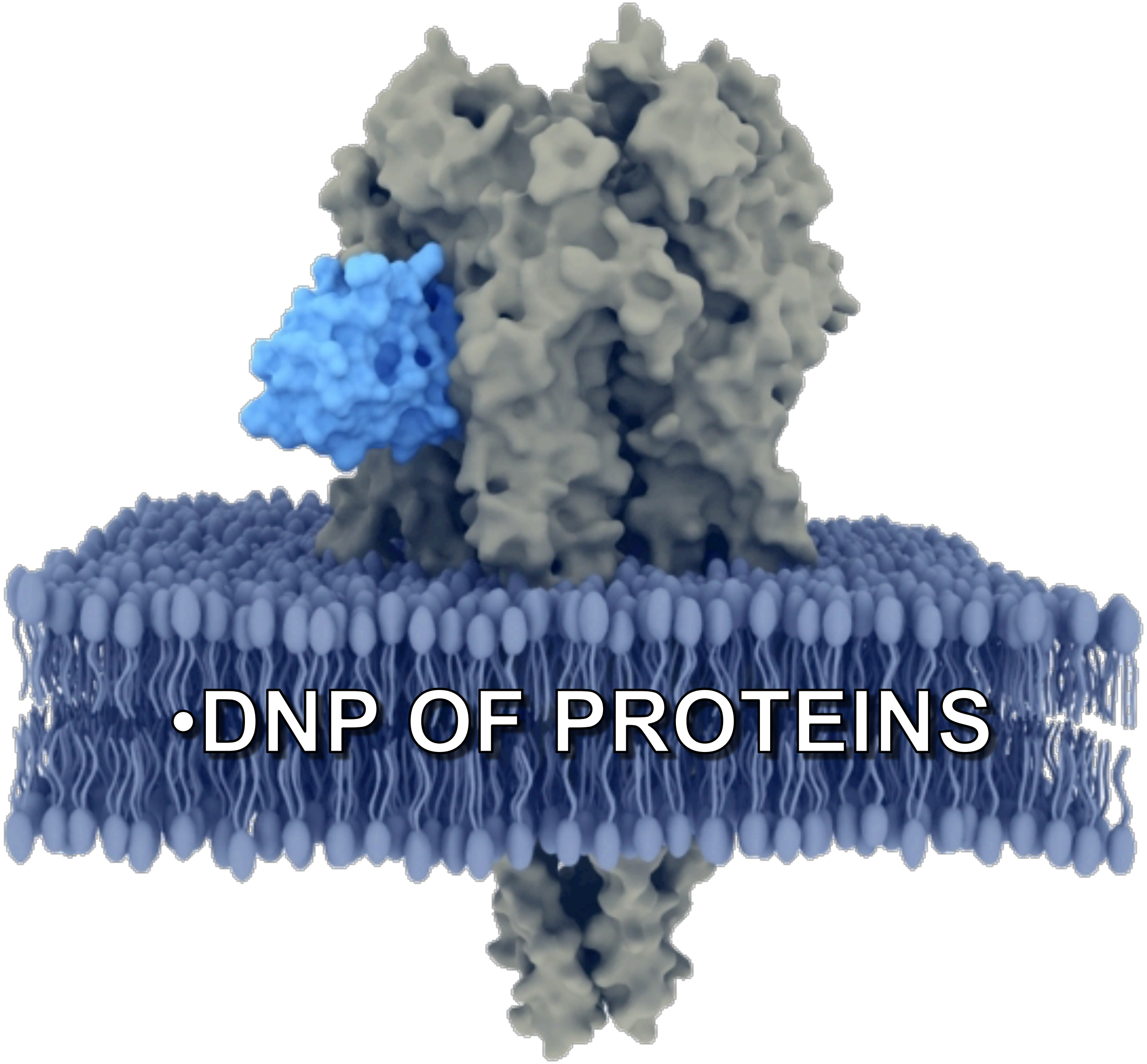
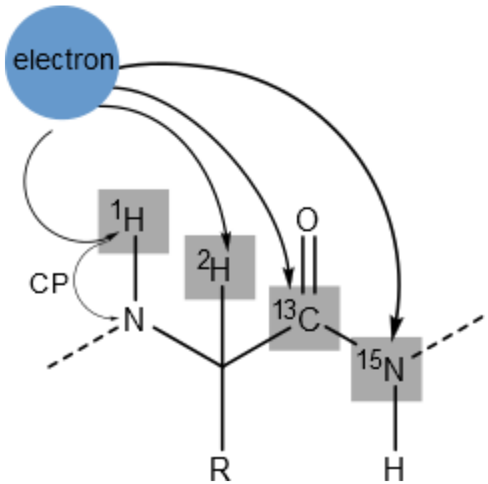
Dynamic Nuclear Polarisation – solid state – NMR

