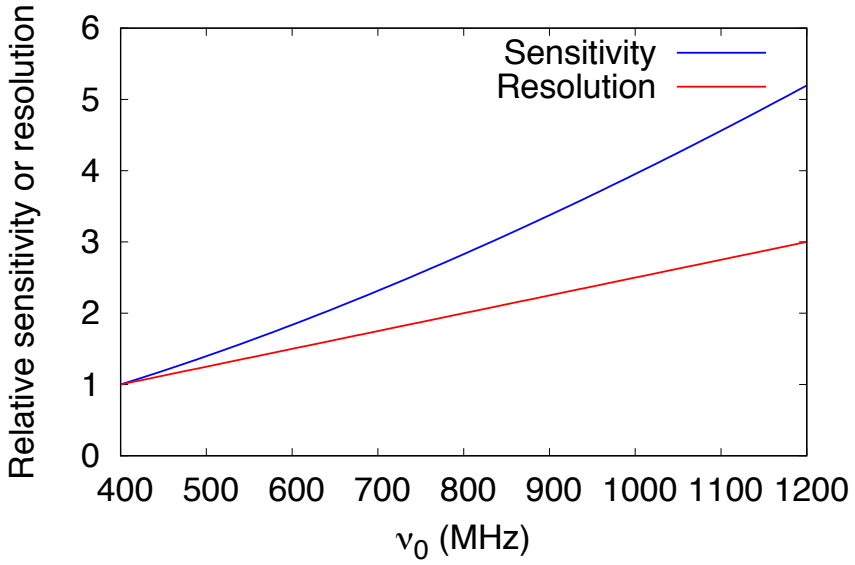
$$\mathcal{S} \sim N\, Q\, \gamma_e\, \gamma_d^{3/2}\, B_0^{3/2}\, T_2^{1/2}\, T^{-3/2}\, (t_{\mathrm{aq}}/T_c)^{1/2}$$

  - N: number of spins
  - Q: probe coil quality factor
  - γ_e: gyromagnetic ratio of *excited* nucleus
  - γ_d: gyromagnetic ratio of *detected* nucleus
  - T_2: transverse relaxation time
  - T: absolute temperature
  - t_{aq}: acquisition sampling time
  - T_c: total time between acquisitions (t_{aq} + recycle delay)

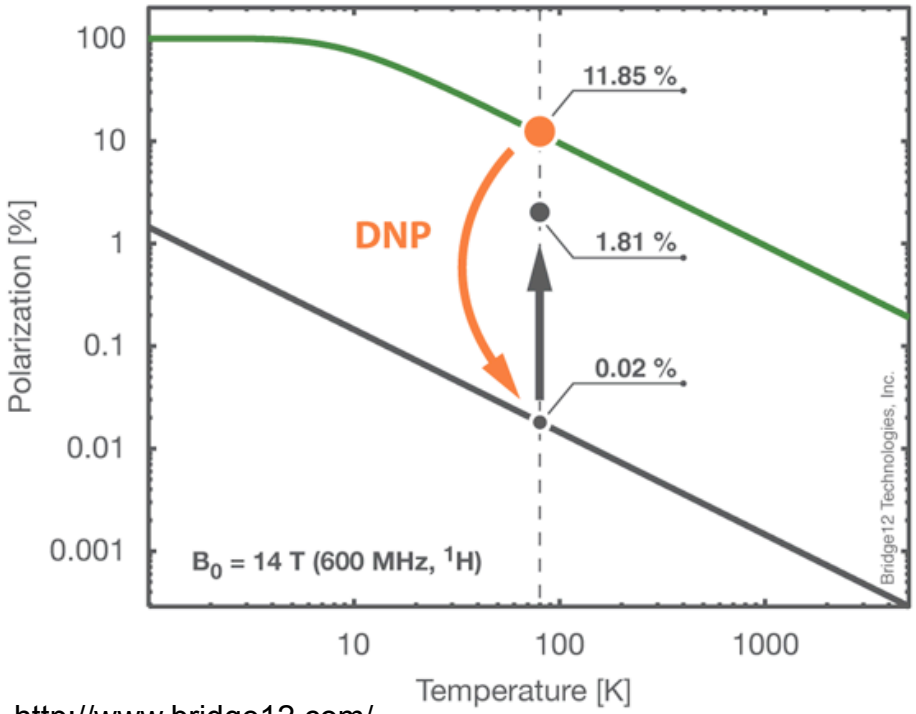
Sensitivity

$$\mathcal{S} \sim N\, Q\, \gamma_e\, \gamma_d^{3/2}\, B_0^{3/2}\, T_2^{1/2}\, T^{-3/2}\, (t_{\mathrm{aq}}/T_c)^{1/2}$$

