NMR of large protein systems:
Solid state and dynamic nuclear polarization

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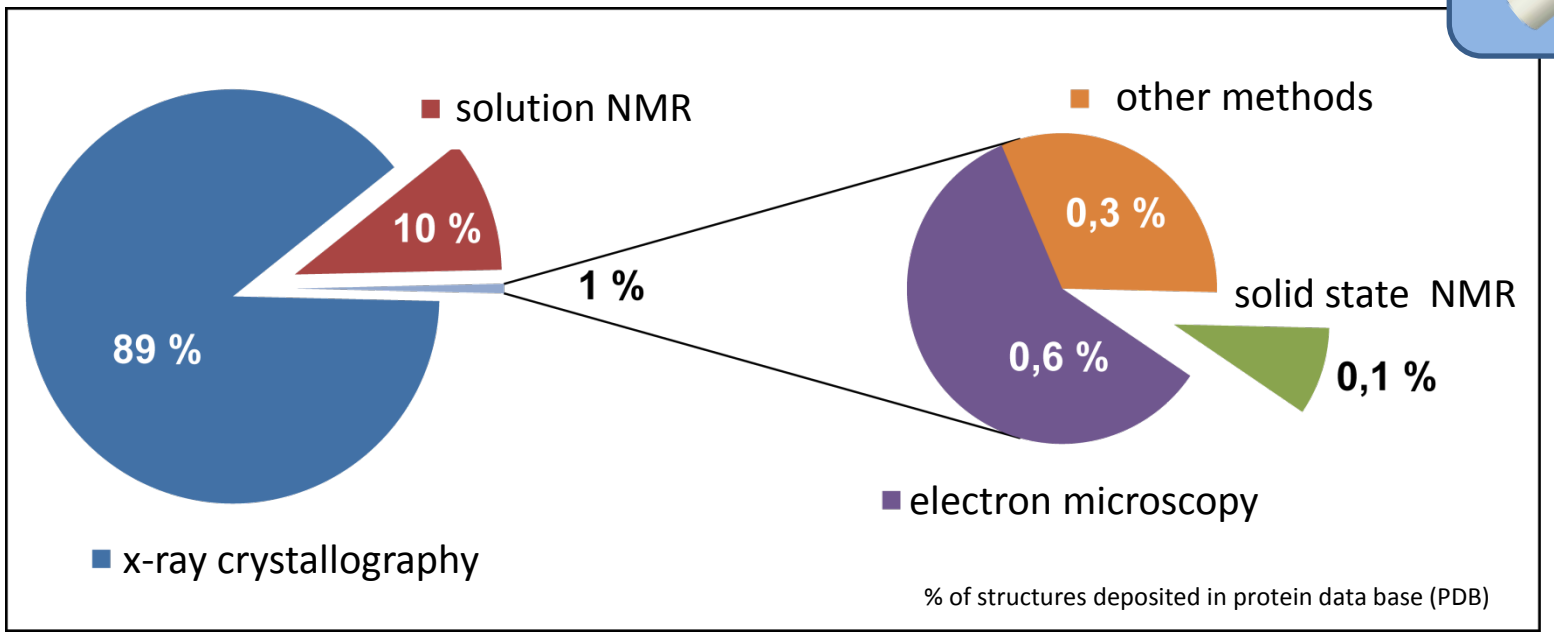
Freie Universität Berlin

BioSupraMol

Core Facility

The Aim of the Game



<u>solution-state NMR</u>

requires rapid reorientation of soluble biomolecules

<u>X-ray crystallography</u>

requires high-quality single crystal…

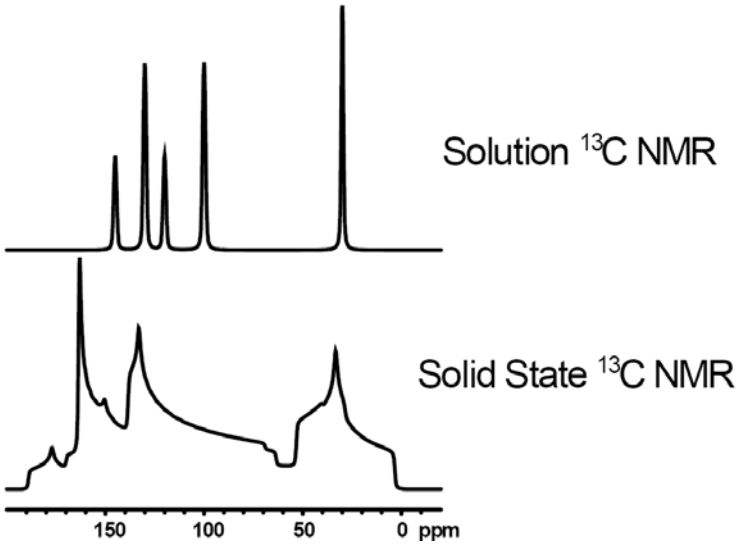
<u>solid-state NMR</u>

no need for large well-ordered crystals or highly-purified proteins

works for immobilised proteins, no inherent limitation on complex size

Anisotropic effects in solid state NMR

why is anisotropy difficult?



liquids: rapid random tumbling averages anisotropic chemical shifts and couplings

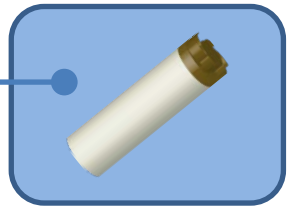
→ small lines, high signal!

solids: no tumbling, interactions depend on orientation of the single molecules (anisotropic)

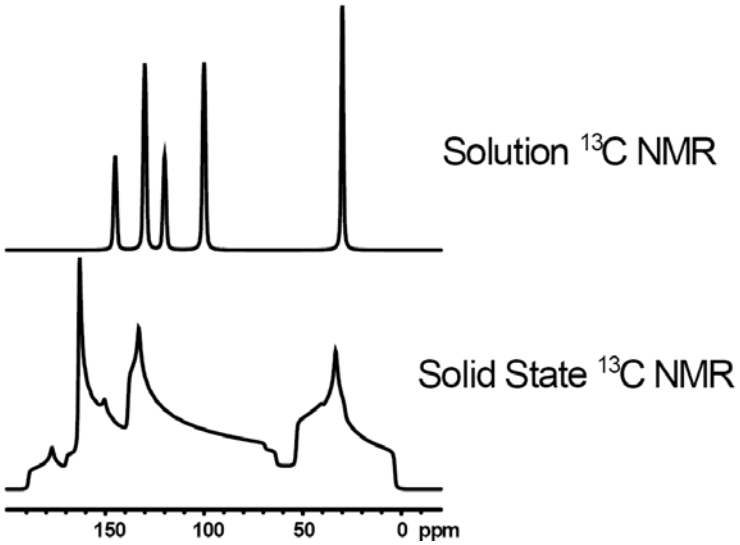
→ very broad lines, low signal!

anisotropic interactions lead to massive line broadening!

Anisotropic effects in solid state NMR



why is anisotropy difficult?



anisotropy of

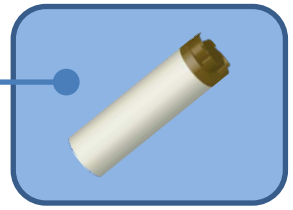
→ heteronuclear dipolar interaction

→ homonuclear dipolar interactions

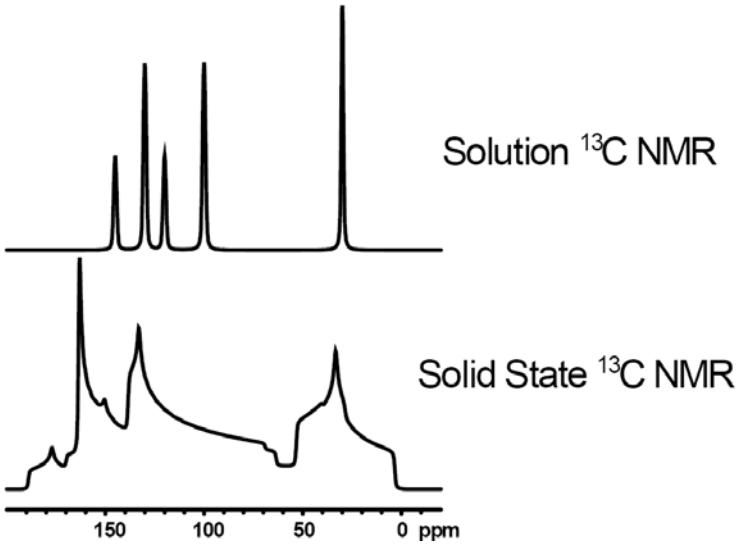
→ chemical shift anisotropy

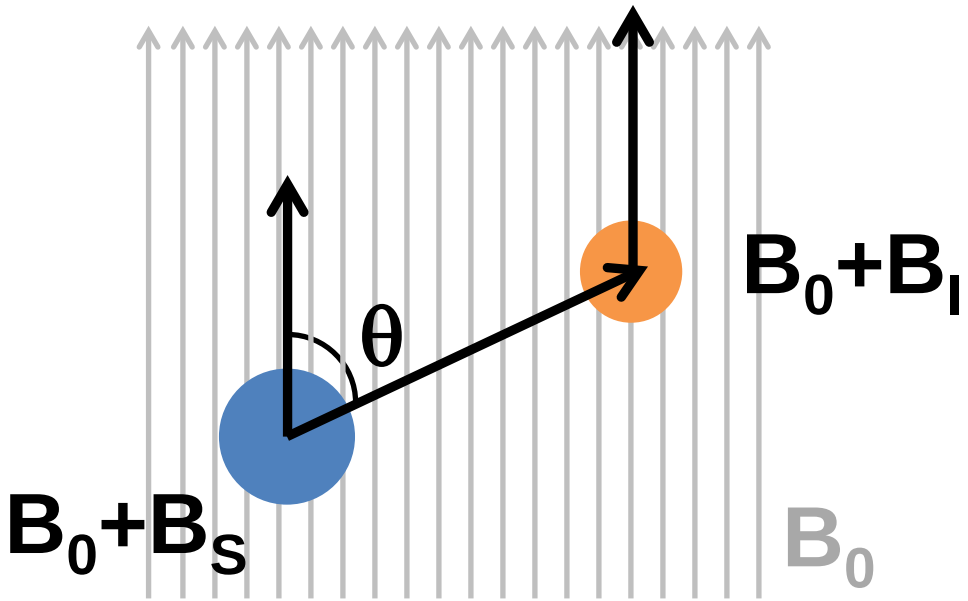
anisotropic interactions lead to massive line broadening!

Anisotropic effects in solid state NMR



→ **heteronuclear dipolar interaction**



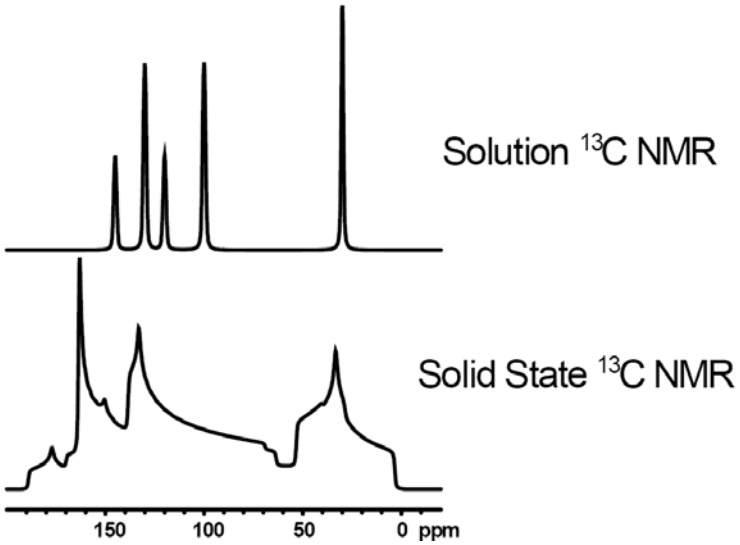


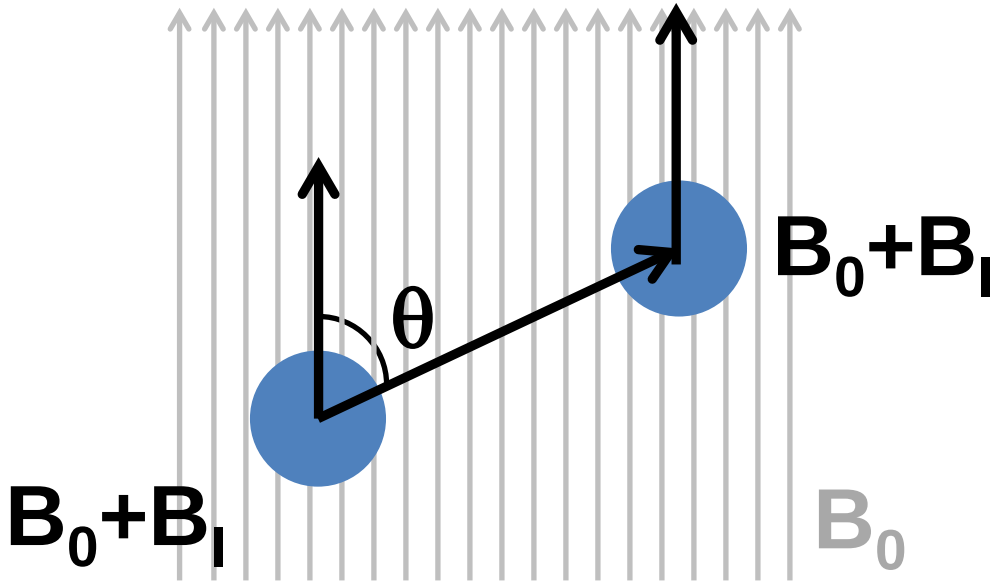
$$H_{IS} = -d\left(3\cos^2\theta - 1\right)I_z S_z$$

$$d = \left(\frac{\mu_0}{4\pi}\right)\frac{\hbar\gamma_I\gamma_S}{r_{IS}^3}$$

Anisotropic effects in solid state NMR

→ **homonuclear dipolar interactions**



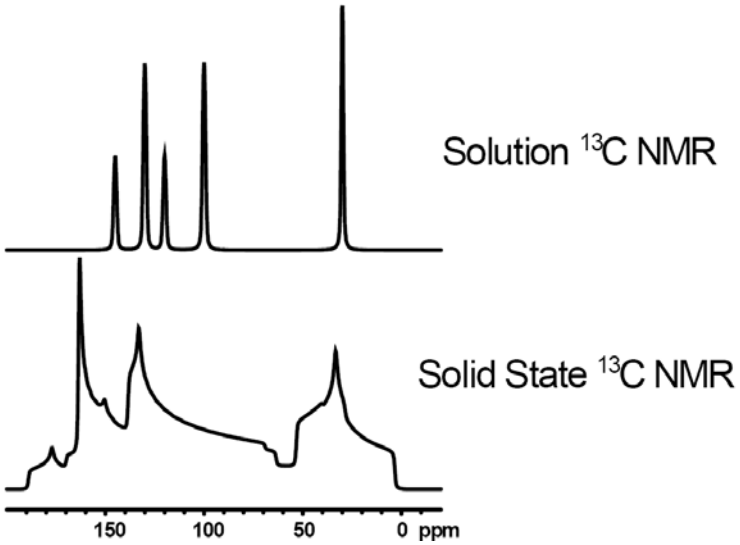


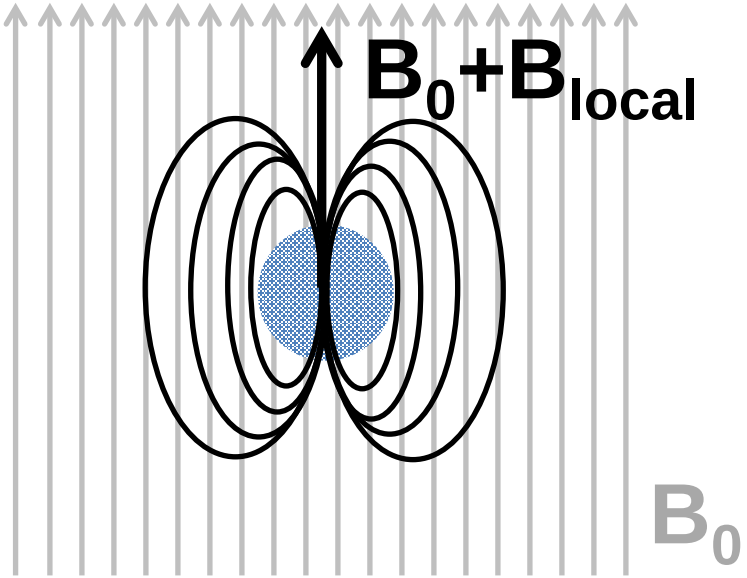
$$H_{II} = -d\,½\left(3\cos^2\theta - 1\right)\left[2I_{1z}I_{2z} - ½\left(I_1^+I_2^- + I_1^-I_2^+\right)\right]$$