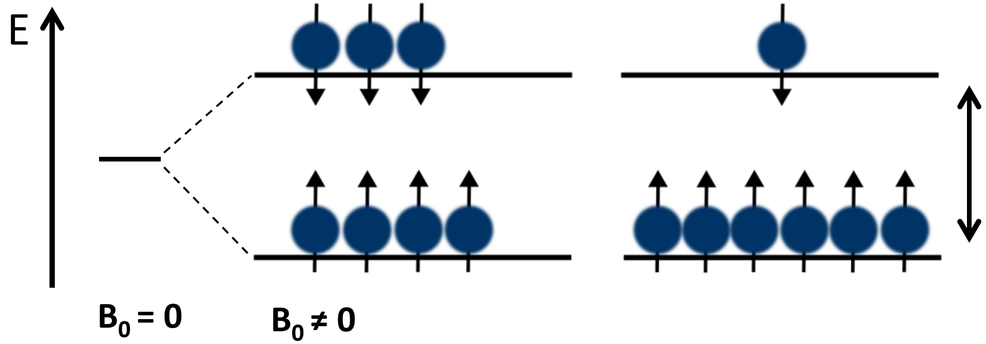
•DNP OF PROTEINS



technique – nACh-Receptor

Dynamic Nuclear Polarization





Polarization

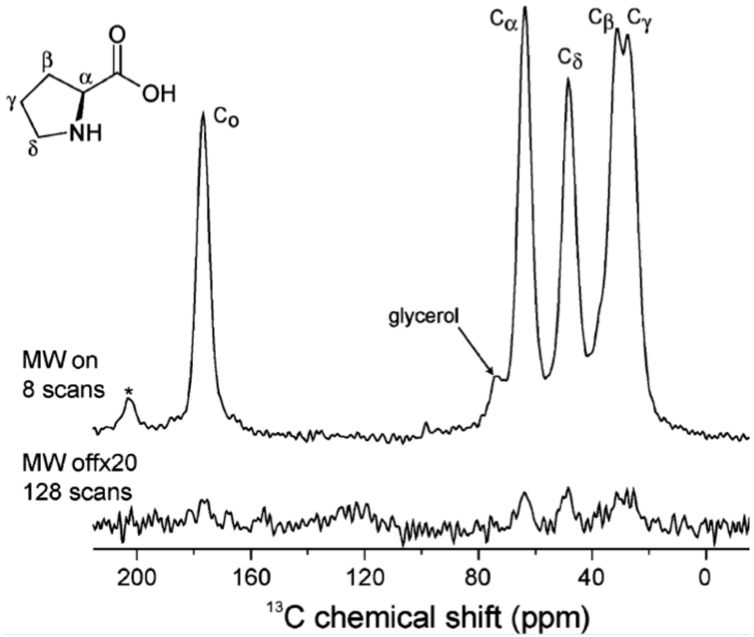
$$P = \frac{n^+ - n^-}{n^+ + n^-} = \tanh\left(\frac{\gamma \hbar B_0}{2kT}\right)$$

P ↑ T ↓ B_0 ↑

$$\varepsilon = 40$$

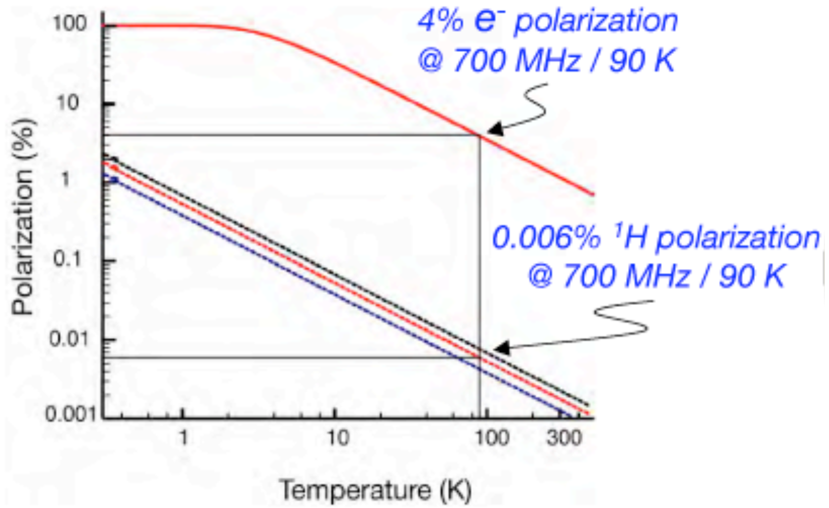
$$\varepsilon^2 = 1600$$

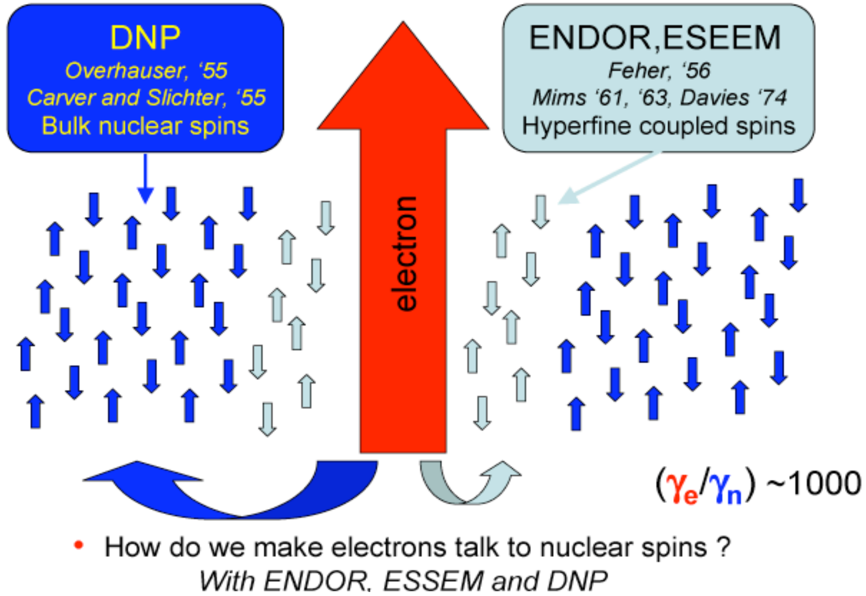
1 h 66 Days



Song *et al.*, *J. Am. Chem. Soc.* **2006**, 128, 11385-11390

Dynamic Nuclear Polarisation (DNP): uses electron spin polarisation

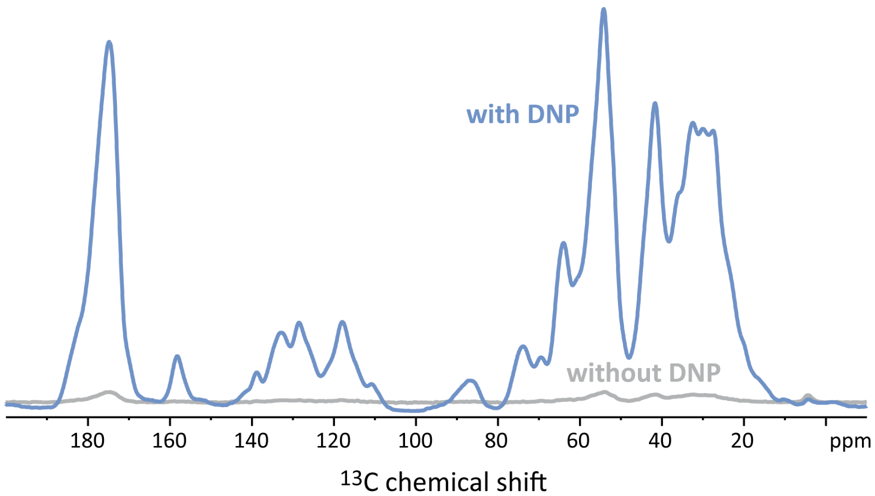




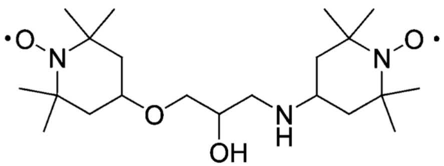
→Structural investigations at picomolar concentration