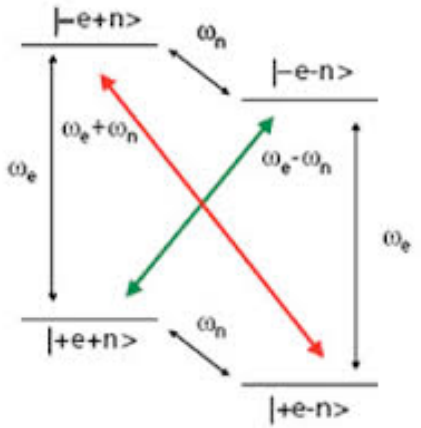
- However, improvements in sensitivity by increasing B_0 are rather incremental... and extremely expensive!
- Is there another way to increase sensitivity, maybe more drastically?



Dynamic Nuclear Polarization (DNP)

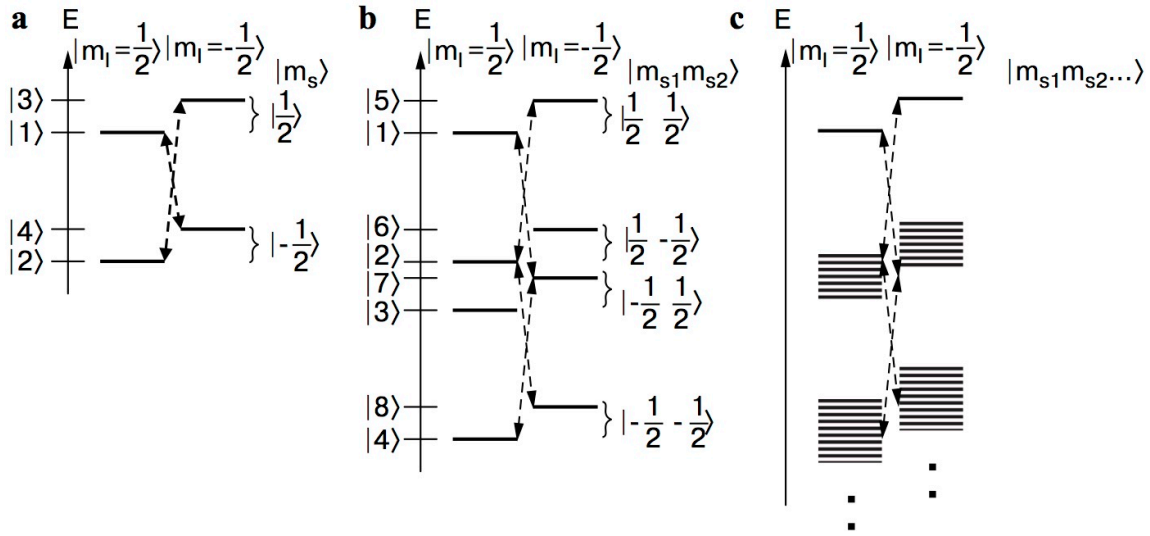


http://www.bridge12.com/
what-is-dynamic-nuclear-polarization-dnp-nmr/



- Transfer the **much larger electron spin polarization** to **nuclei** to enhance their signal!
- Theoretical enhancement factor of **~660** (ratio of electron and proton gyromagnetic ratios)!
- Principle: **saturate transition** of an **unpaired electron (radical)** via **microwave** irradiation; e^- – *n* polarization transfer via **hyperfine coupling**
- first proposed by **A. Overhauser** (yes, that one!) in 1953