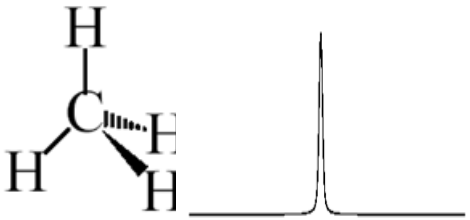
„flip-flop-term“

Anisotropic effects in solid state NMR

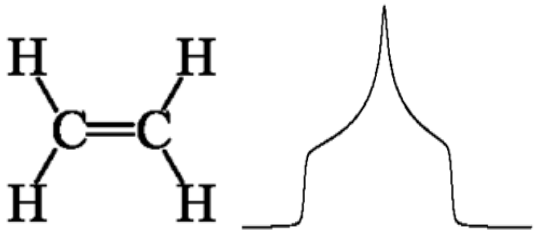
→ Chemical Shift Anisotropy



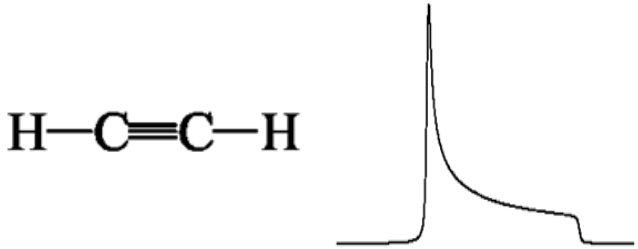




spherical symmetry



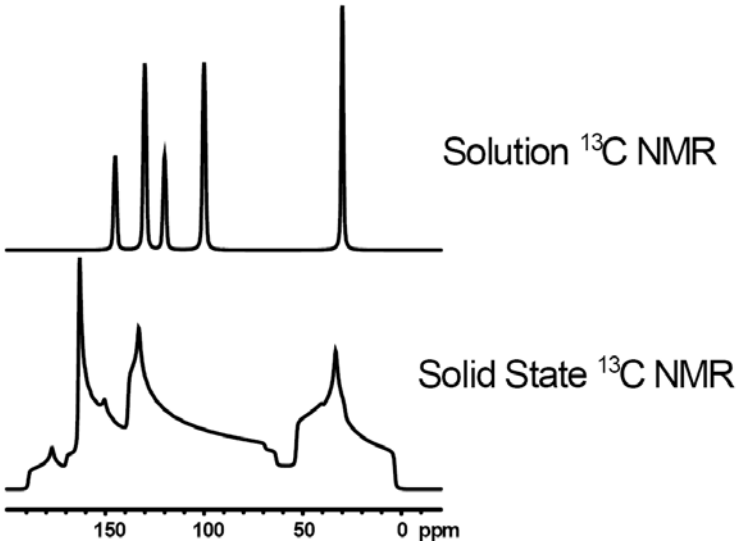
non-axial symmetry

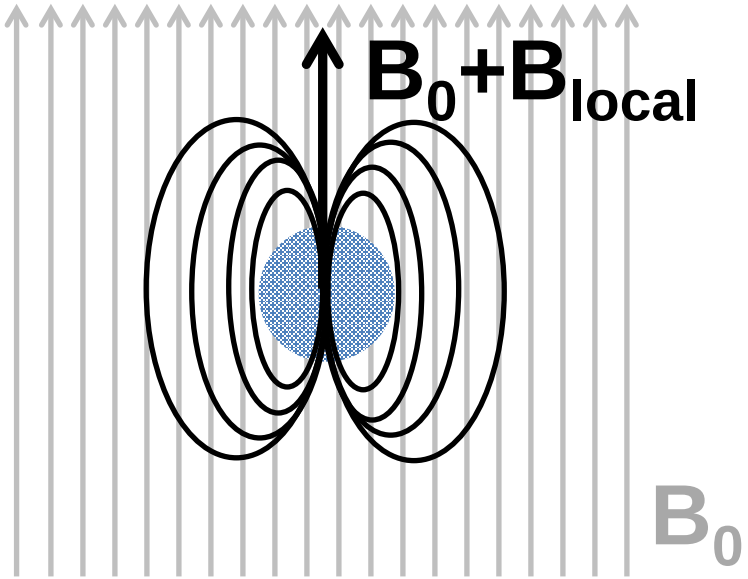


axial symmetry

Anisotropic effects in solid state NMR

→ Chemical Shift Anisotropy





$$H_{CS} = \gamma B_0 I_z \left[\delta_{iso} + \tfrac{1}{2} \delta_{aniso} \left(3\cos^2 \theta - 1 \right) \right]$$

how to get rid of it?

$$H_{IS} = -d\left(3\cos^2\theta - 1\right)I_z S_z$$

$$H_{II} = -d\,\tfrac{1}{2}\left(3\cos^2\theta - 1\right)\left[2I_{1z}I_{2z} - \tfrac{1}{2}\left(I_1^+ I_2^- + I_1^- I_2^+\right)\right]$$

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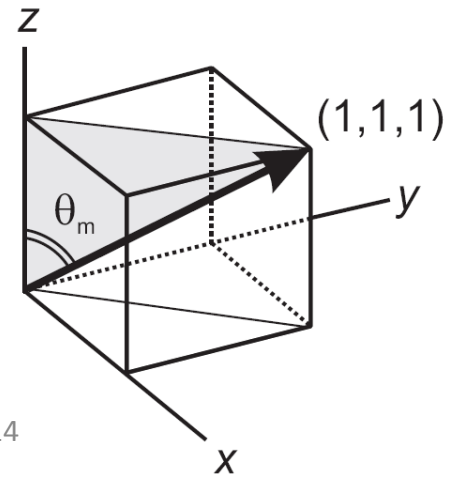

$3\cos^2 54.7 - 1 = 0$

54.7 = the magic angle!

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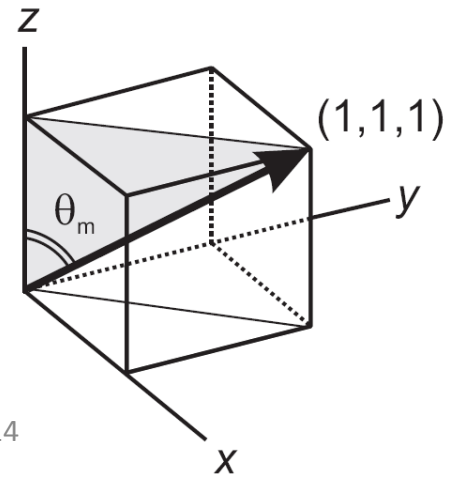

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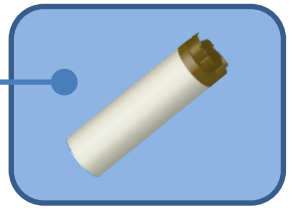

how to get rid of it?

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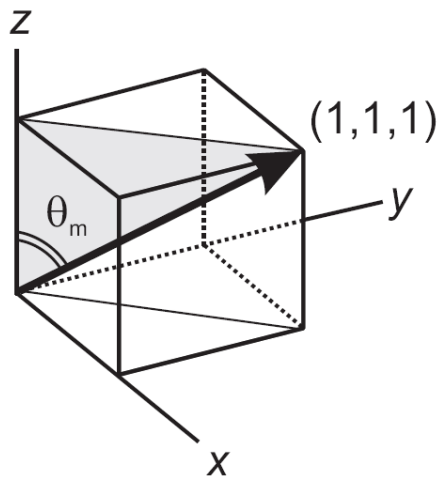

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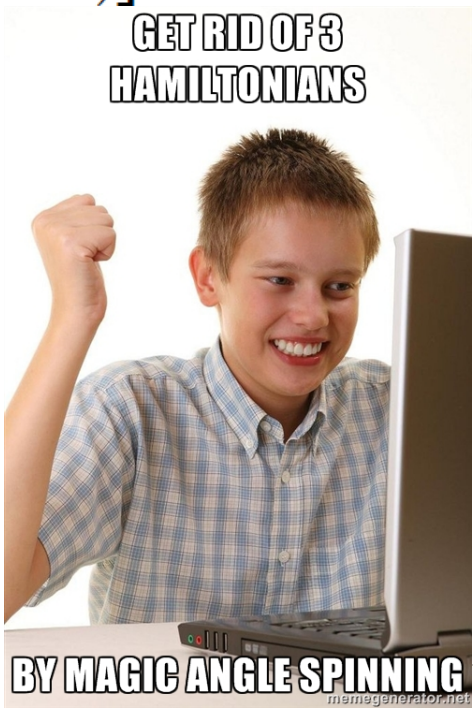
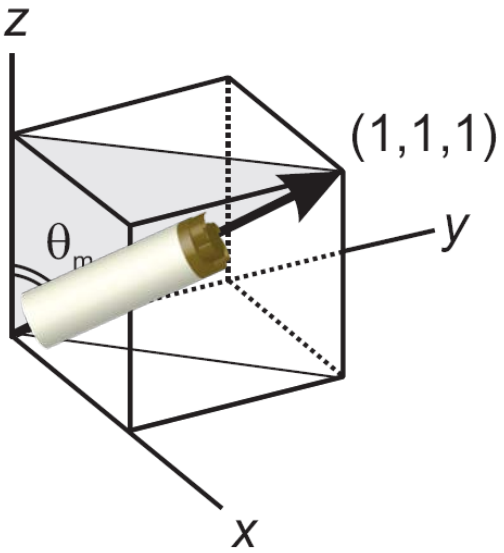
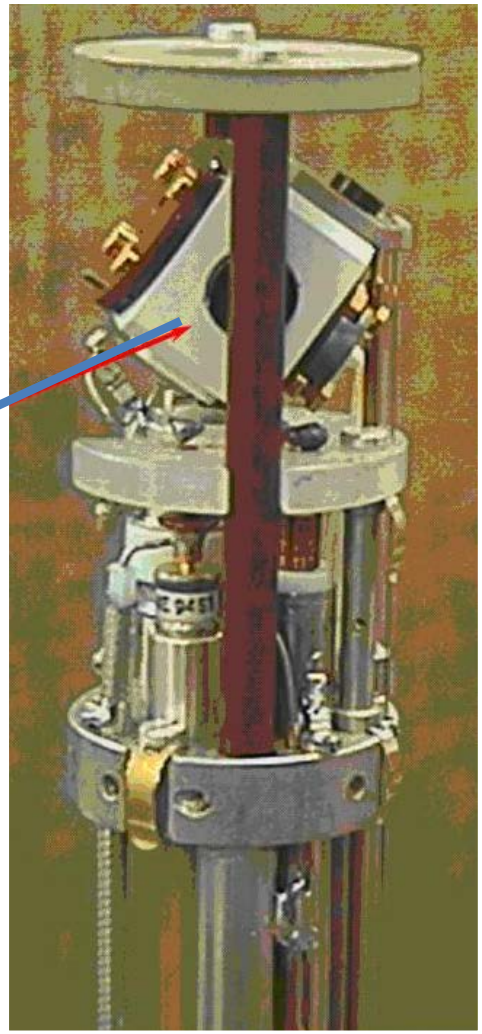


how to get rid of it?

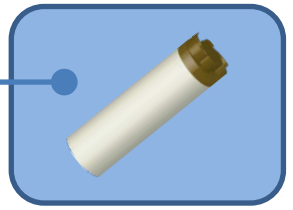
…but the information is NOT LOST FOEVER!

(more information at 5pm by Barth-Jan van Rossum)



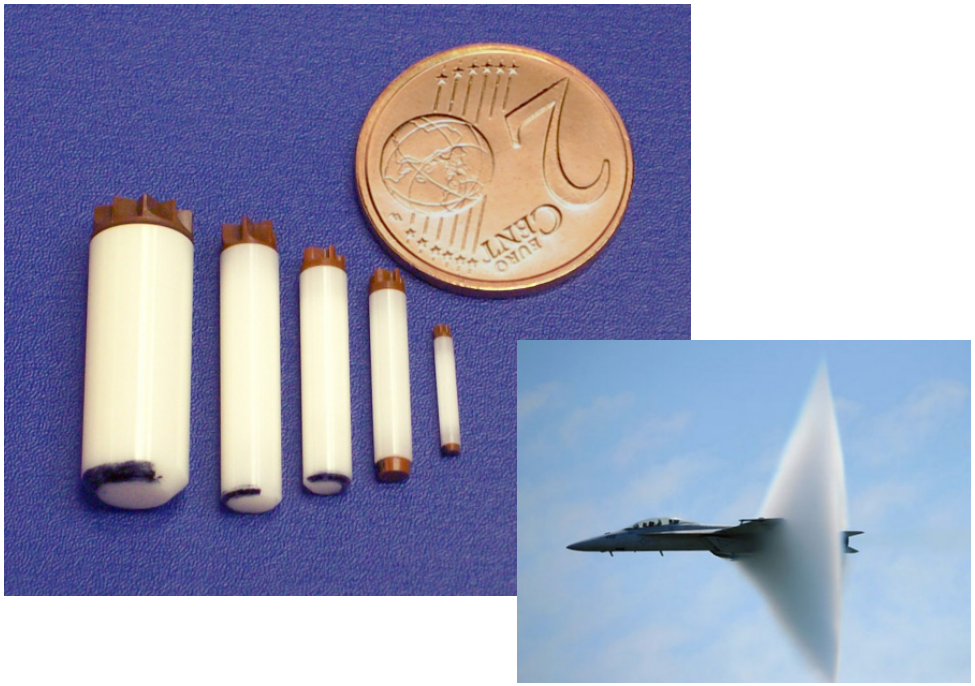


Magic Angle Spinning (MAS)



Maximum spinning frequency depends on rotor diameter

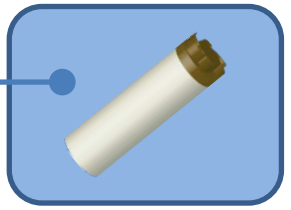
some typical diameters:	4.0 mm	→	15 kHz	(1,400,000 x *g*)
	3.2 mm	→	25 kHz	(2,700,000 x *g*)
	2.5 mm	→	35 kHz	(3,500,000 x *g*)



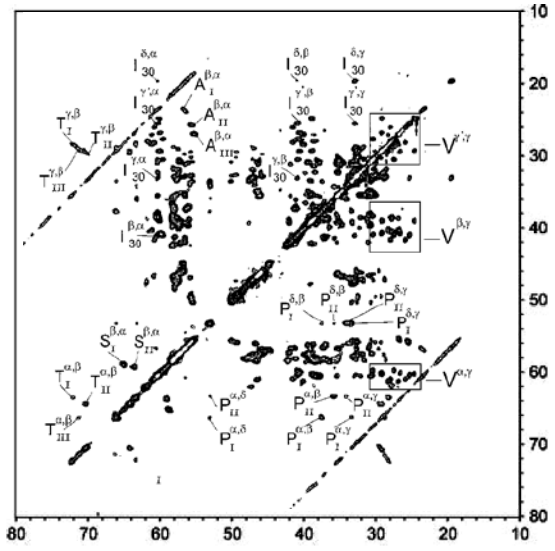
(80.000 x *g*)…

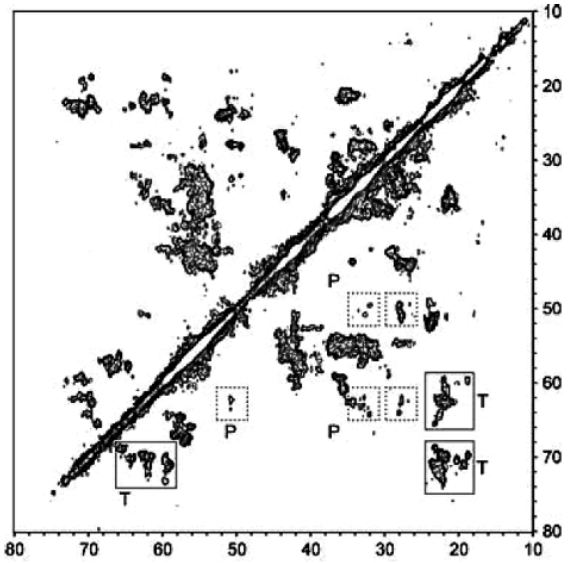
Solid-state NMR is brute force…

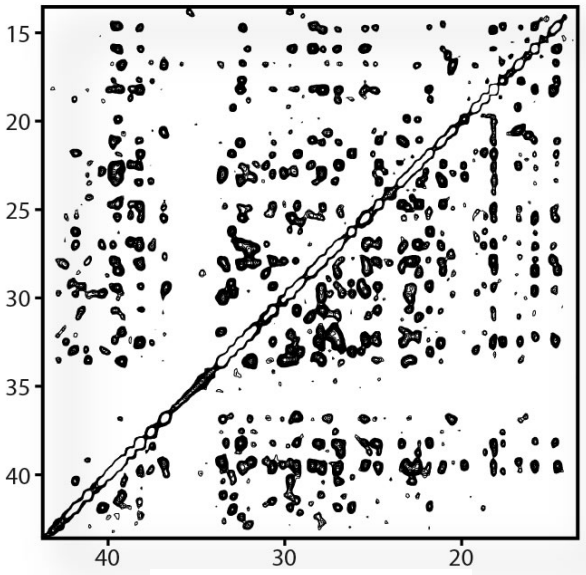
Magic Angle Spinning (MAS)

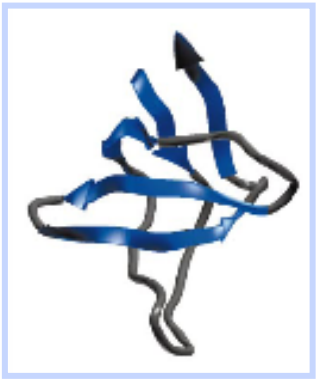


‘no inherent limitation on complex size’ : What does it mean?