DNP principle

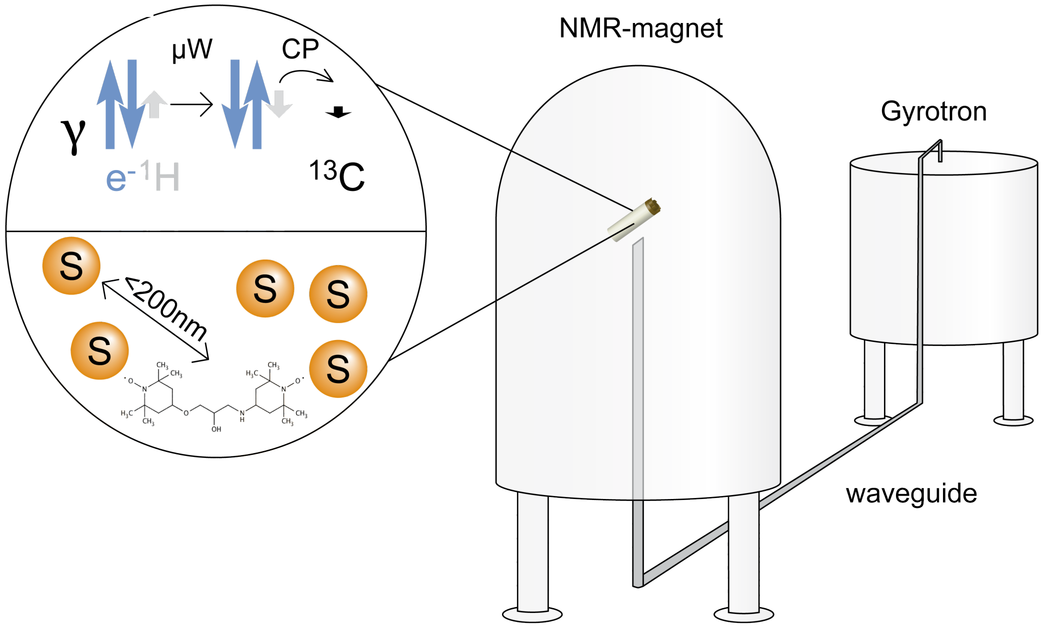
Polarisation transferred from electron to nuclei of interest



needed:
- biradical (TOTAPOL)
- cryogenic temperature (100K)
- microwave irradiation



TOTAPOL



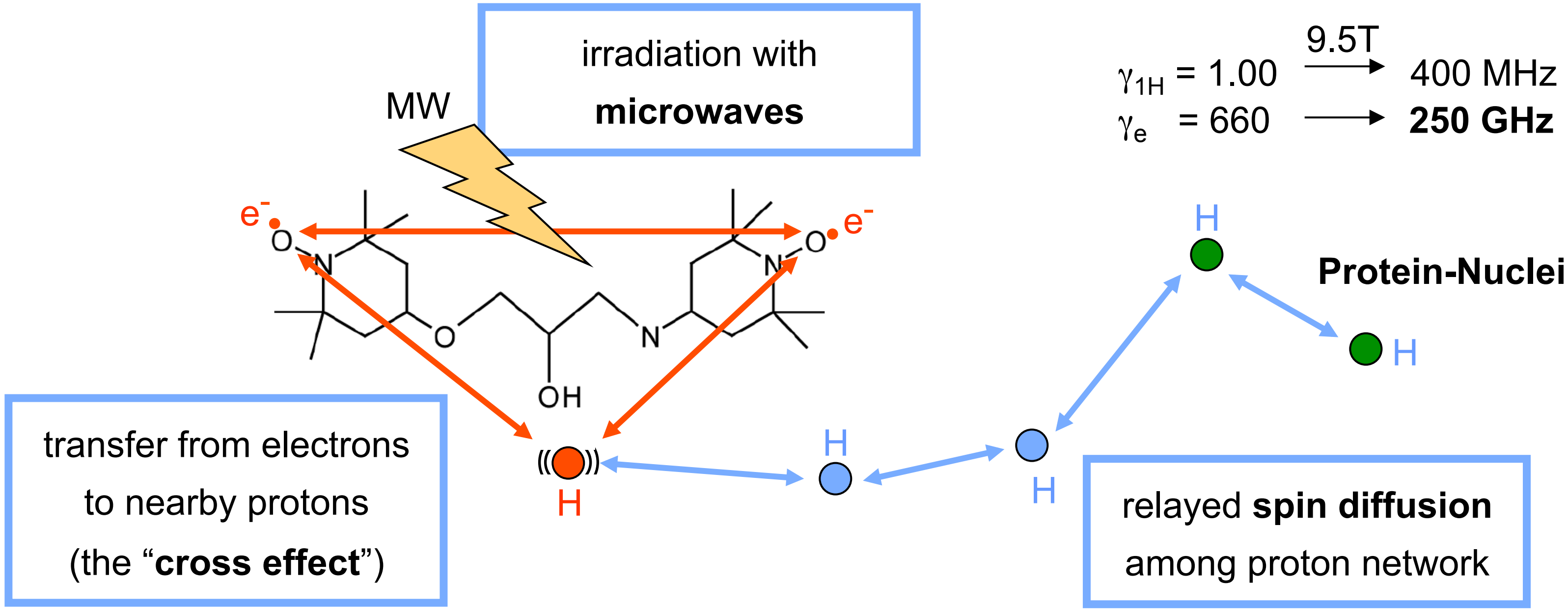
DNP mechanisms

…and electron T1

A few comments to the solid and cross effect mechanisms

DNP principle

Polarisation transferred from electron to nuclei of interest

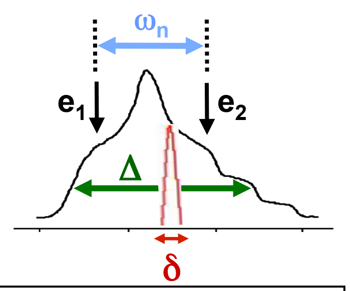


mixture of 60% deuterated glycerol, 30% D_2O, 10% H_2O → provides glass forming matrix

non-glass-forming matrix → crystals formed upon freezing
→ much lower DNP enhancement

(partially) deuterated matrix → more complete polarization of smaller proton reservoir

DNP mechanisms: solid + cross effects



…abbreviated SE and CE

SE: possible when **homogeneous linewidth δ**

and **breadth of spectrum Δ < ω_n**

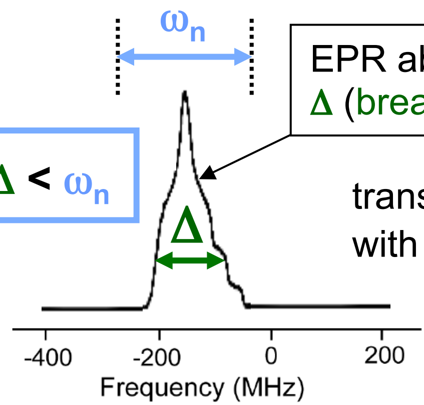
1 **e⁻** ←→ 1 **n**

'forbidden transitions'