DNP: flip-flop, flip-flip, flip-flop-flip

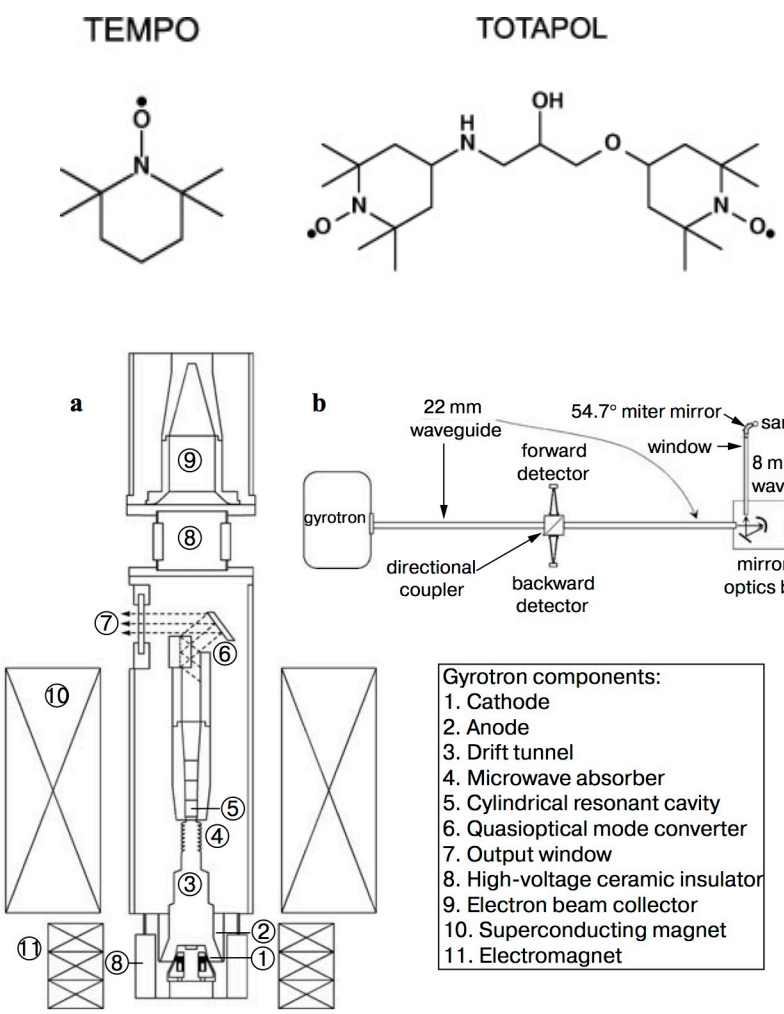


- Originally proposed mechanism is a **cross-relaxation** mechanism and relies on **dynamics** (hence the name!)
  - inefficient for larger molecules / at high field
- perform DNP in the **solid state** at **cryogenic temperatures** (≤ 100 K) to
  - transfer via the more efficient **cross effect** (or **solid effect** / **thermal mixing**)
  - have **much longer electron** (and nuclear) **T_1 relaxation times**
  - (as well as larger initial polarization of both)