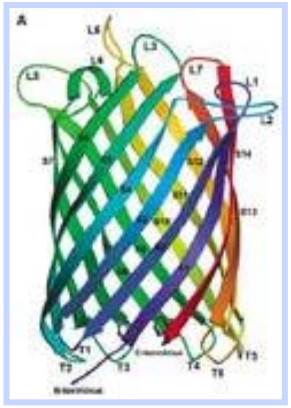




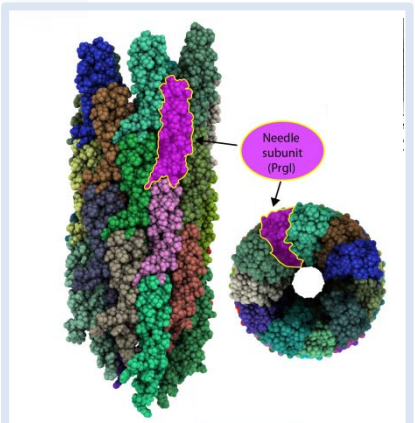




SH3 domain
62 residues (~7 kDa)

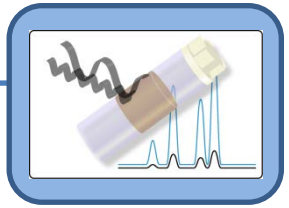


OmpG
281 residues (~34 kDa)



type III secretion system
80 residues)

Dynamic Nuclear Polarization (DNP)



low sensitivity is one of the biggest bottlenecks in solid state NMR

$$\frac{N_\beta}{N_\alpha} = e^{\frac{-\Delta E}{kT}} = e^{\frac{-h\upsilon_0}{kT}} \qquad \Delta E = \frac{\gamma \hbar B_0}{2\pi}$$

sensitivity depends on:

→ gyromagnetic ratio γ of the nuclei → the **higher** the better (e.g. ^{1}H vs. ^{13}C)

→ energy difference (i.e. magnetic field strength B_0) → the **stronger** the better

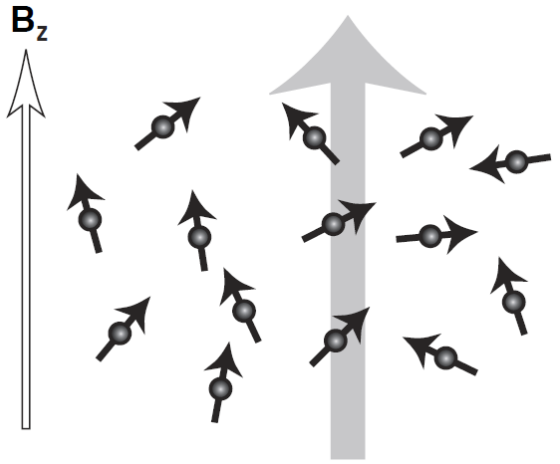
→ sample temperature → the **cooler** the better

Dynamic Nuclear Polarization (DNP)

Why is (solid state) NMR so insensitive

Small net magnetic moment (polarization) aligned with B_z

$$\frac{N_\beta}{N_\alpha} = e^{\frac{-\Delta E}{kT}} = e^{\frac{-h\upsilon_0}{kT}}$$



1H nuclei @ RT @ 16.5 T

ΔE = hv = 5.6 x 10^{-25} J

h = 6.626×10^{-34} Js

$k_b T$ = 4.1 x 10-21 J

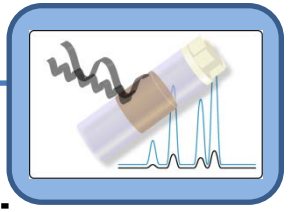
k_b = 1.381×10^{-23} JK^{-1}

$$\frac{N_\beta}{N_\alpha} = 1.0001$$

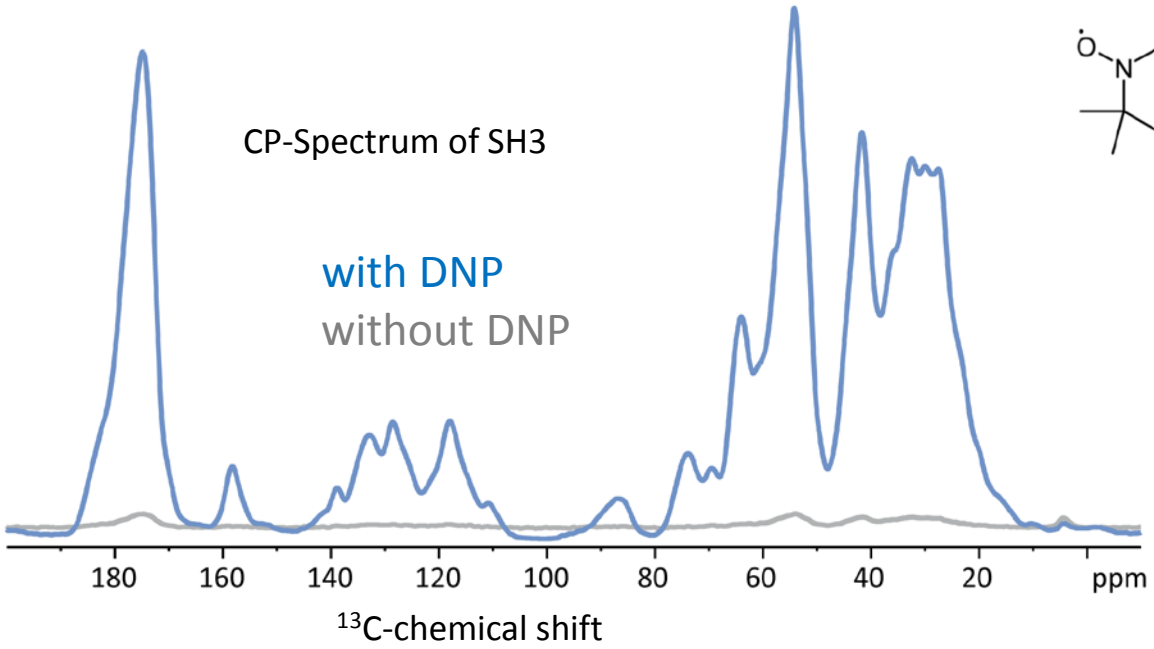
10,000 ^{1}H spins up (I_z is aligned with B_z)

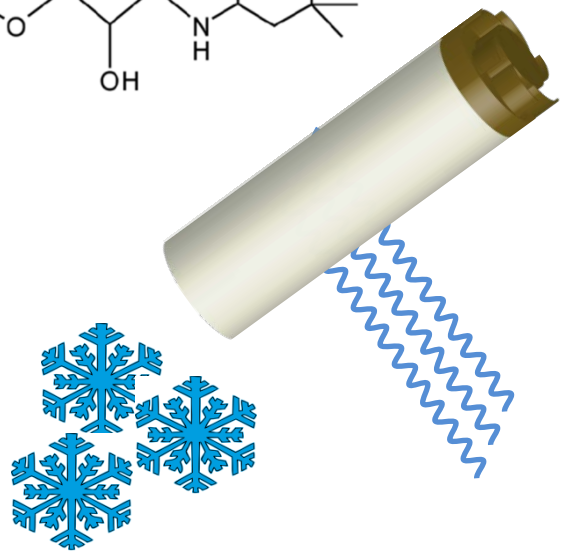
9,999 ^{1}H spins down (I_z is aligned against B_z)

Dynamic Nuclear Polarization (DNP)



DNP = transfer of the high electron polarization to nearby nuclei





90 – 110 K

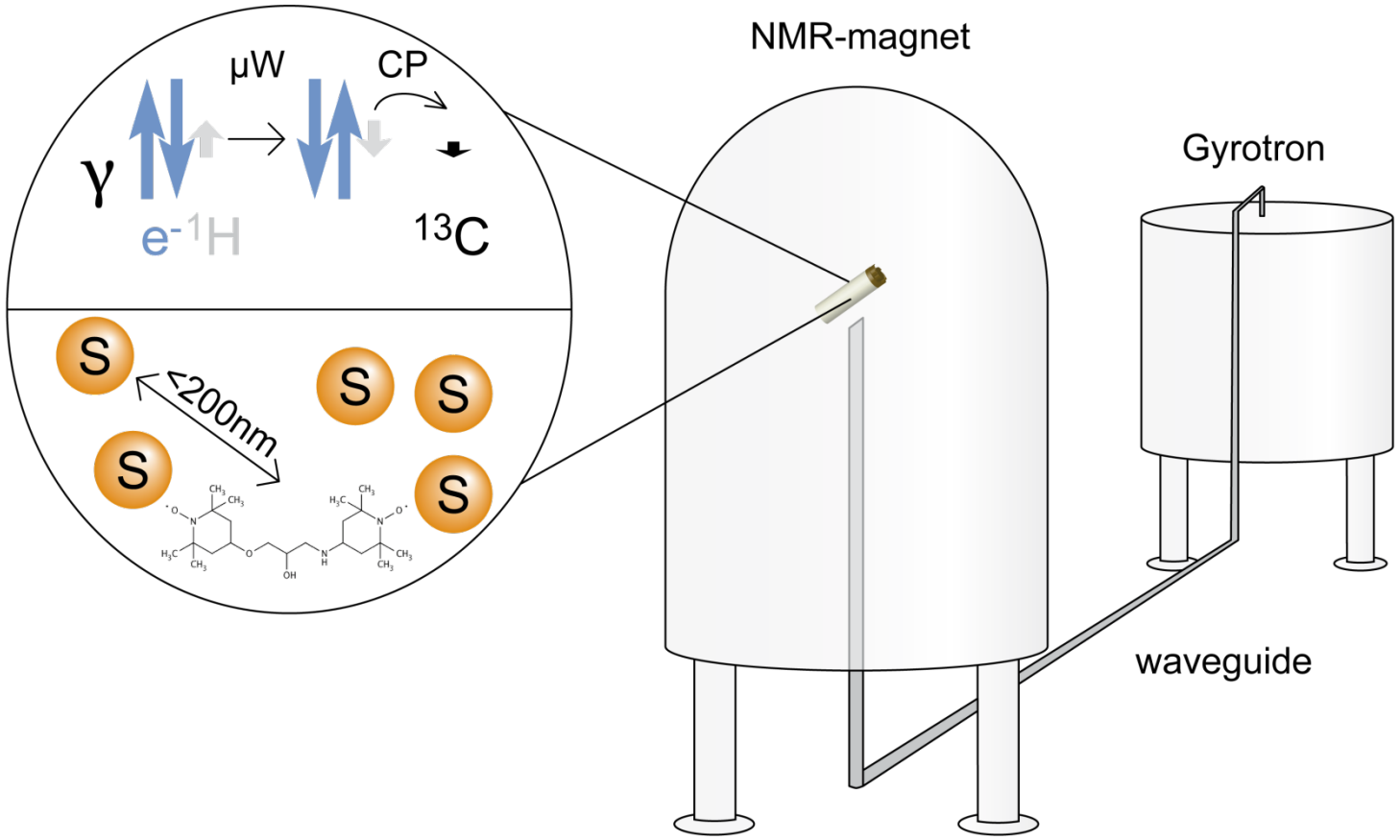
$$\frac{N_\beta}{N_\alpha} = e^{\frac{-\Delta E}{kT}} = e^{\frac{-h\upsilon_0}{kT}}$$

theoretical enhancement $= \frac{\gamma_e}{\gamma_n}$

e.g. for Protons: $\frac{\gamma_e}{\gamma_{1H}} \approx 660$

Dynamic Nuclear Polarization (DNP)

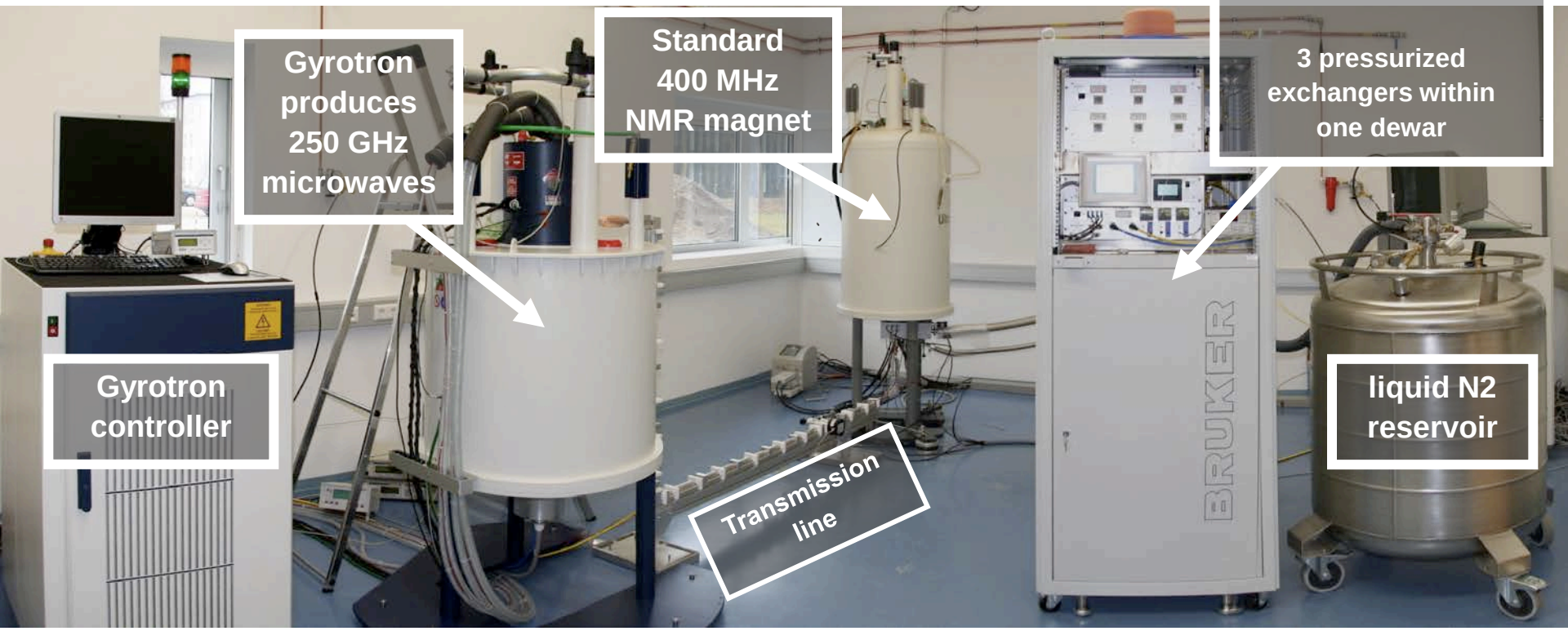
The DNP-Spectrometer



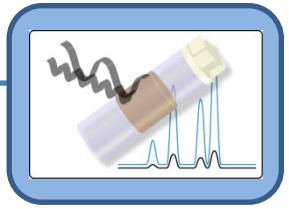
needed amount **5 nmol** / 25 µl (**0.2 mM**)

Dynamic Nuclear Polarization (DNP)

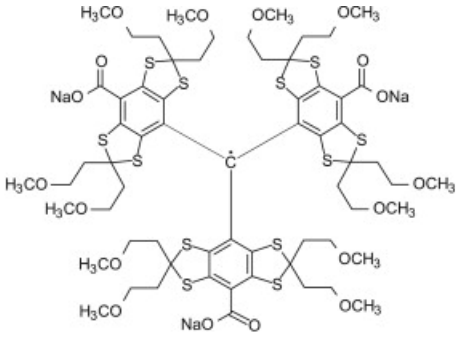
The DNP-Spectrometer



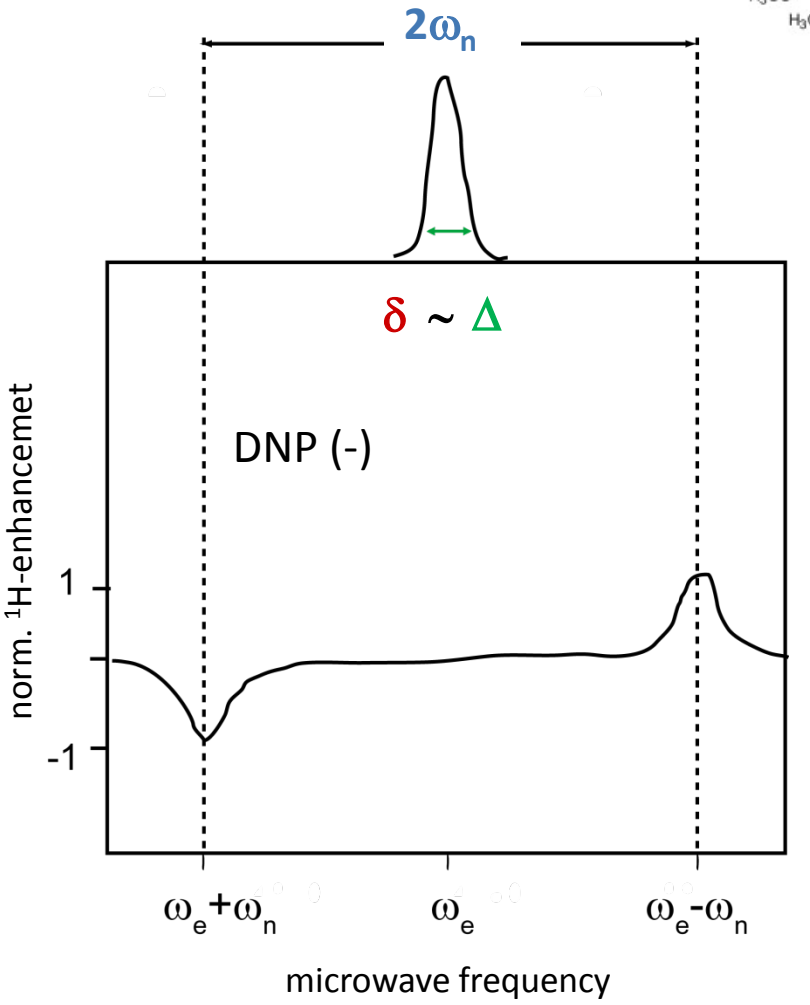
DNP-Mechanism: 1. The Solid Effect

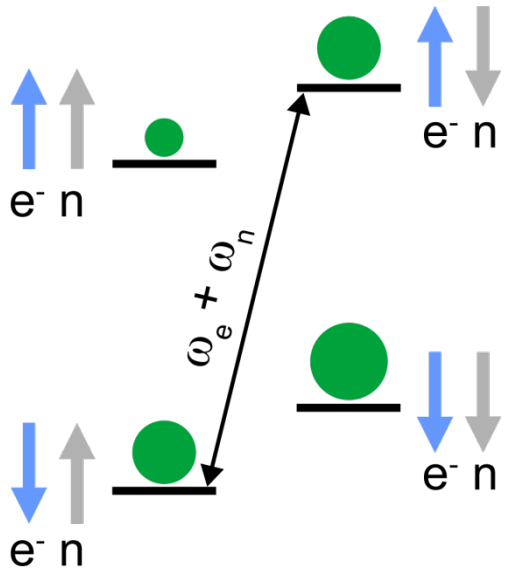


$\delta \sim \Delta < \omega_n$



Trityl-radical





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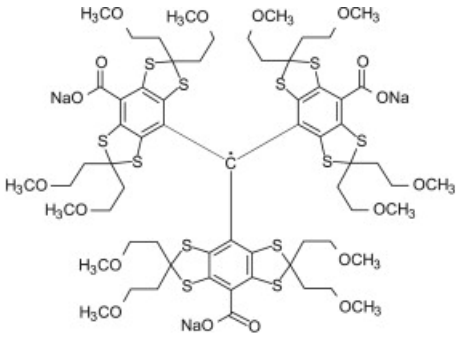