(second-order perturbation theory)

transition probability always low

$\delta \sim \Delta < \omega_n$



EPR absorption line shape
Δ (breadth of spectrum) ~ δ

transition probability scales with $1/(\omega_n)^2 = 1/(B_0)^2$

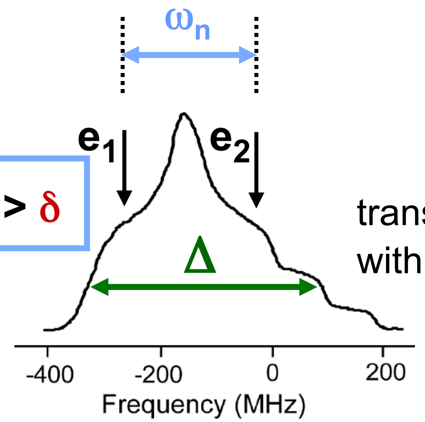
CE: possible when **breadth Δ > ω_n > δ**

2 **e⁻** ←→ 1 **n**

two paired electrons, EPR frequencies separated by Larmor frequency

transition probability can be high at match → energy-conserved flip-flop process

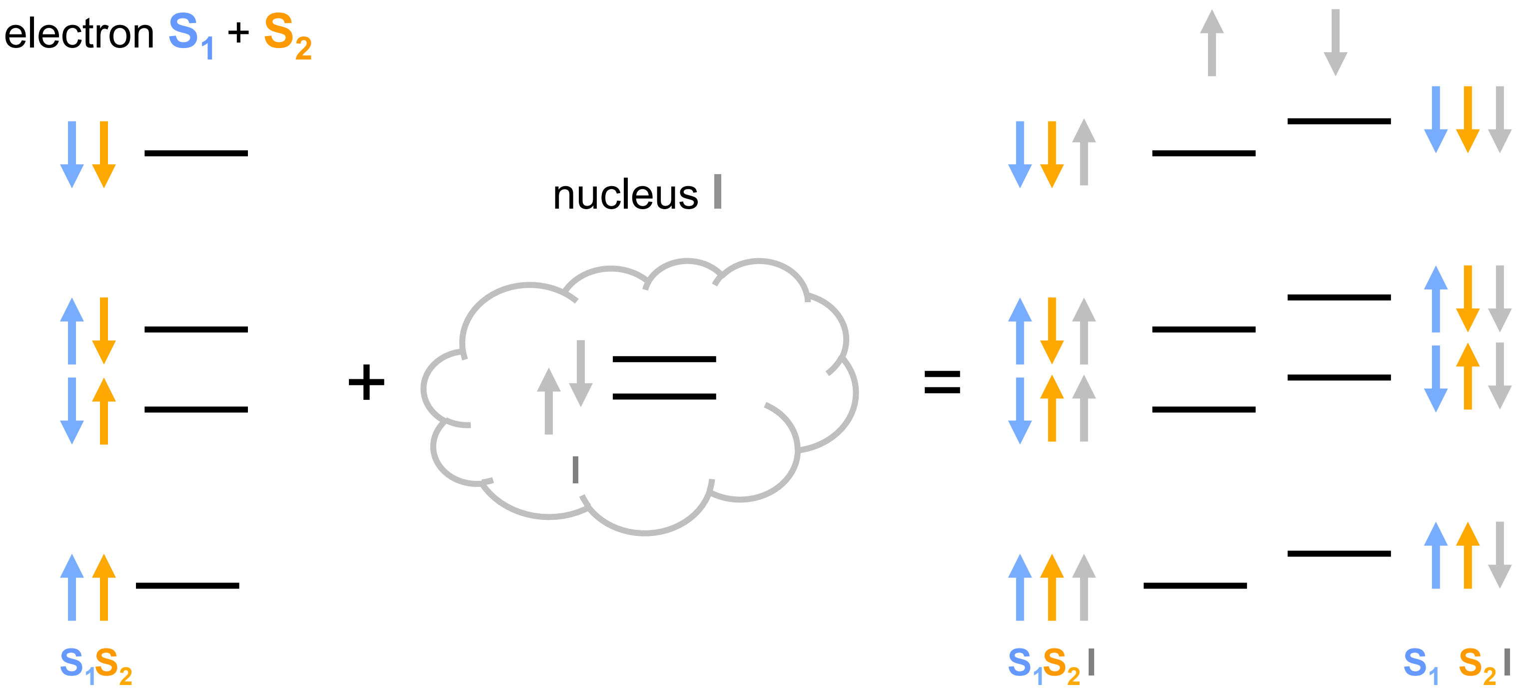
$\Delta > \omega_n > \delta$



transition probability scales with $1/(\omega_n) = 1/(B_0)$

Cross effect DNP

Using a biradical



Cross effect DNP

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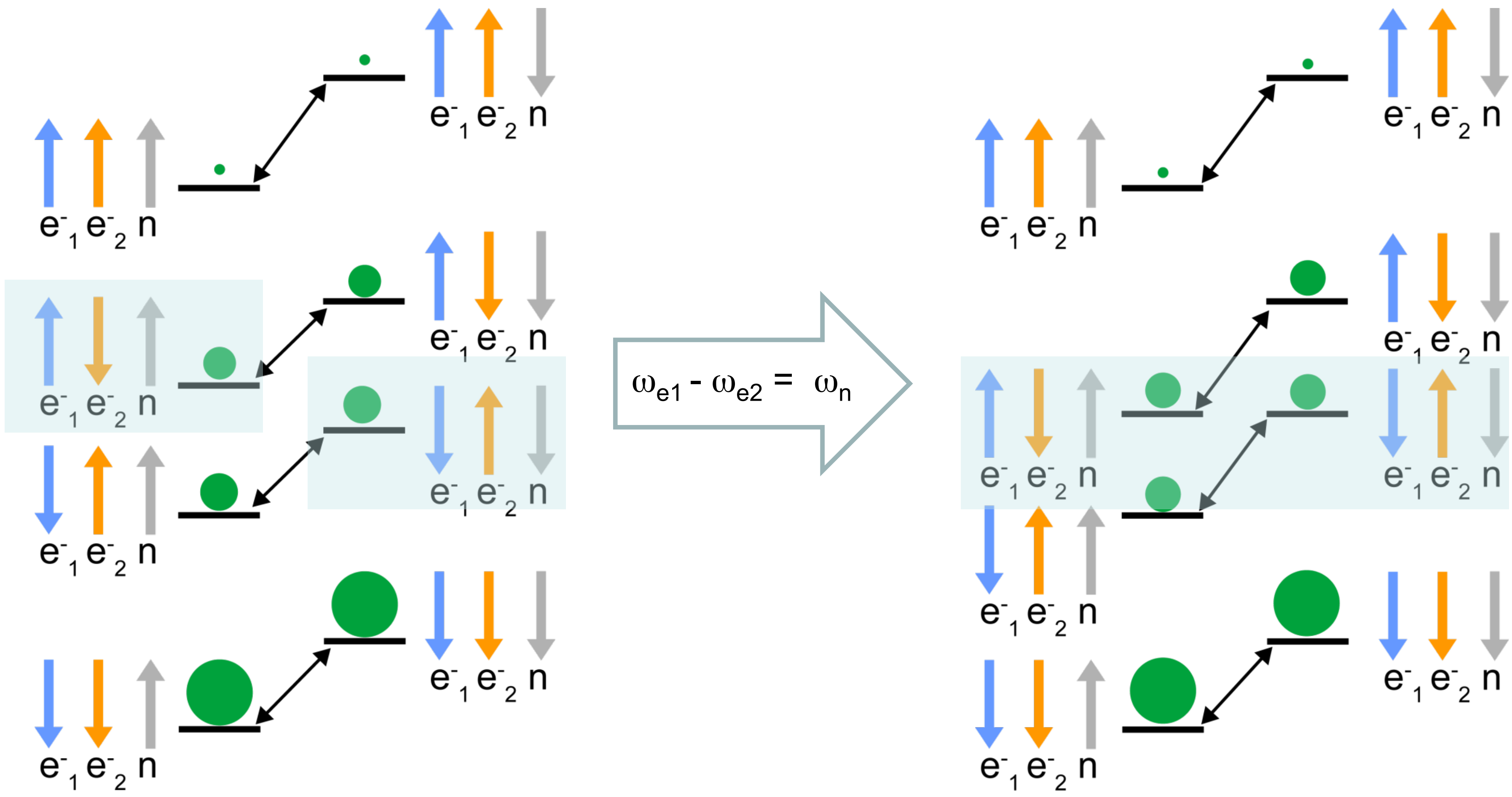

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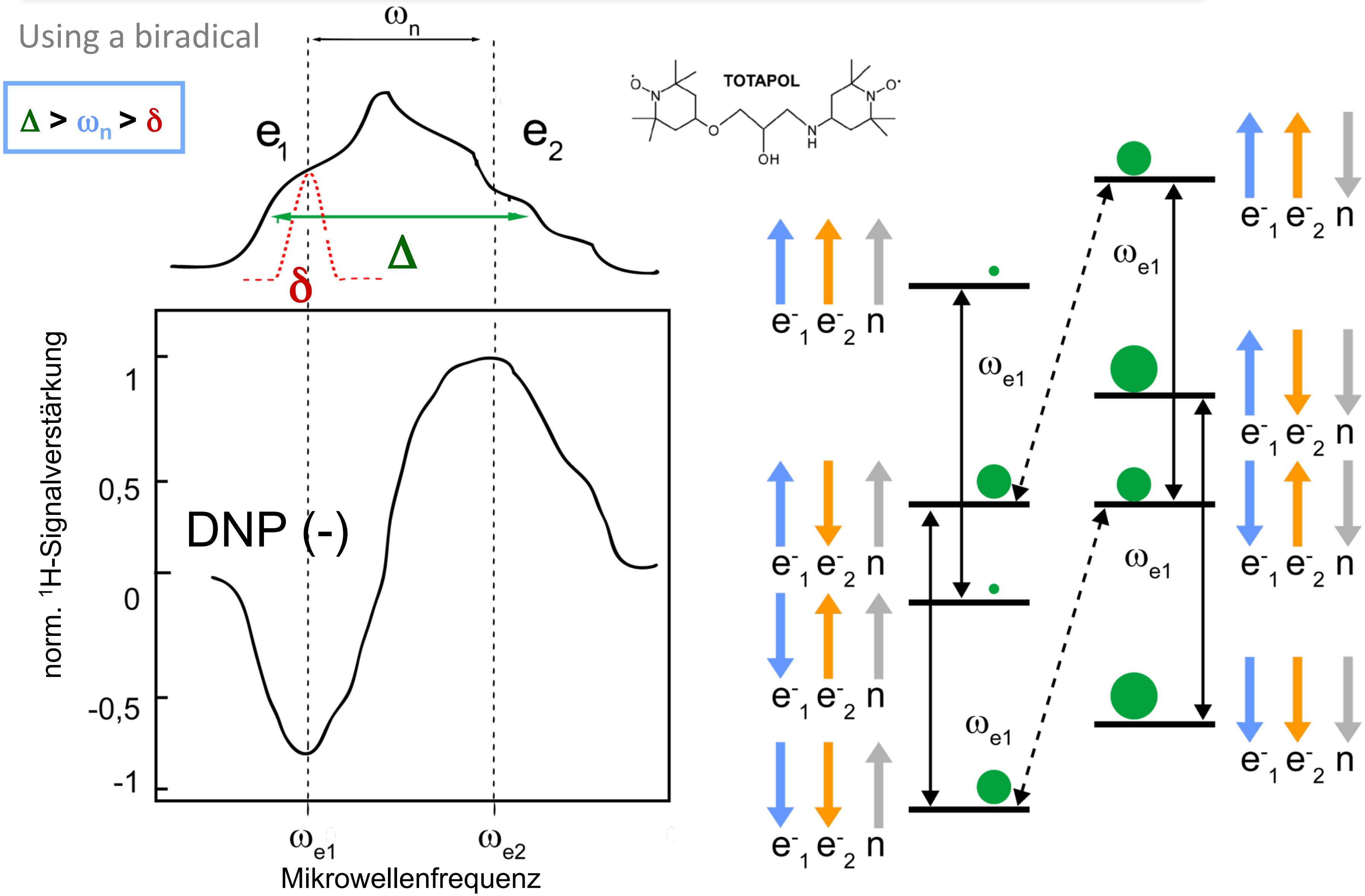
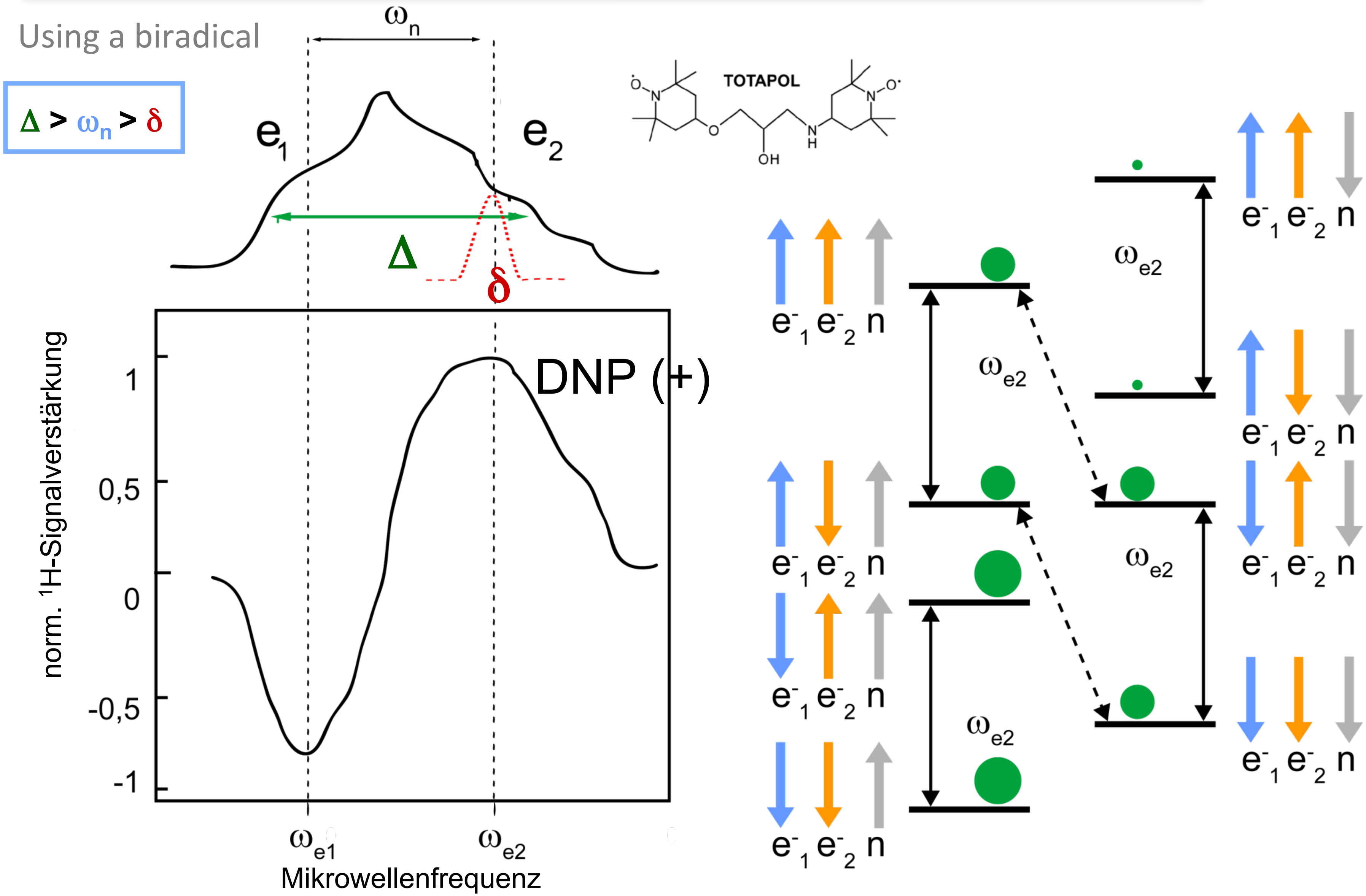
Cross effect DNP