Barnes et al., Appl Magn Reson 34, 237, 2008

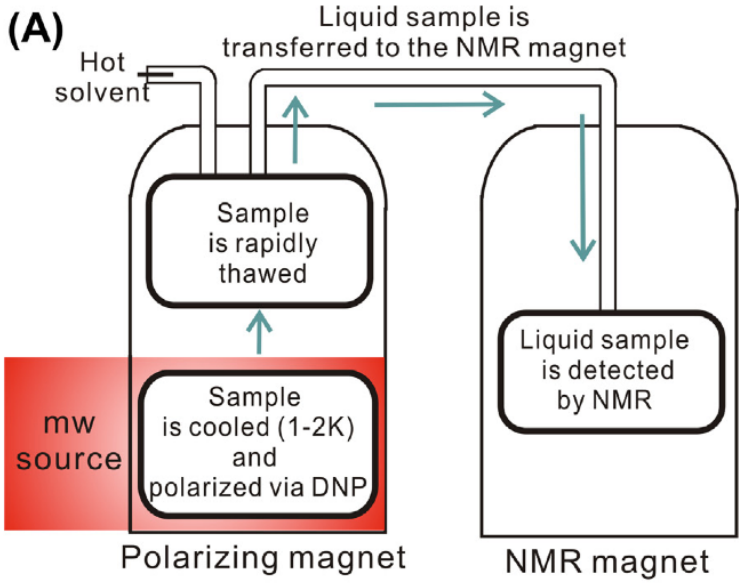
DNP: Howto

- Dissolve sample in **glycerol / D_2O / H_2O** (60/30/10) doped with organic **(bi)radicals** (~ mM)
  - glass-forming matrix ensuring homogeneous radical distribution and absence of water crystals in the frozen state
- Polarize at ≤ 100 K using high-frequency **microwave** radiation at several W of power (→ **gyrotron** source; e.g. 263 GHz for DNP at 9.4 T)
  - ideally at **low B_0 field** since efficiency of cross / solid effect drops as B_0^{-1} / B_0^{-2}, respectively!
  - Polarization **spreads** in sample via ^1H **spin diffusion**!
- ... and then?



Akbey & Oschkinat, J Magn Reson 269, 213, 2016
Barnes et al., Appl Magn Reson 34, 237, 2008

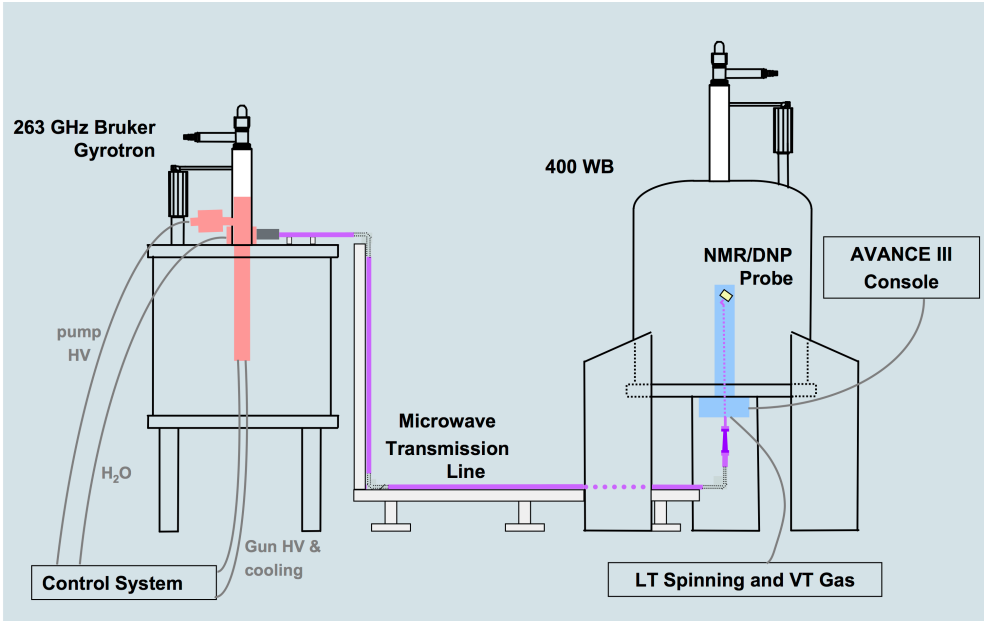
DNP for solution NMR?

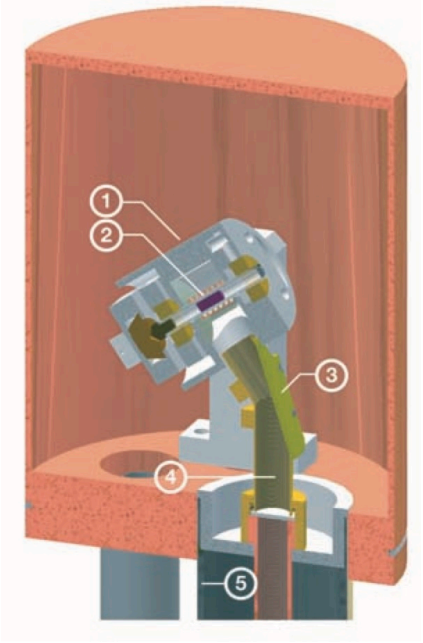


- **Dissolution DNP**: After hyperpolarization, **rapidly dissolve** sample (hot buffer!), **transfer** to NMR magnet, detect!
  - one-shot experiment!
- Alternatives which can **repeat** the experiment:
  - **temperature-jump DNP (**rapidly **melt** sample via **laser**)
  - **shuttle DNP** (in solution, use Overhauser effect at low field)
- Other hyperpolarization techniques accessible in solution exist:
  - parahydrogen induced polarization; optical pumping; (photo-)CIDNP