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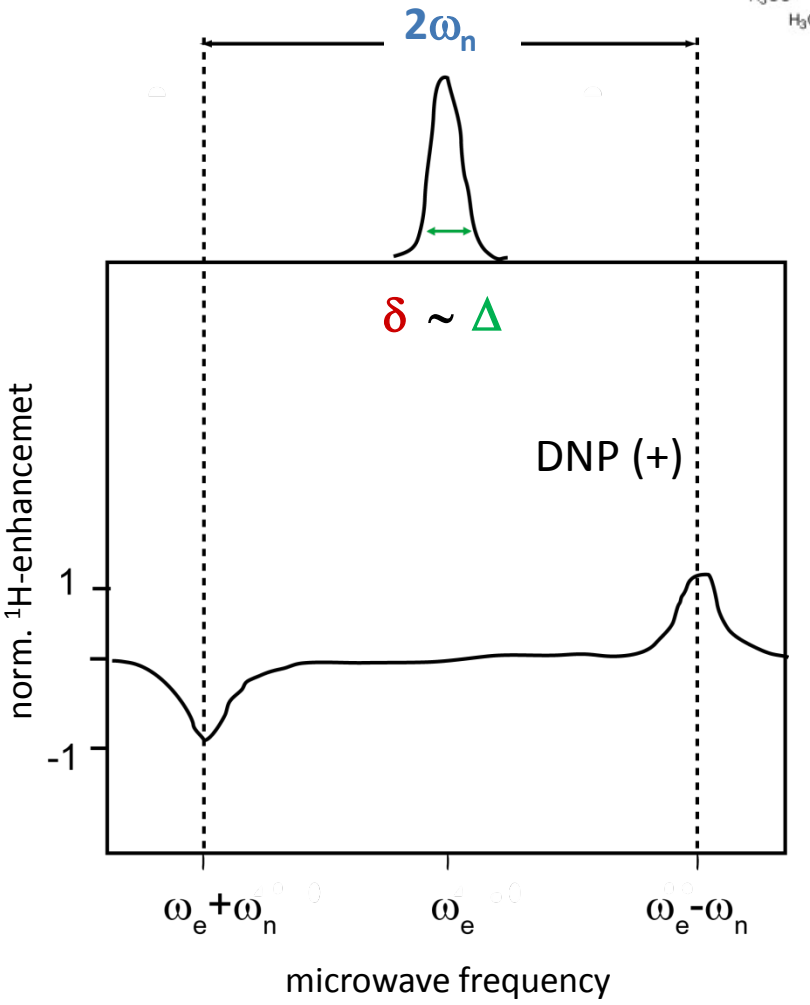
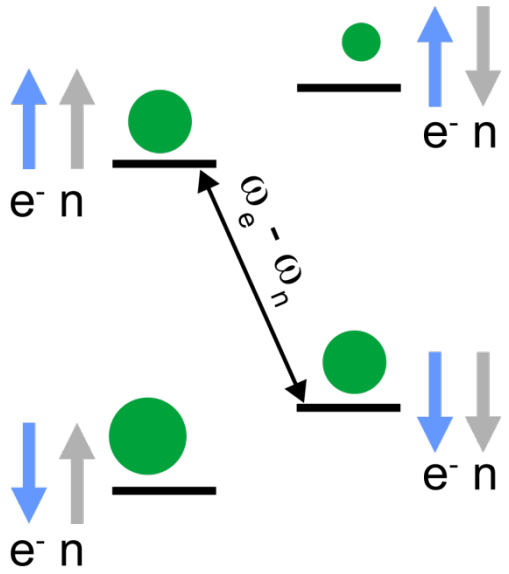
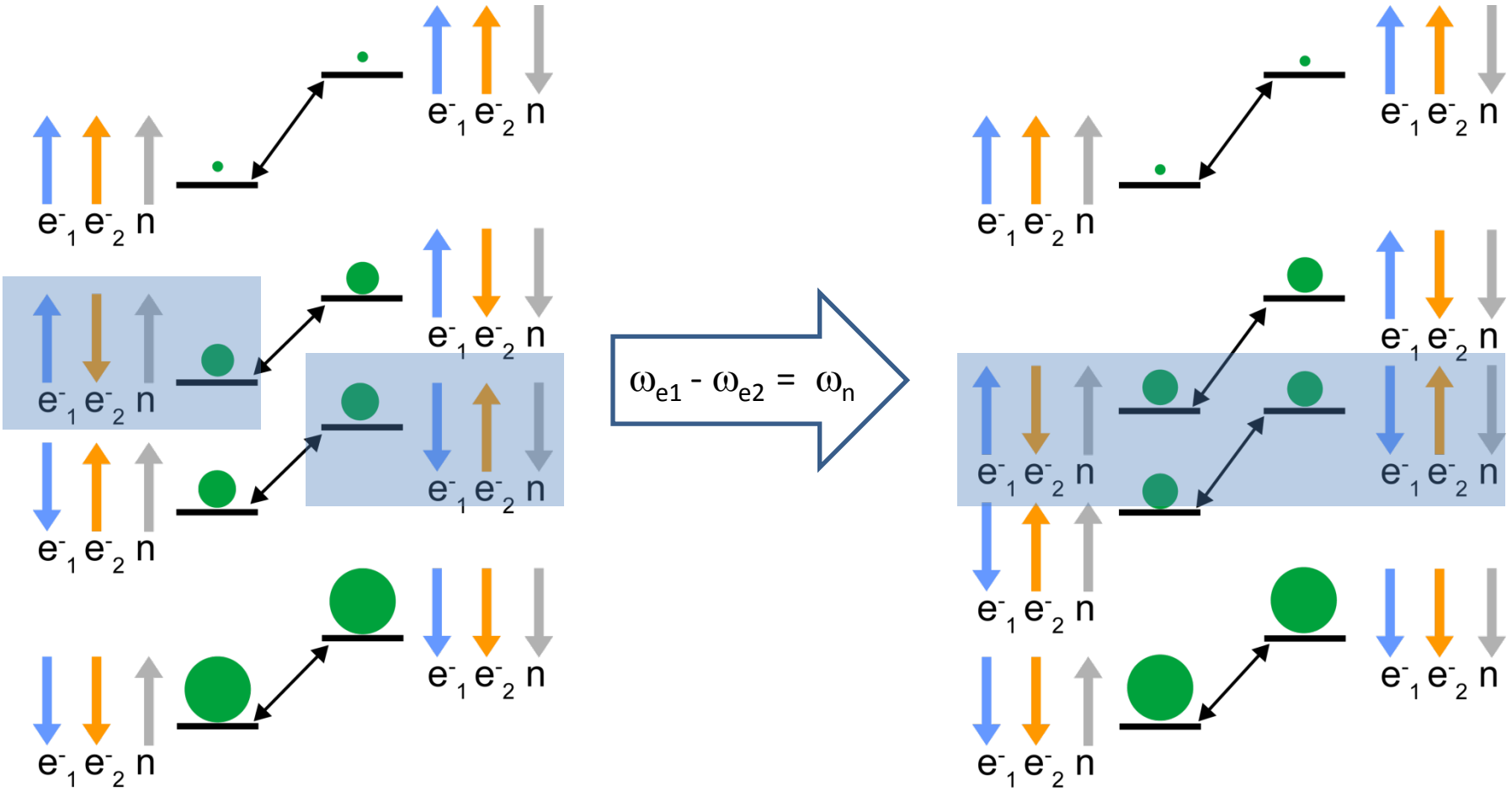
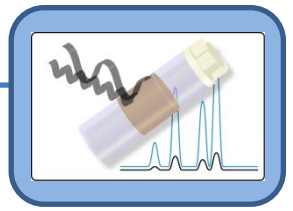




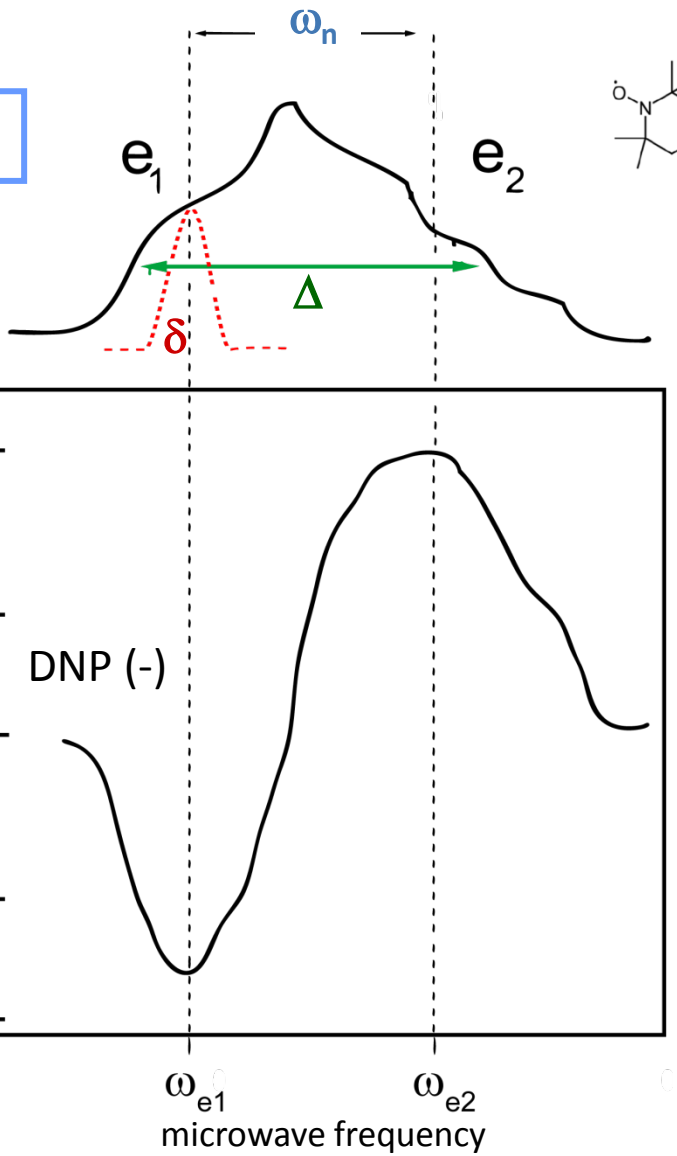
DNP-Mechanism: 2. The Cross Effect

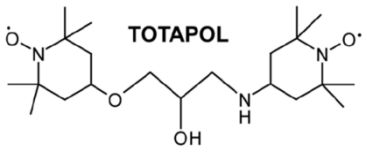


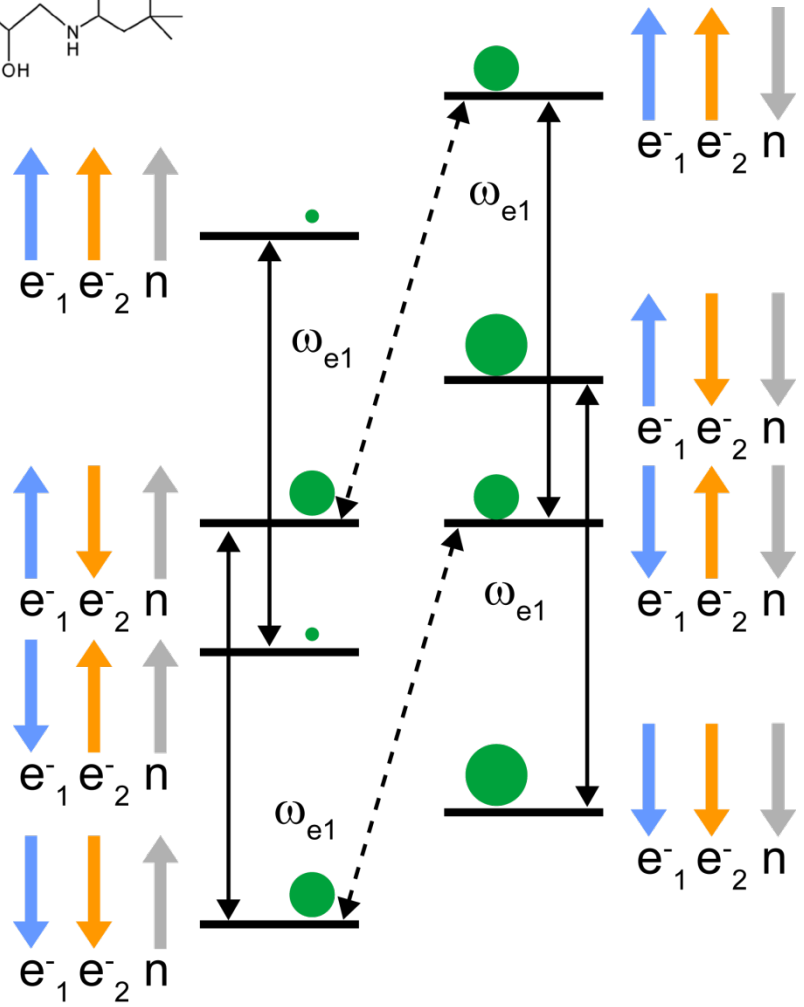
DNP-Mechanism: 2. The Cross Effect

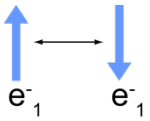
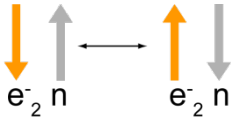


$\Delta > \omega_n > \delta$

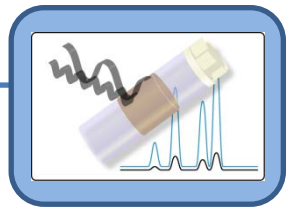




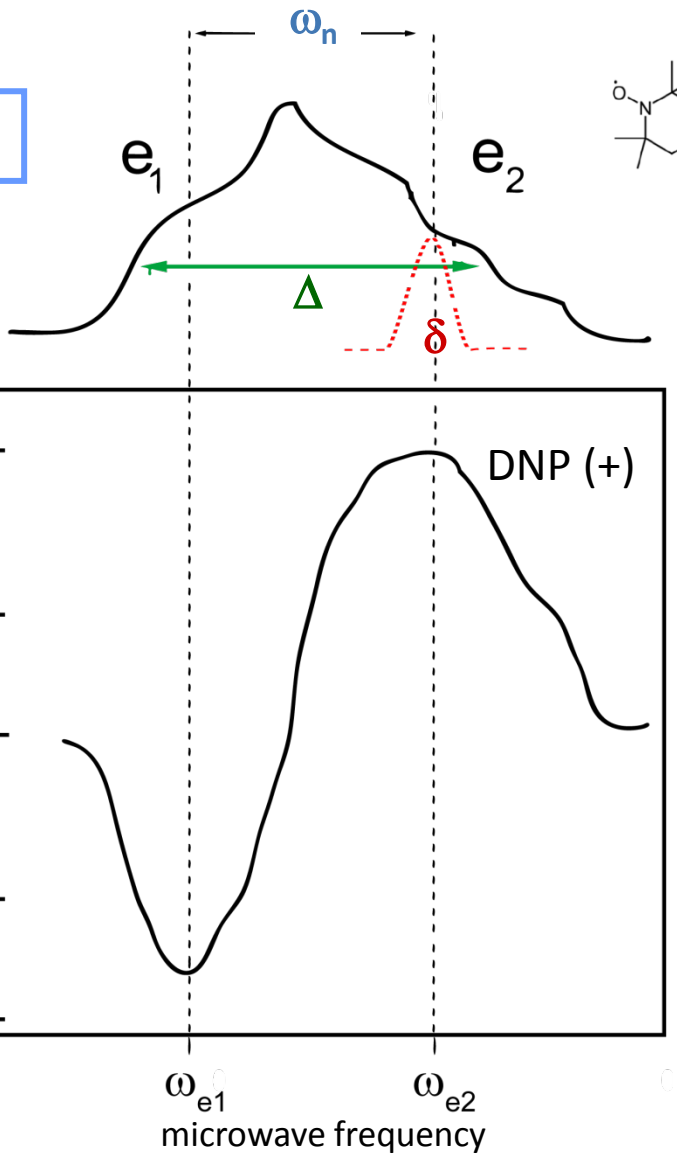


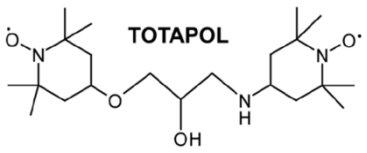
Irradiation with ω_{e1} leads to  at the same time: 