Using a biradical



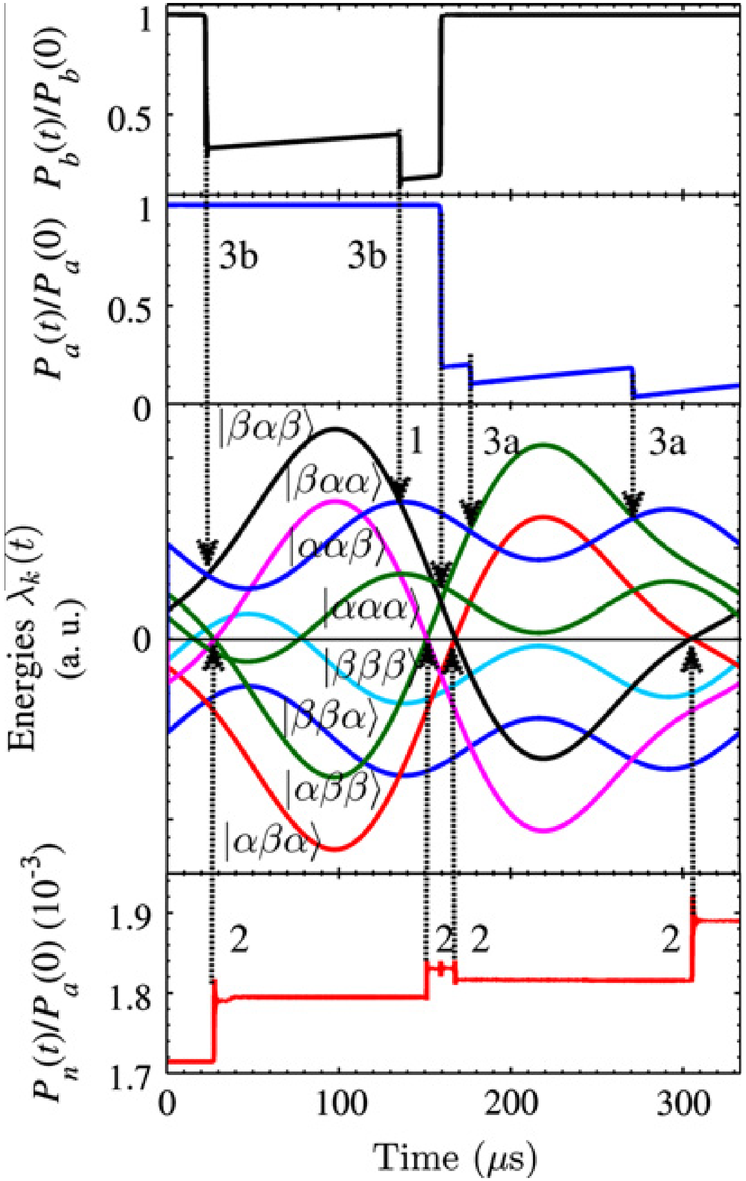
Cross effect DNP

Using a biradical



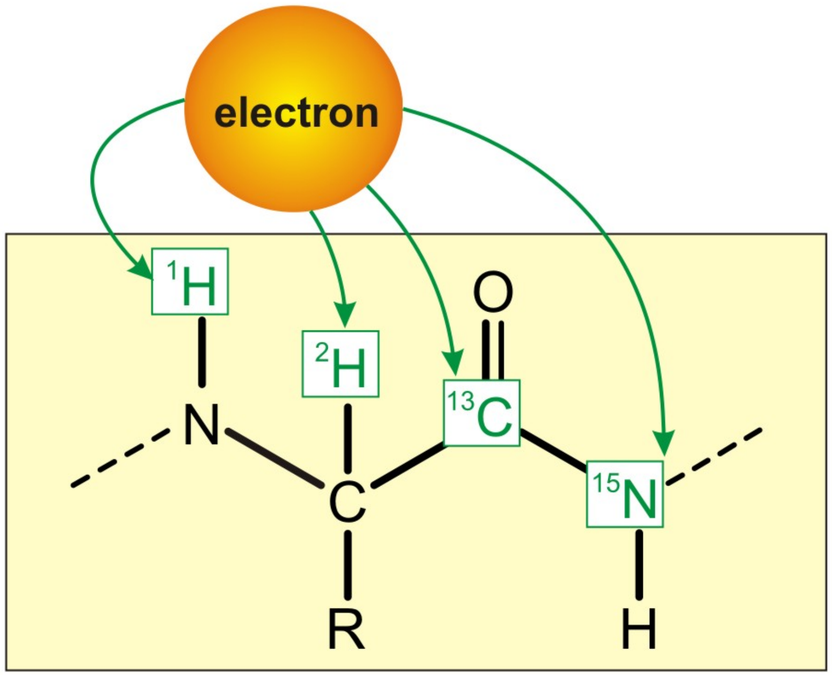
Cross effect transfer upon rotation

Varying matching conditions