Ardenkjær-Larsen et al. PNAS 100, 10158, 2003

Solid-state DNP NMR

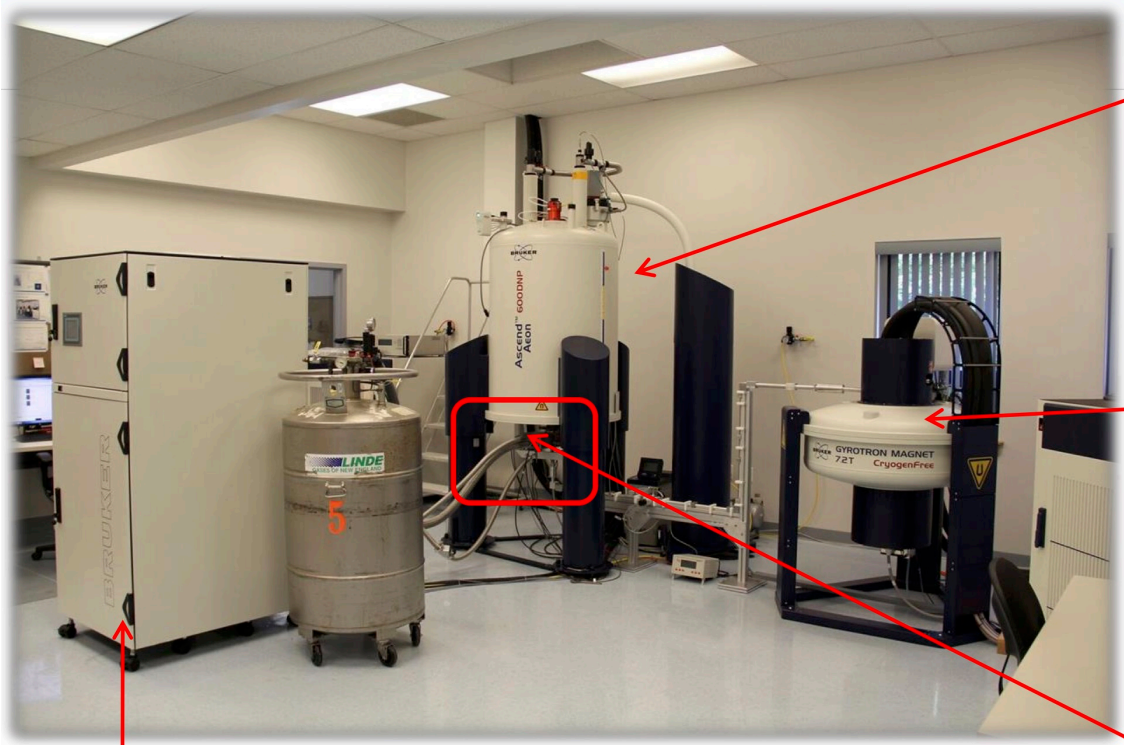




- Remain in solid state at cryogenic temperatures for better polarization and relaxation properties!
- Polarize "**in situ**" in the NMR magnet
  - use the cross effect and optimized biradicals to still obtain reasonable enhancements despite field dependence
  - waveguides from gyrotron to sample in the NMR probe
- Perform **magic angle spinning** to improve resolution
  - need **cryogenic N_2** (or He!) gas for spinning!
  - rotors "translucent" to microwaves (sapphire!)