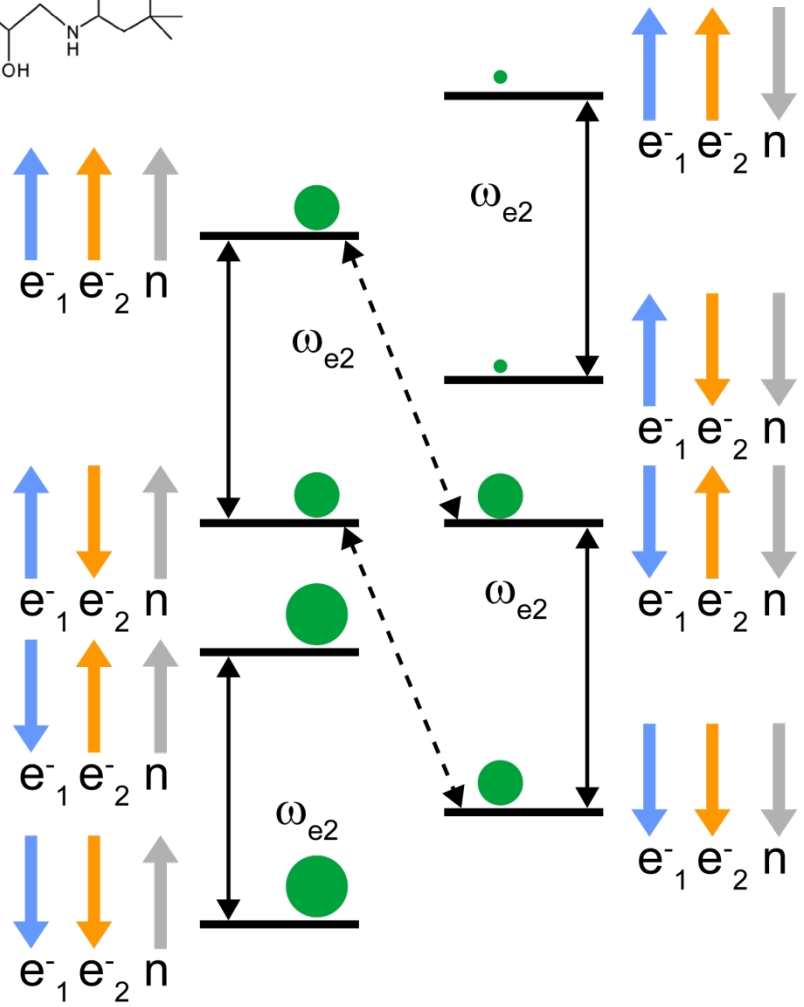
DNP-Mechanism: 2. The Cross Effect

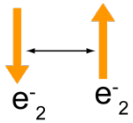
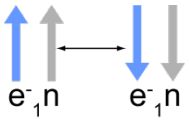


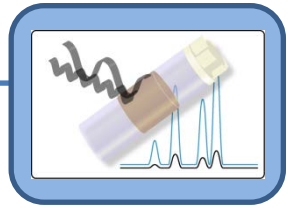
$\Delta > \omega_n > \delta$







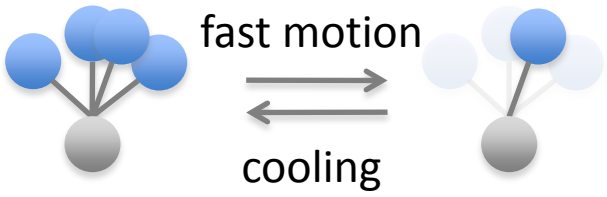
Irradiation with ω_{e2} leads to  at the same time: 

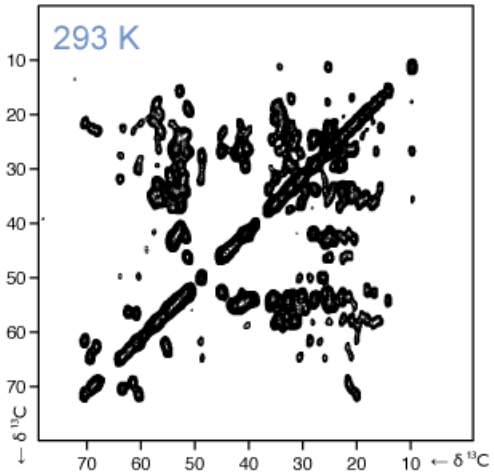


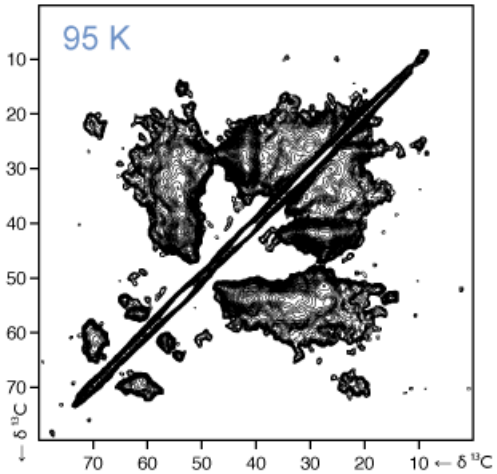
two main drawbacks of DNP

→ inhomogeneous broadening due to cooling

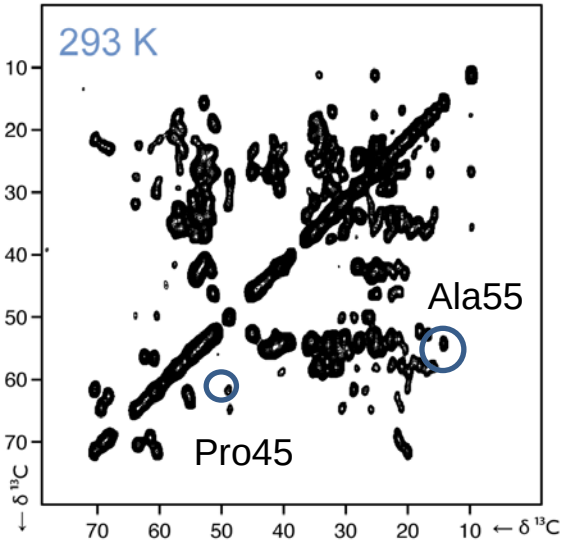
→ homogeneous broadening due to addition of radicals

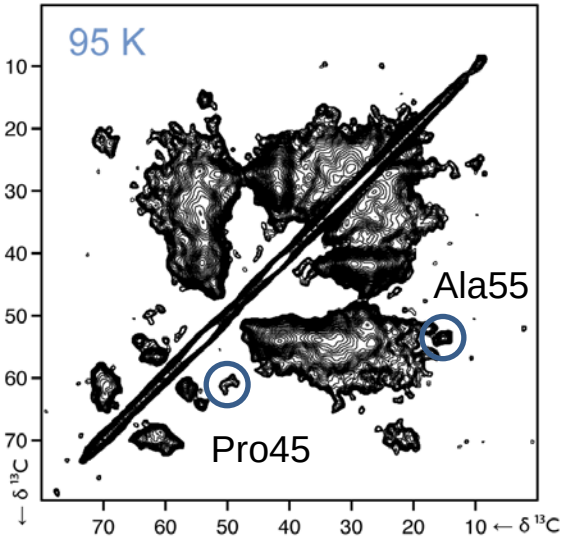






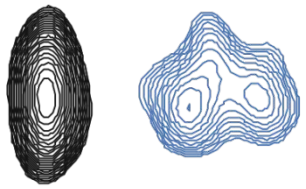
DNP as a Tool for Structural Biology





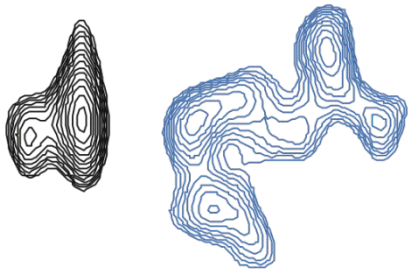
at low temperatures we can detect different conformers!

Ala55
Cα - Cβ



293 K 95 K

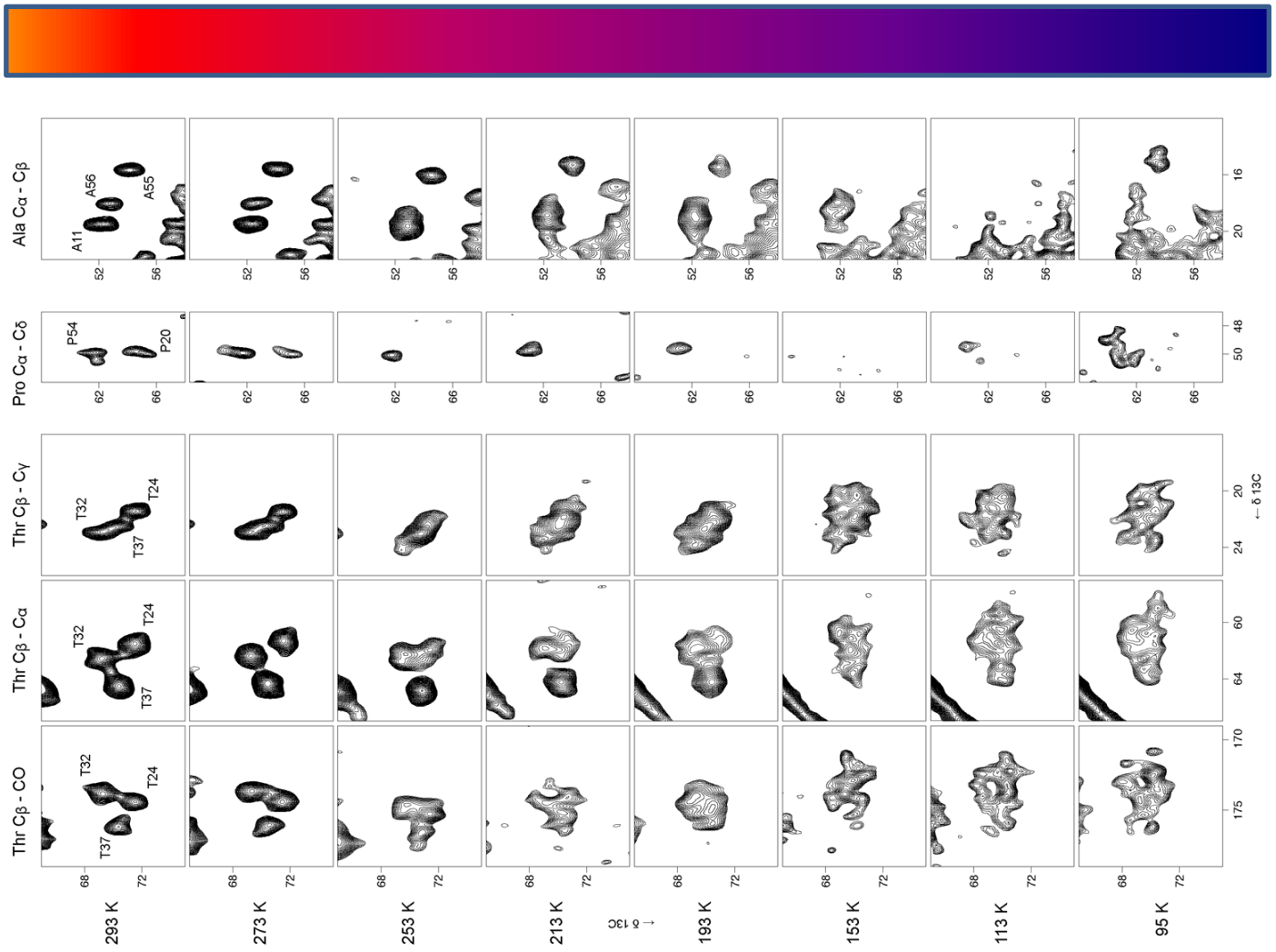
Pro45
Cα - Cδ



293 K 95 K

Modell system: SH3

… and we can determine coalescence temperatures!

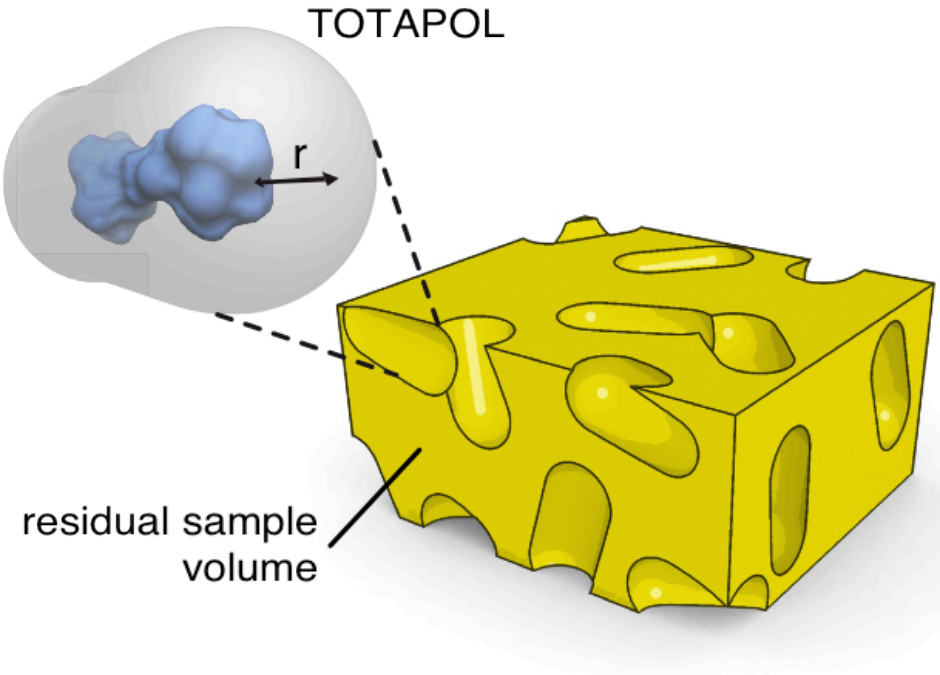


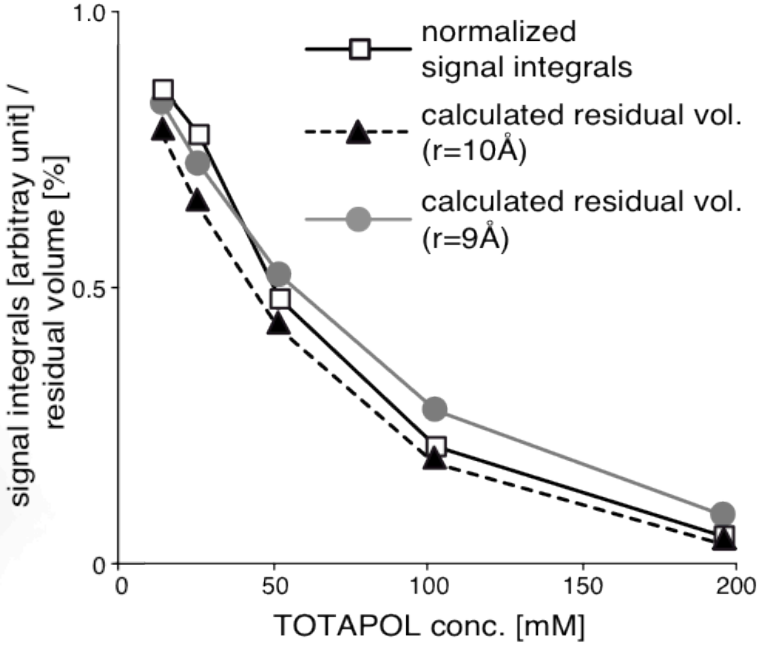
Modell system: SH3

two main drawbacks of DNP

→ inhomogeneous broadening due to cooling

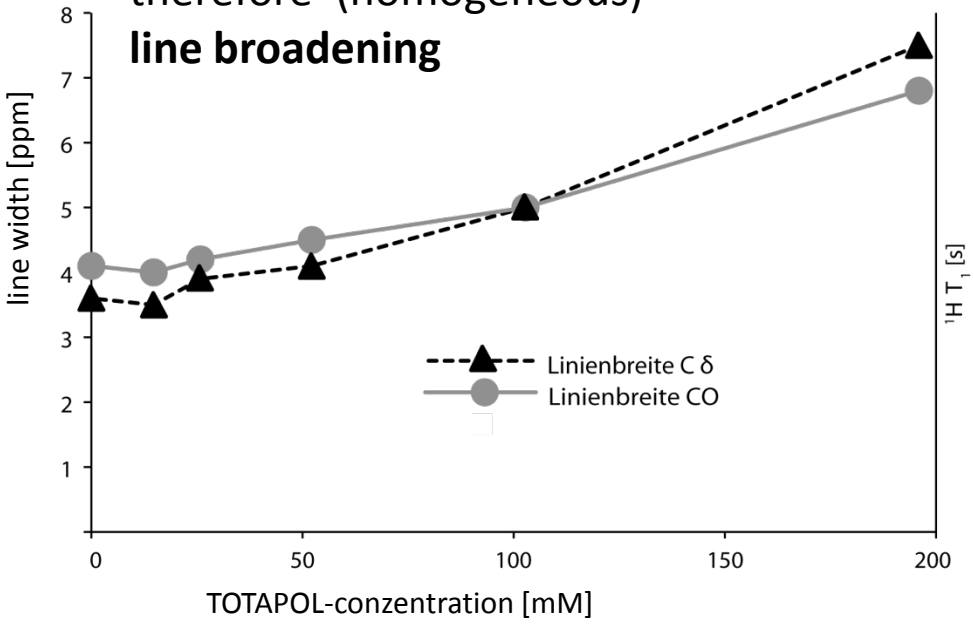
→ homogeneous broadening due to addition of radicals



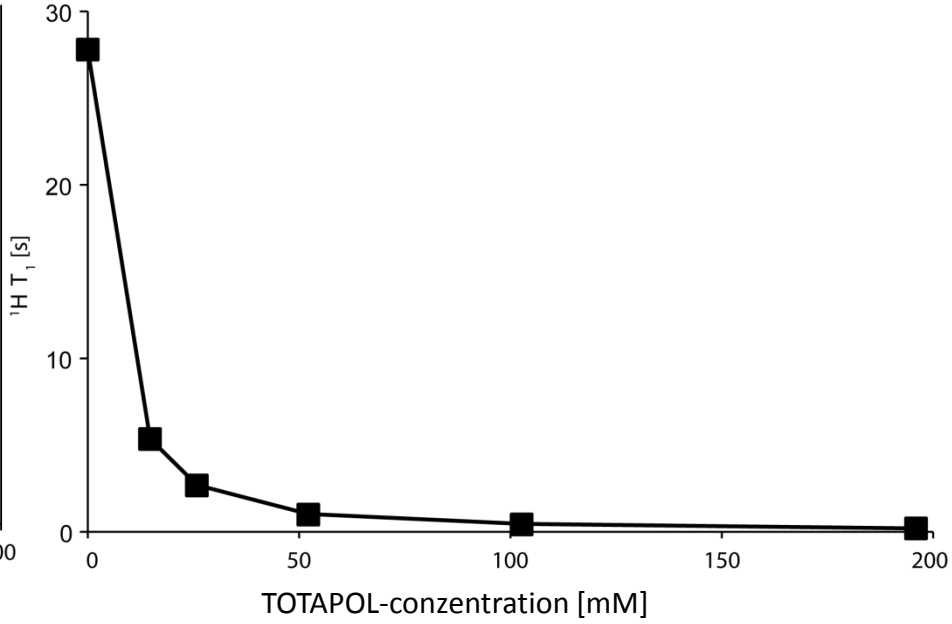


the radical is causing homogeneous line broadening

TOTAPOL increases **T_2-Relaxation** and therefore (homogeneous) **line broadening**



TOTAPOL increases **T_1-Relaxation**

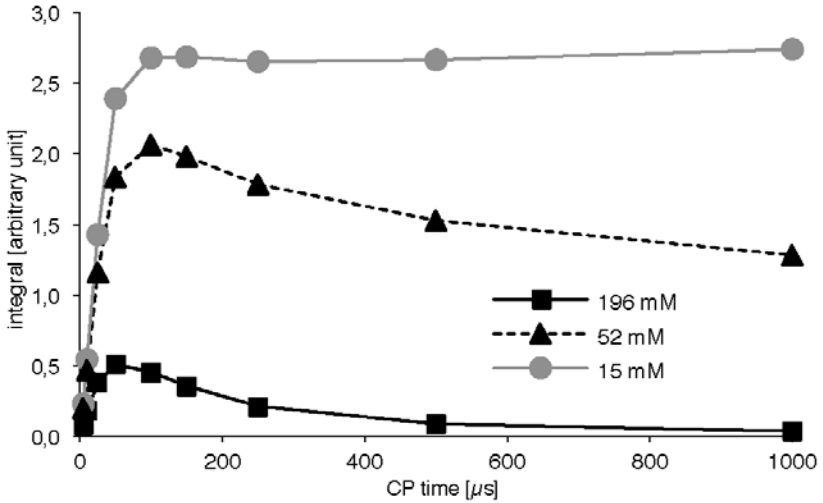


e.g. broader lines, causing **lower S/N**

e.g. shorter repetition times are possible (**higher S/N per time unit**)

the radical is shortening effectively CP times

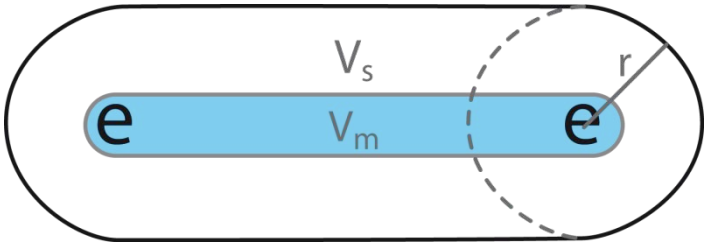
TOTAPOL causes shorter effective CP times

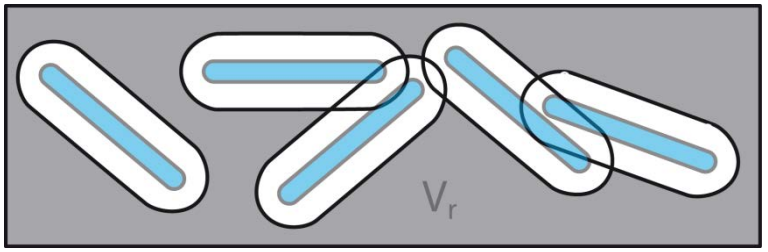


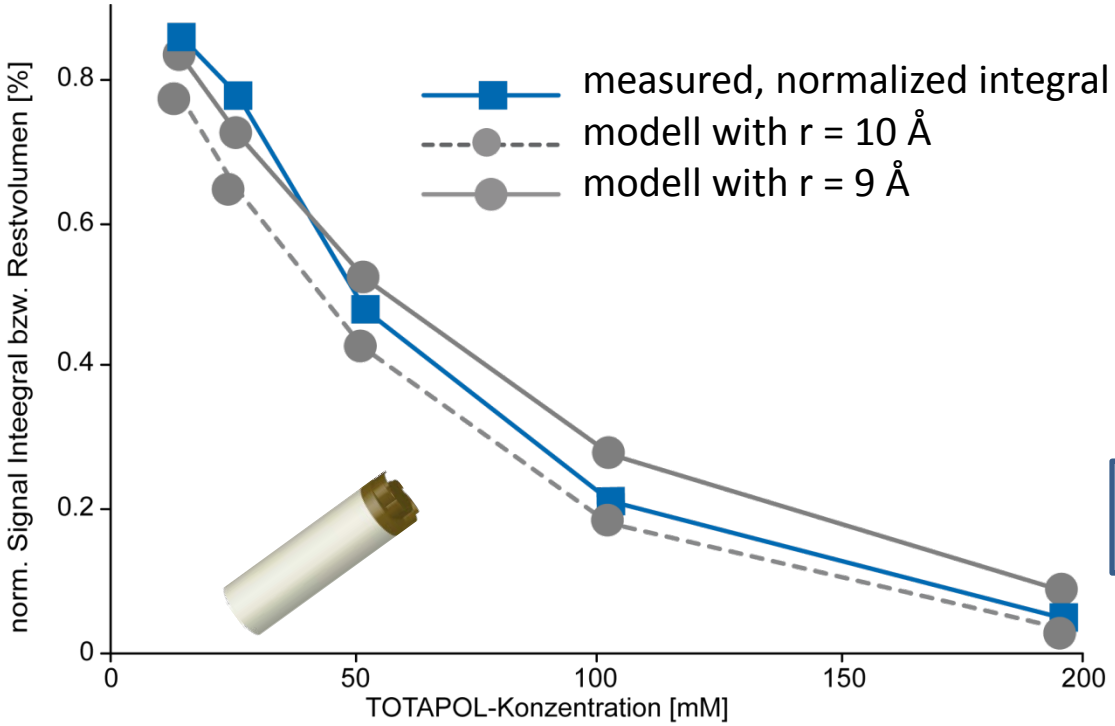
TOTAPOL-conzentration [mM]

multi dimensional experiments difficult
lower signal intensities

TOTAPOL leads to a **reduction of detectable nuclei**:







$$V_{b_N} = \sum_{i=1}^{N} V_m + (V_s \cdot a_i)$$

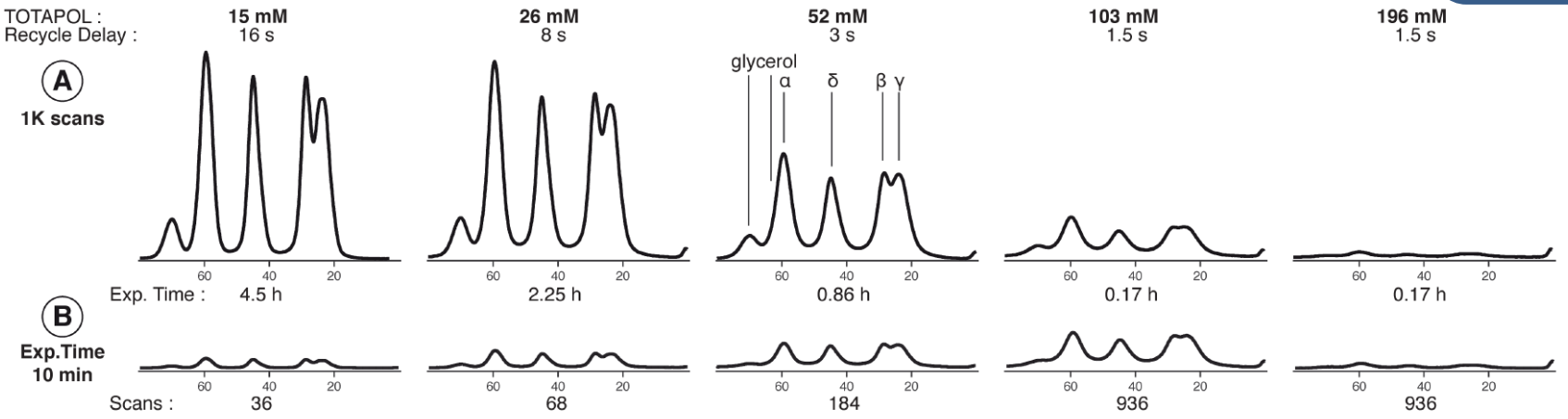
a_i – overlapping factor

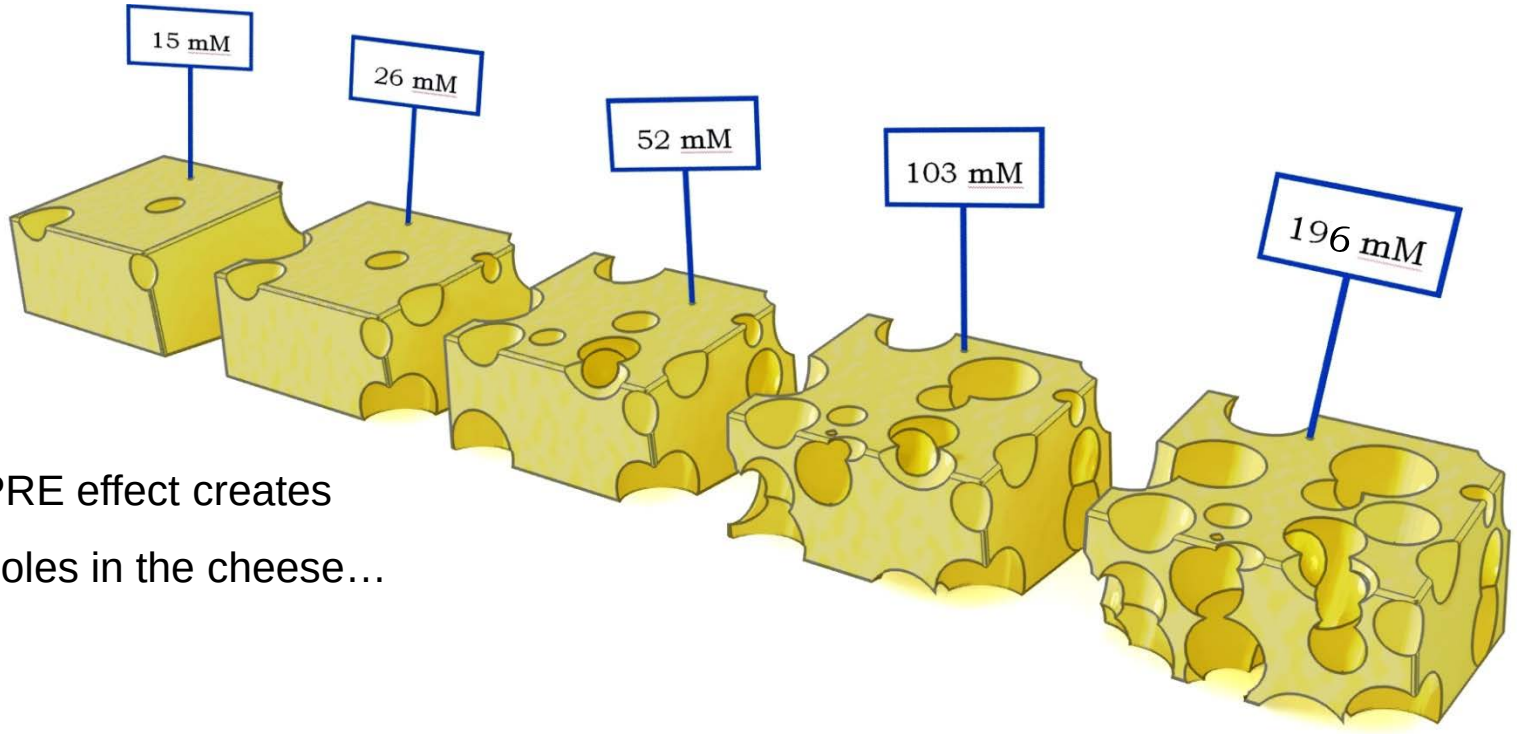
$$a_i = \frac{V - (i \cdot V_m) - \sum_{j=1}^{i-1} D_j}{V - (i \cdot V_m)}$$

$$V_r = V - V_{bN} \approx V - e^{r \cdot [TOT]}$$

[TOT] = TOTAPOL-concentration

DNP as a Tool for Structural Biology



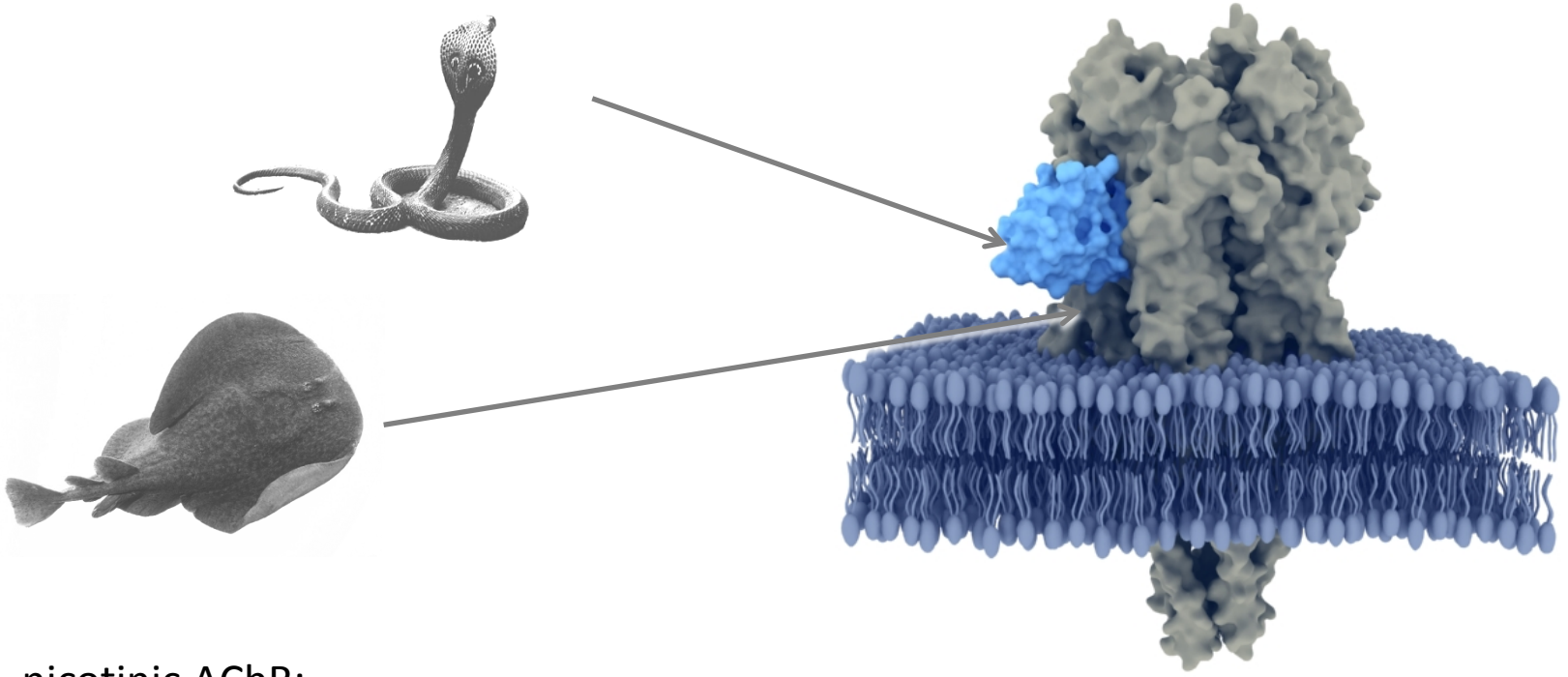


PRE effect creates

holes in the cheese…

DNP as a Tool for Structural Biology, ACh- Receptor

Neurotoxin II (Naja naja oxiana; NOR1) on nAChR (Torpedo californica)