Bruker Biospin
Barnes et al., Appl Magn Reson 34, 237, 2008

Solid-state DNP NMR setup

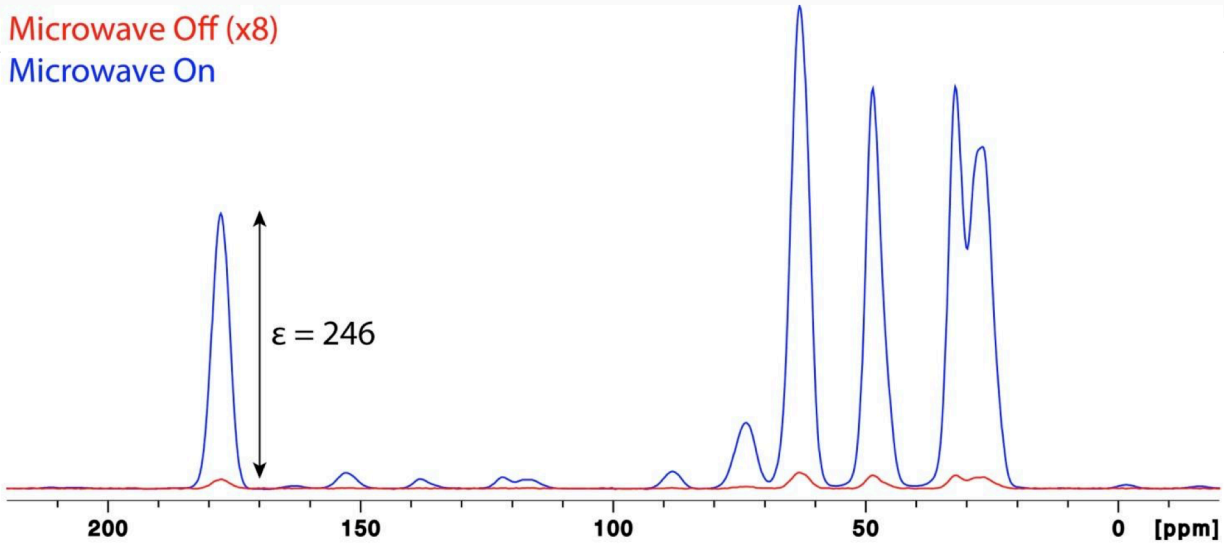


- NMR Magnet
- Microwave source: Gyrotron
- DNP Probe
- Low-temperature Magic Angle Spinning (LTMAS) Cabinet

- Magnet setup includes a supplementary **supraconducting sweep coil** to **change the B_0 field** (!) to the optimal resonance condition for the radical used

Bruker Biospin (https://www.bruker.com/service/education-training/webinars/nmr-webinars/introduction-to-solid-state-dnp-nmr.html)

Result: Enhancement!



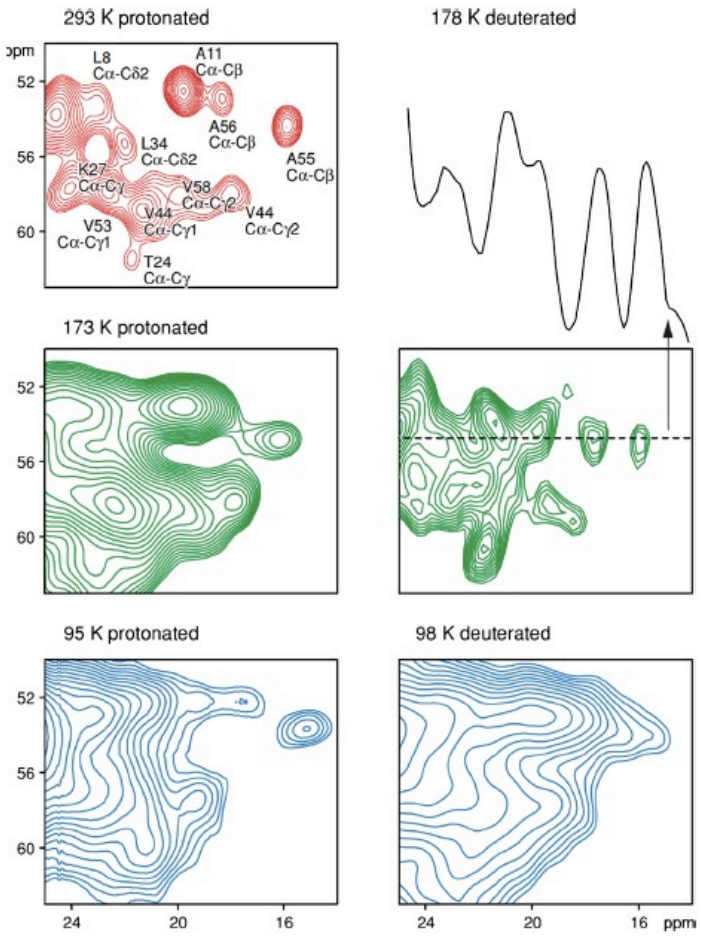
400 MHz / 263 GHz DNP: 12 kHz MAS
0.25 M U-^{13}C,^{15}N-Proline with 10 mM AMUPol
in glycerol-d_8/D_2O/H_2O 6:3:1 mixture

- Enhancement ~240 at 400 MHz, ~130 at 600 MHz!
- An enhancement factor *x* in sensitivity permits a reduction in experiment time by a factor of x^2 !
- Perform **"normal" ssNMR experiments** after initial enhanced excitation

Bruker Biospin (https://www.bruker.com/service/education-training/webinars/nmr-webinars/introduction-to-solid-state-dnp-nmr.html)

Challenges and problems

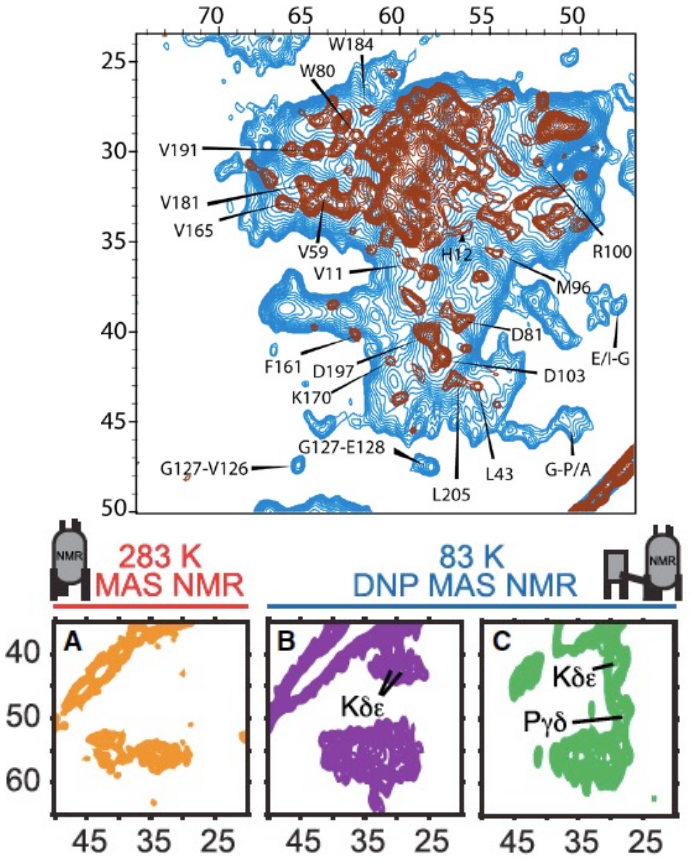
- Actual enhancement factor can **vary widely** depending on the system studied
  - **optimization** necessary
  - influence of **deuteration level**, MAS, (bi)radical, ...
- Large inhomogeneous **broadening** at low temperature!
  - depends on **heterogeneity** of sample (and deuteration!)
- Broadening due to radical-induced **paramagnetic relaxation** (-> PRE)
- **Absolute signal enhancement** (considering reduced amount of sample, different broadening effects, ...) may be **way less** than the conventional “MW on − MW off” enhancement!



Akbey et al., Appl Magn Reson 43, 81, 2012

Applications in structural biology

- Reasonable resolution obtained for some **protein fibrils** (e.g. Aβ40)
  - apparently remain quite **homogeneous** when frozen out!
  - collection of through-space restraints facilitated by S/N gain
- Study a low abundance target molecule in a complex environment
  - **bacteriorhodopsin** photocycle (^{15}N-Lys labeling; trap intermediates at low temperature)
  - nascent chain in **ribosome**, DNA in **bacteriophage**
  - protein in **cells,** cell walls, cellular lysates (e.g. via radical **ligands**!)
- Observe (parts of) proteins or RNA that are **too mobile** at room temperature
  - directly visualize conformational sampling of IDPs



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