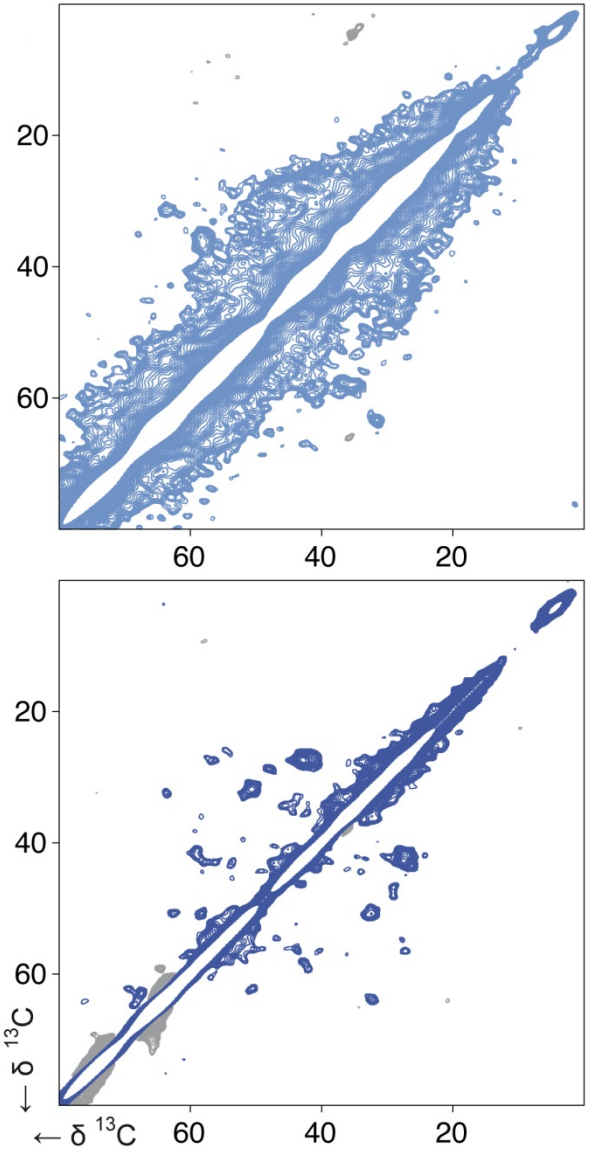


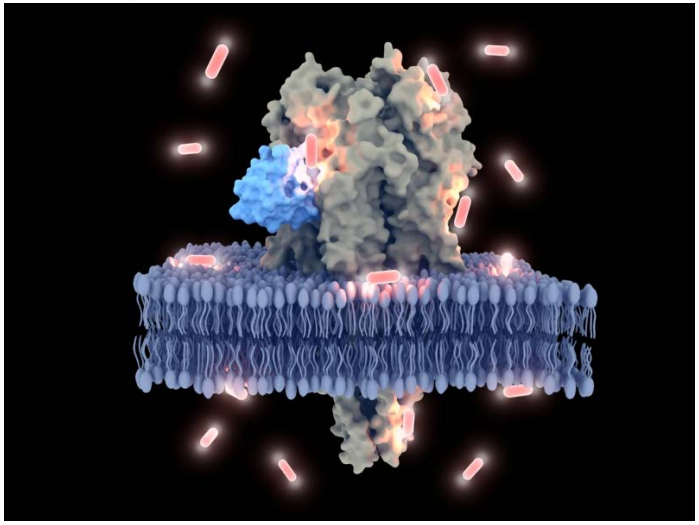
http://www.dkimages.com/discover/previews/824/5020072.JPG http://www.sevcikphoto.com/images/naja_naja_2.jpg

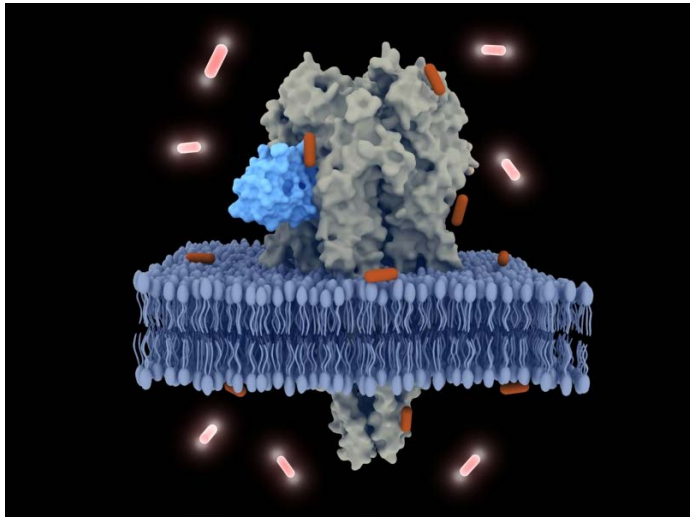
nicotinic AChR:
ionotropic (ligand gated ion-channels)
parasympathetic autonomic nervous system, neuromuscular junction

DNP as a Tool for Structural Biology, ACh- Receptor

inactivation of TOTAPOL in close proximity

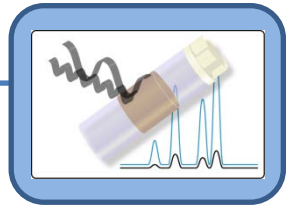




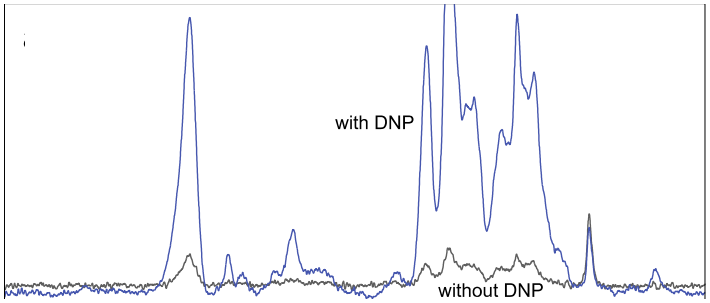


Linden, Oschkinat et al. *J. Am. Chem. Soc.* ***2011***

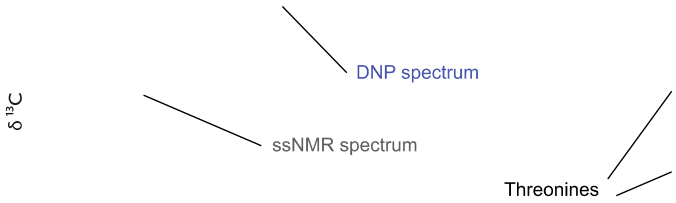
DNP as a Tool for Structural Biology, ACh- Receptor



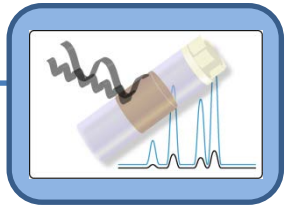
inactivation of TOTAPOL close proximity can help



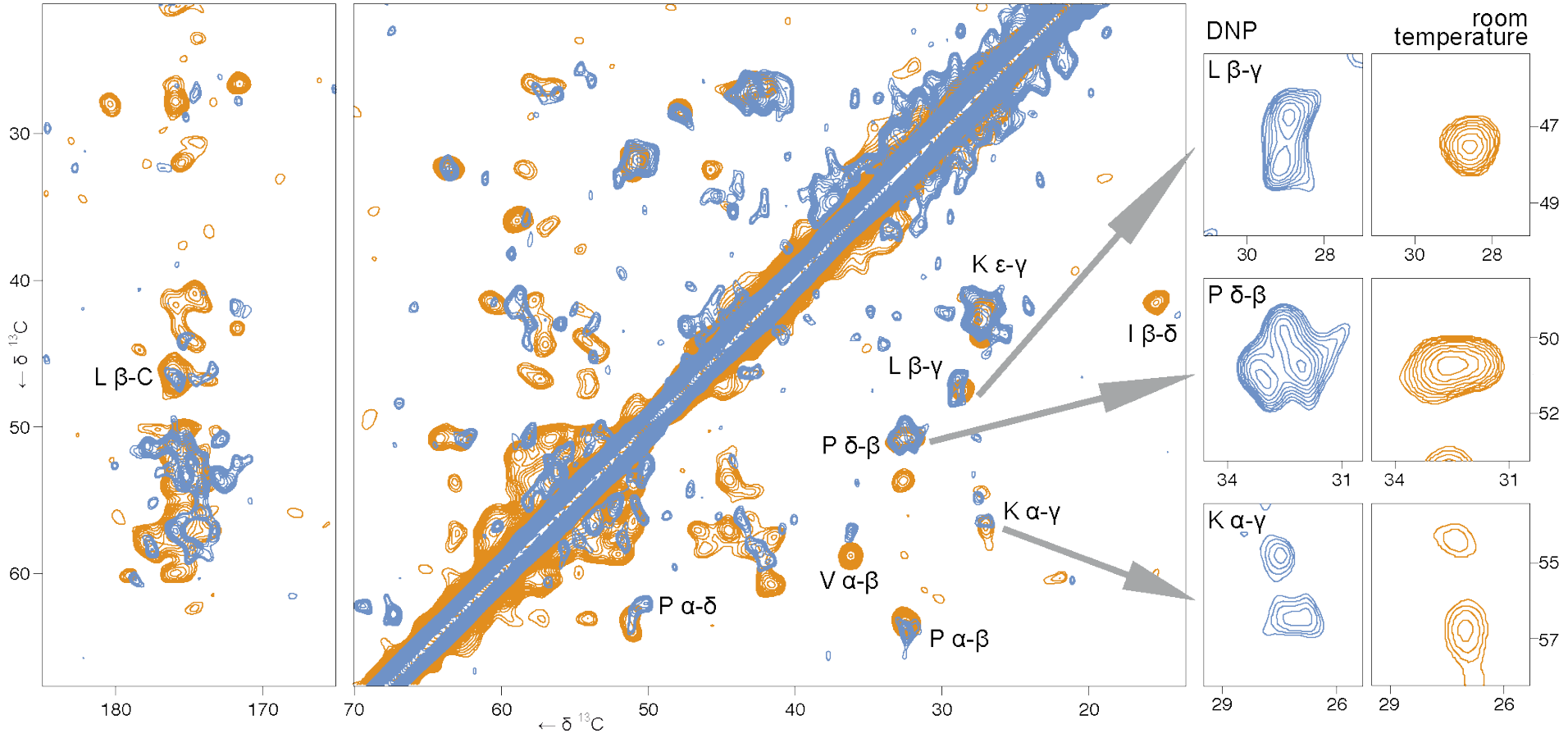
DNP spectrum
ssNMR spectrum



DNP as a Tool for Structural Biology, ACh- Receptor

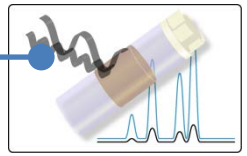


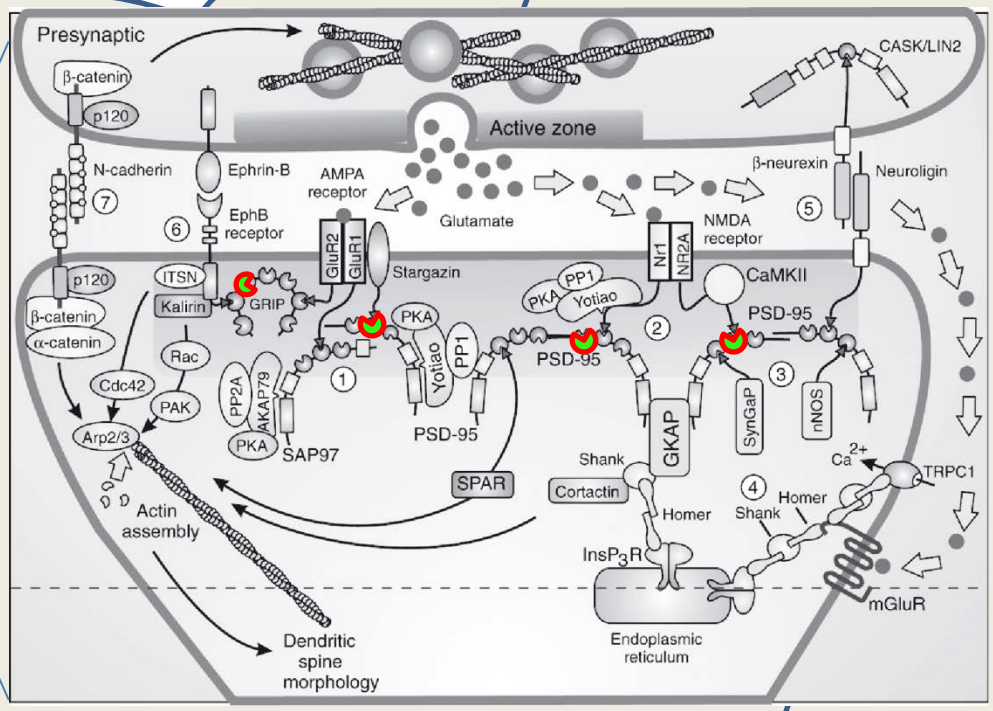
just 6% of the measurement time needed

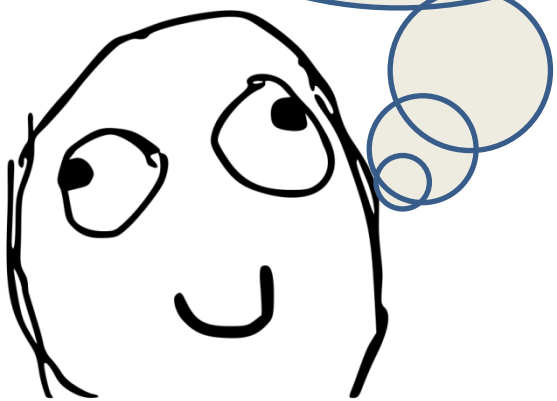


10 days → 10 hours

DNP as a Tool for Structural Biology, RNCs

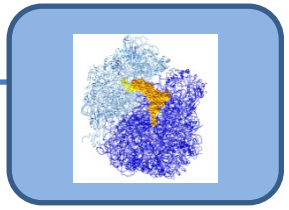


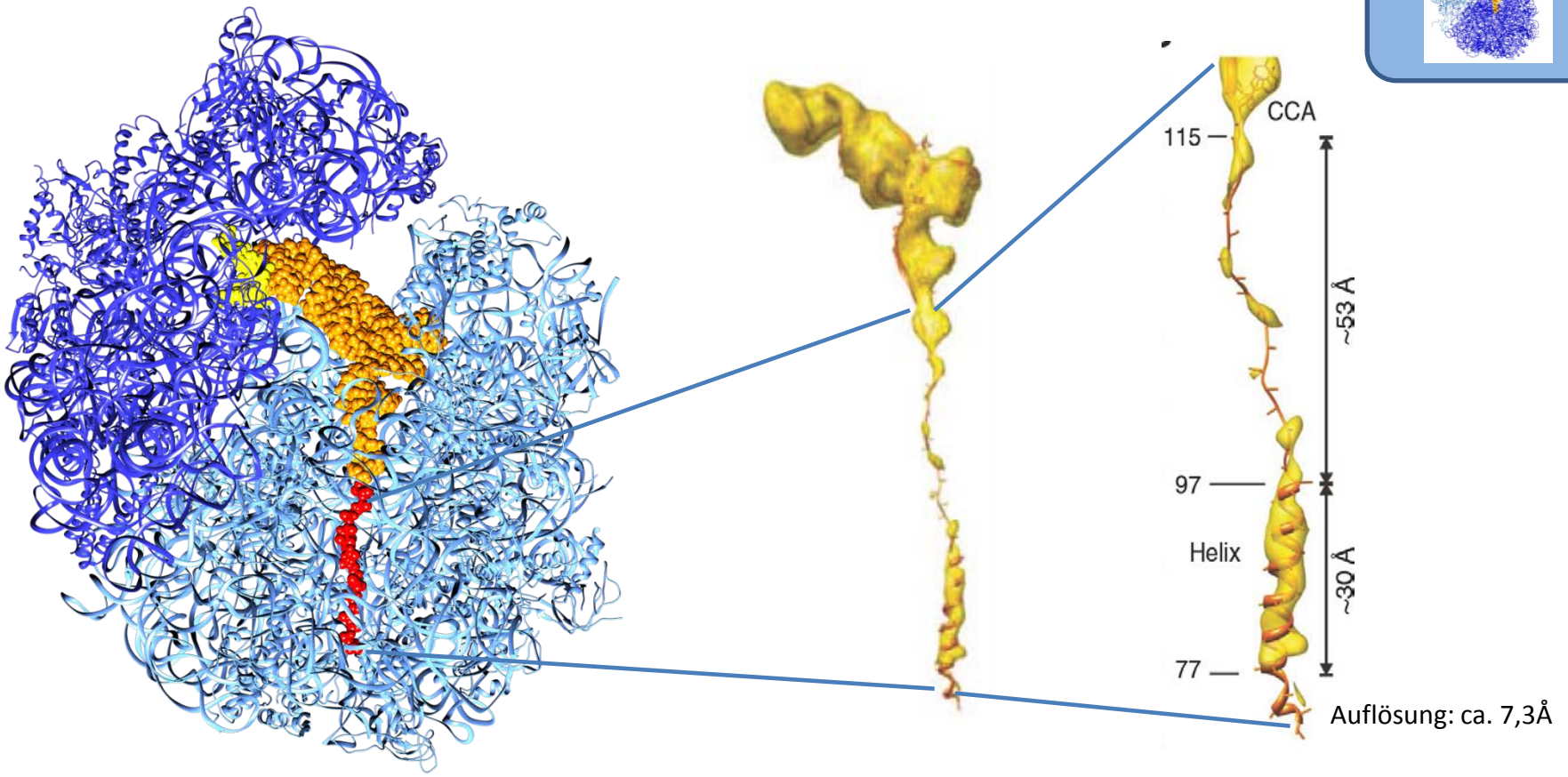




with DNP we could investigate something small in something very big and ‚nature-like‘

DNP as a Tool for Structural Biology, RNCs



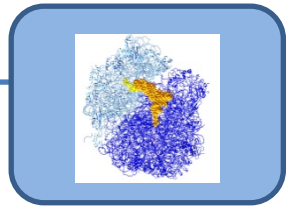


Auflösung: ca. 7,3Å

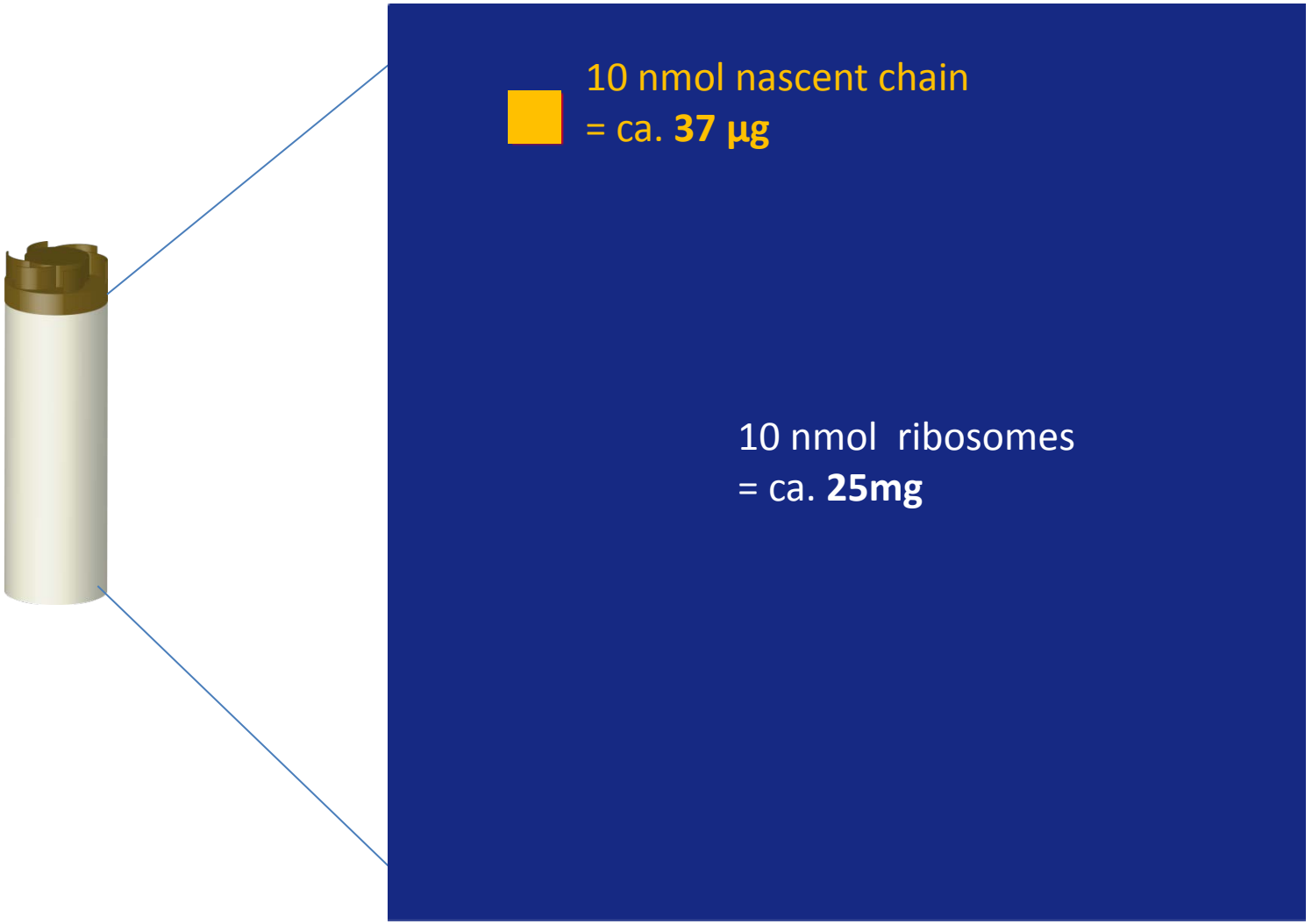
Bhushan, Beckmann et al. Nature Structural & Molecular Biology (2010)

we investigated the folding state of a signal peptide within the ribosomal exit tunnel
→ is there one specific conformation of the nascent chain?
→ what is the helix content?

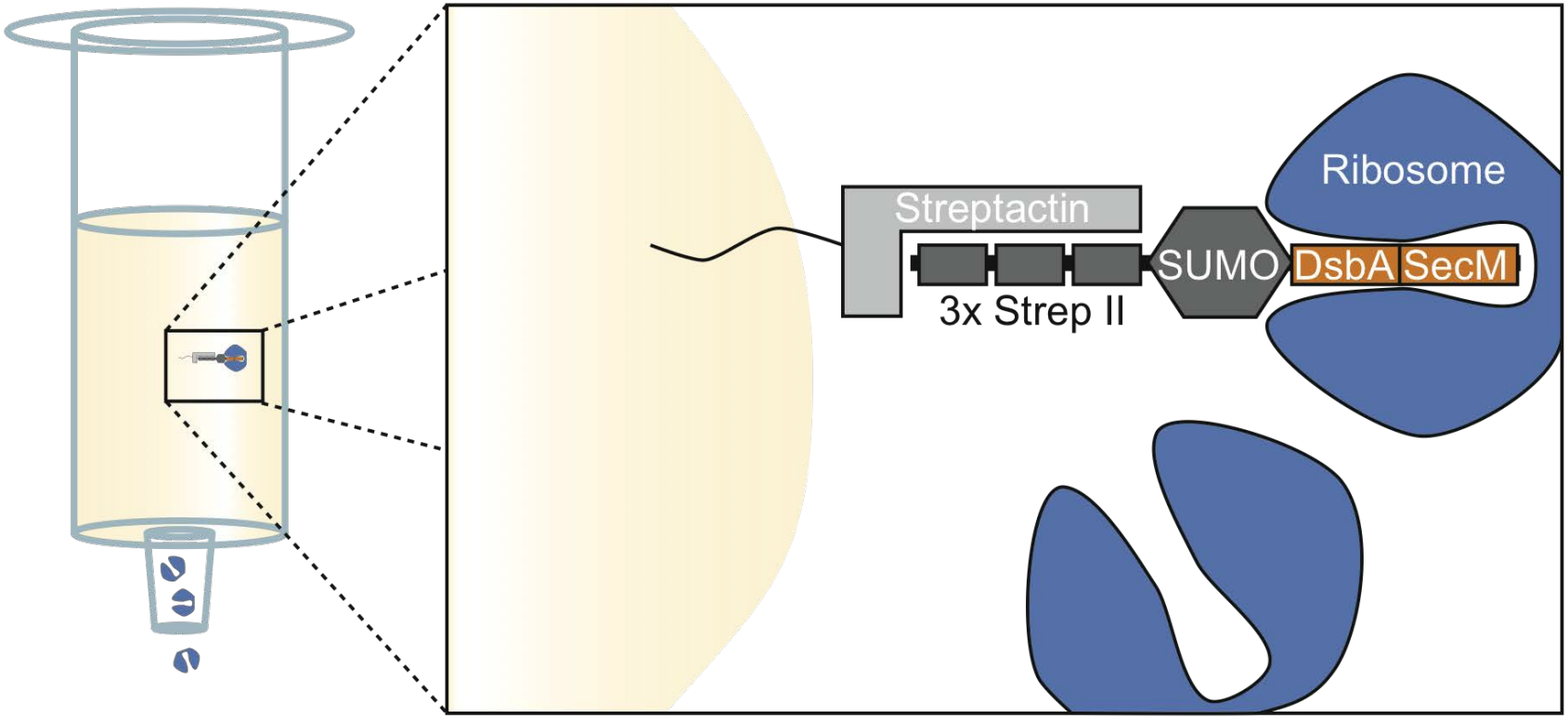
DNP as a Tool for Structural Biology, RNCs



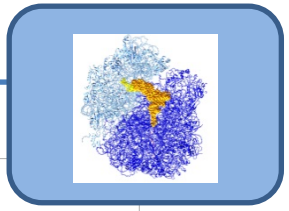
the ribosome is 10.000 times bigger compared to the NC

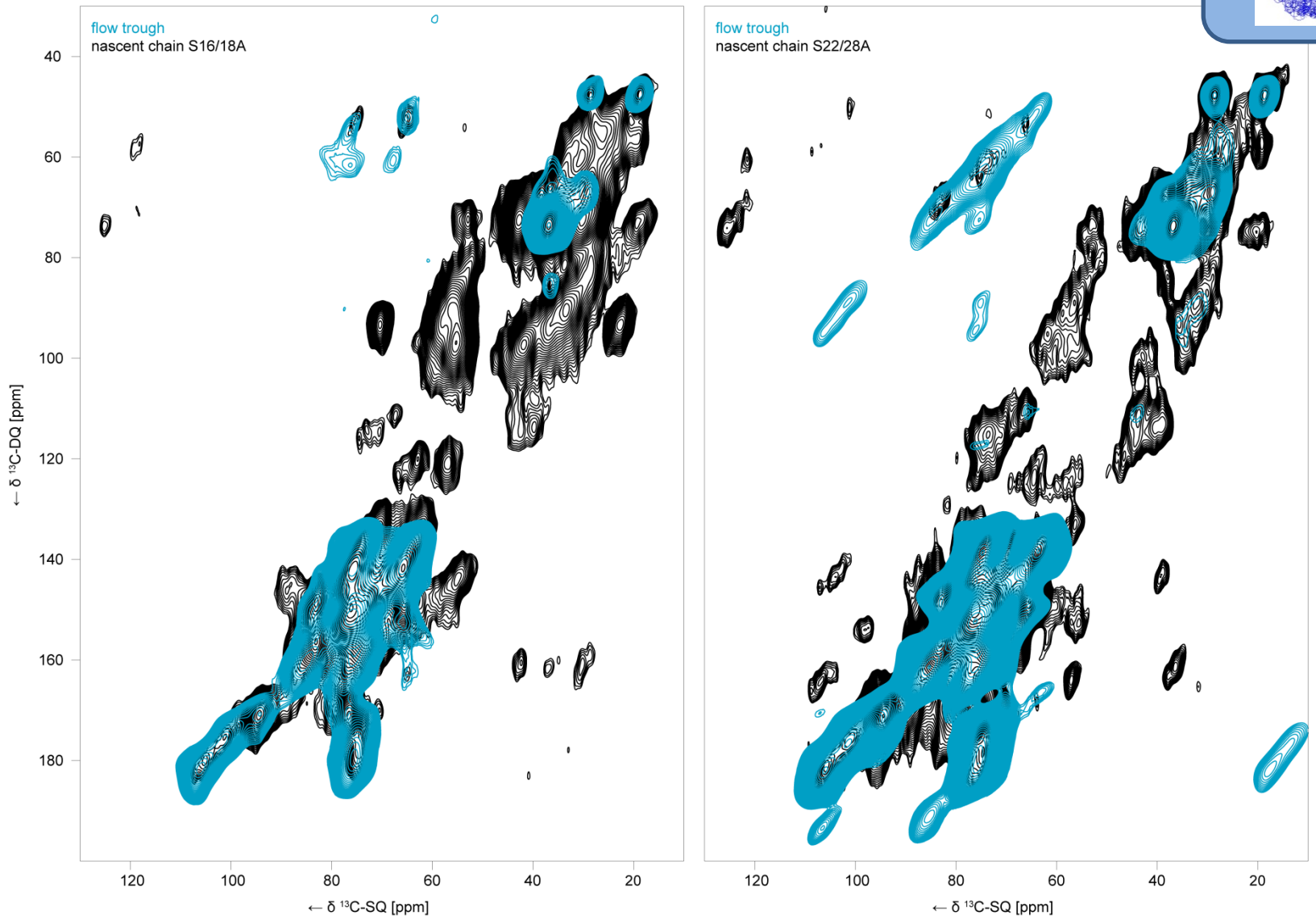


DNP as a Tool for Structural Biology, RNCs

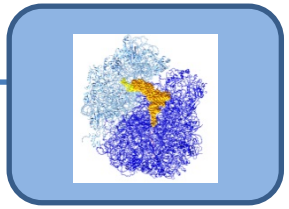


DNP as a Tool for Structural Biology, RNCs

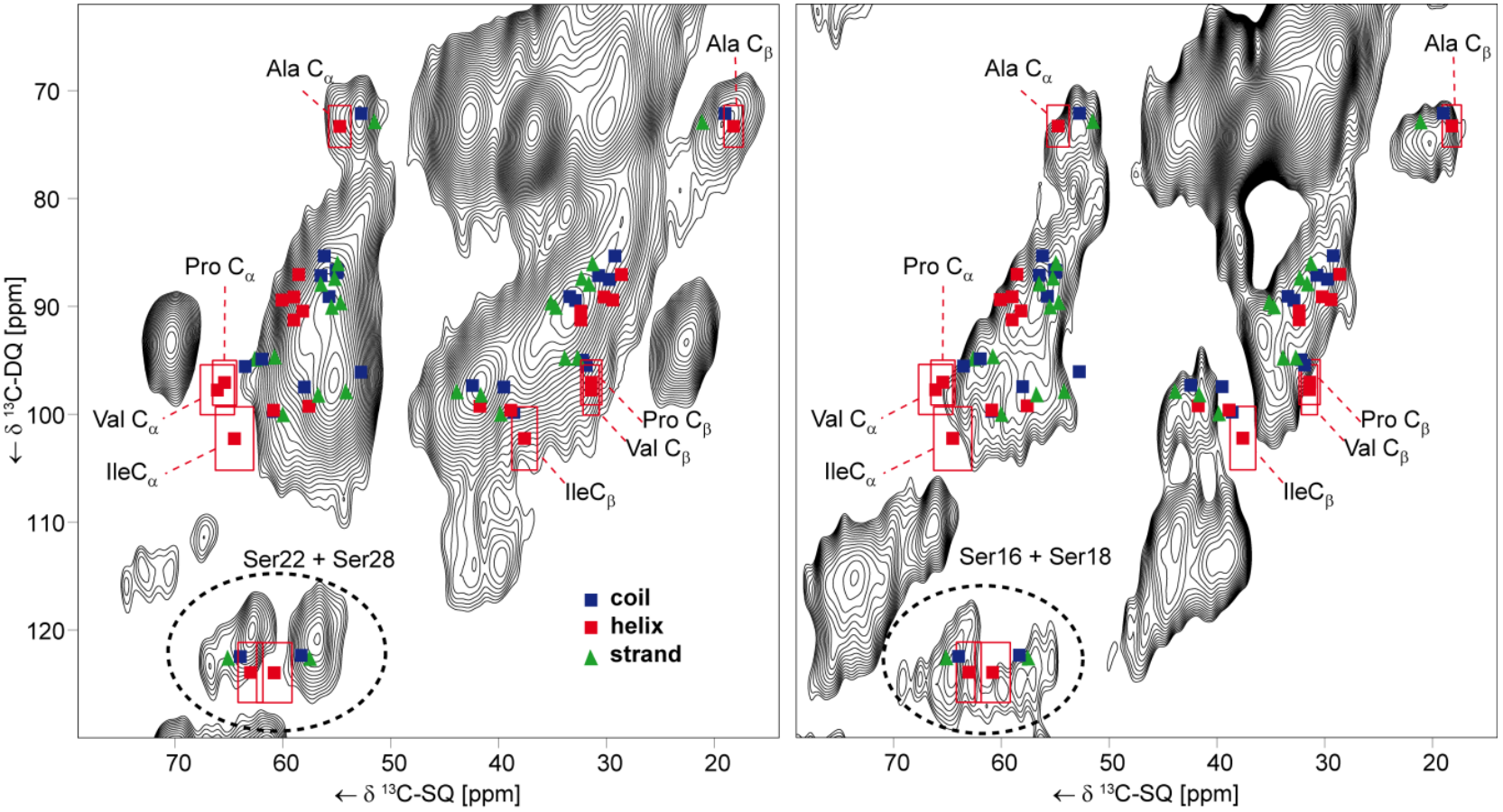




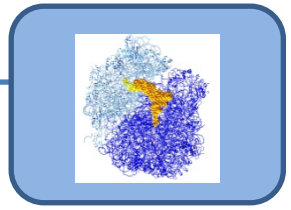
DNP as a Tool for Structural Biology, RNCs



^{1}MKKIWLALAG LVLAF**S**A**S**AA^{20} ^{21}FATPVWI**S**Q AQGIR**S**GP^{37}



DNP as a Tool for Structural Biology ???



there are lot of barriers …

- line broadening is a very big issue (DNP is still blobby)
- short CP times prevent multi-dimensional experiments
- *de novo* assignements ar nearly impossible
- cryo hardware is difficult to maintain

… and construction sites

- new radicals with longer electron relaxation
- deuteration of samples
- sample preparation (glas matrix)
- new systems (more suitable)
- coupling of the radical

…