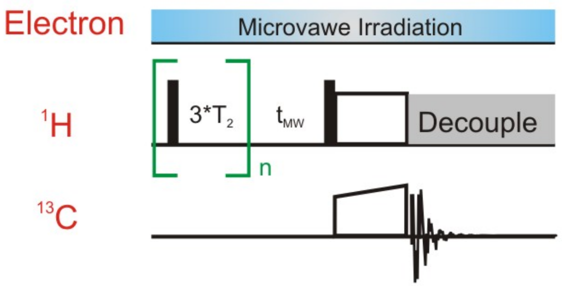


Which nuclei to excite best?

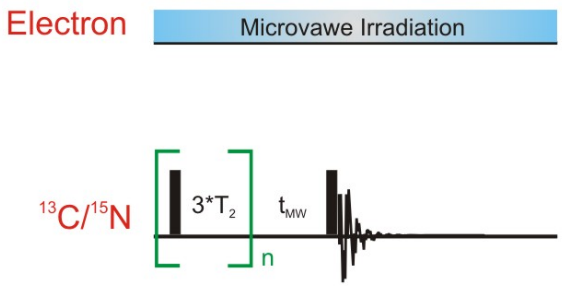
SH3 domain



For Proton:

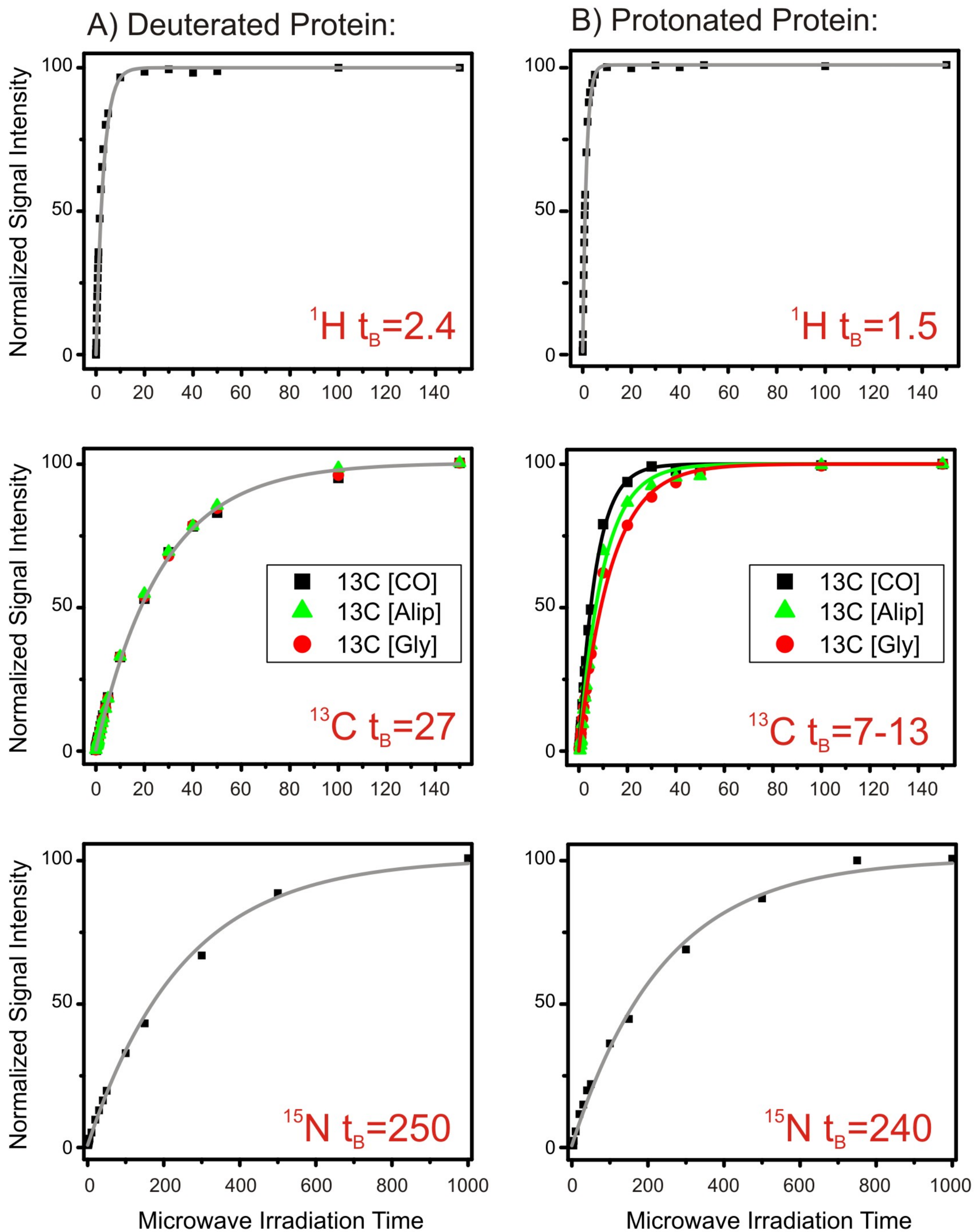


For Heteronuclei:



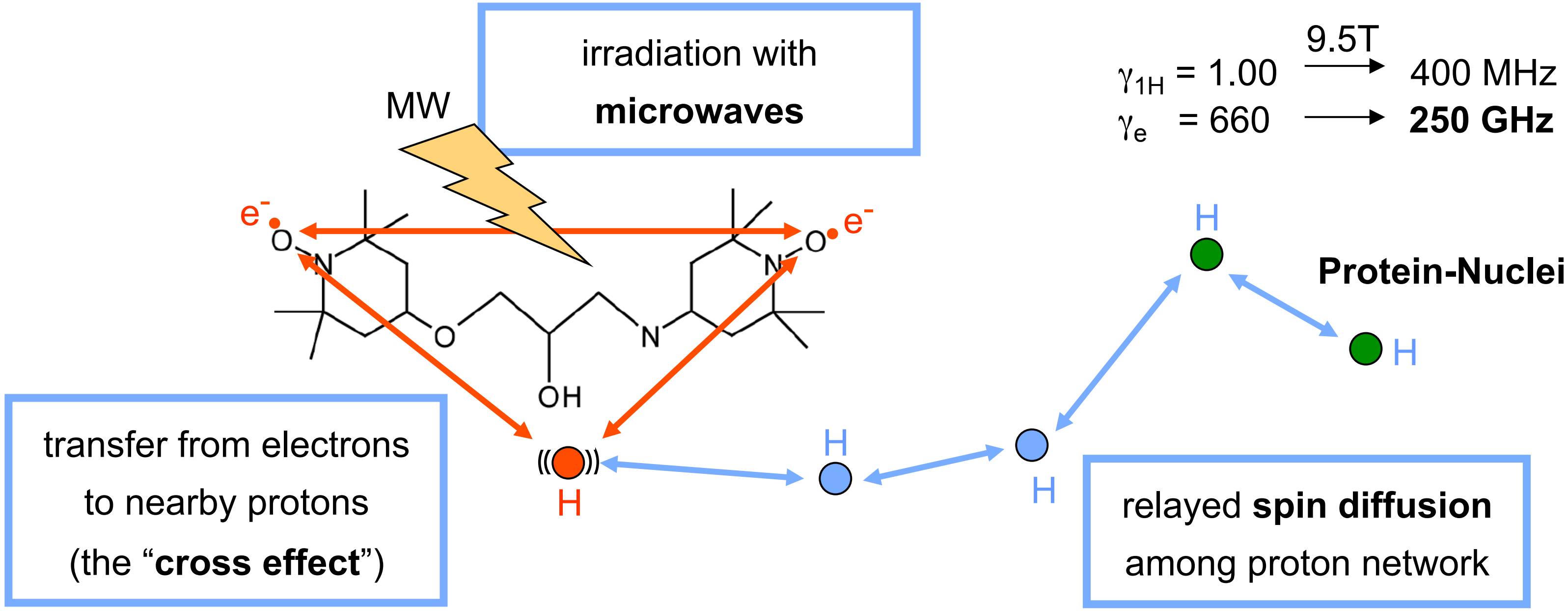
Polarisation build-up in [s]

- Buildup behavior is different for diff. Nuclei
- $^{1}H < ^{13}C << ^{15}N$
- Deuteration increases the τ_B significantly



DNP principle

Polarisation transferred from electron to nuclei of interest



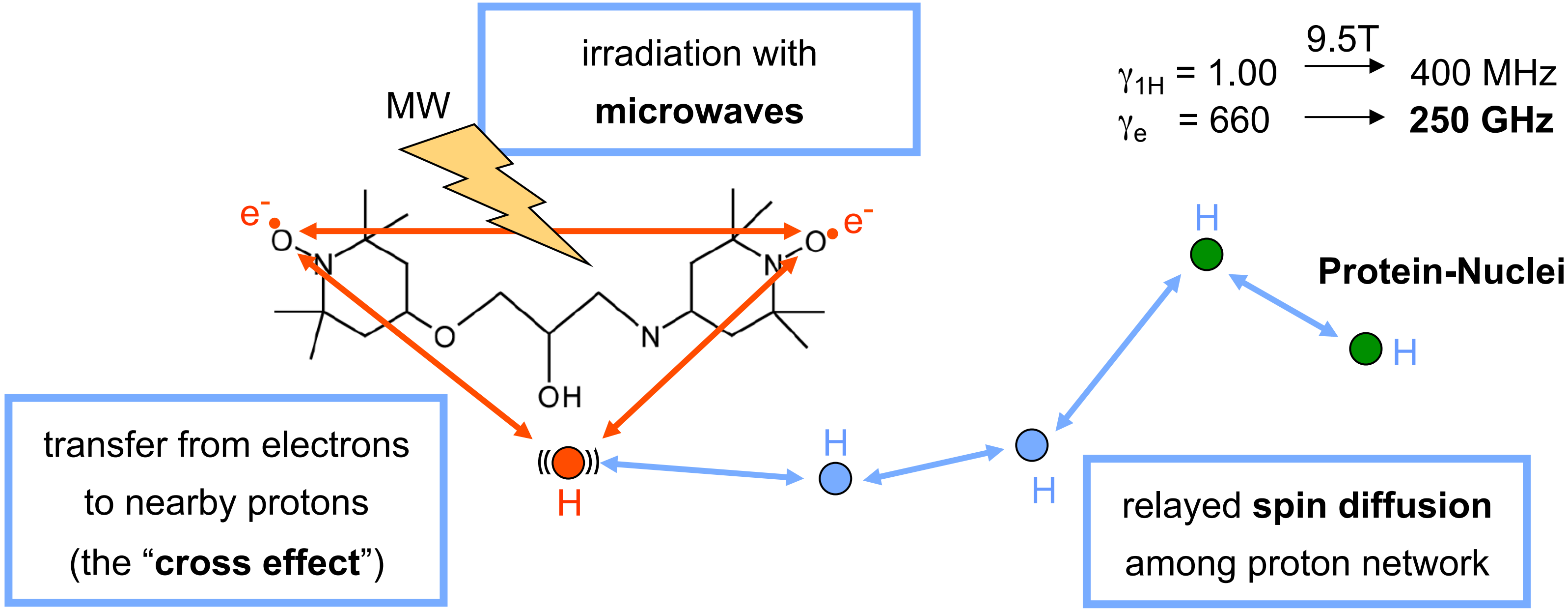
To be optimized: Electron relaxation times for saturation of lines

Nuclear T_1 and T_2 for

- Effectivity of spin diffusion
- The buildup rate of polarisation
- Effectivity of CP
- T_1 for the repetition rate of the experiment

DNP principle

Polarisation transferred from electron to nuclei of interest

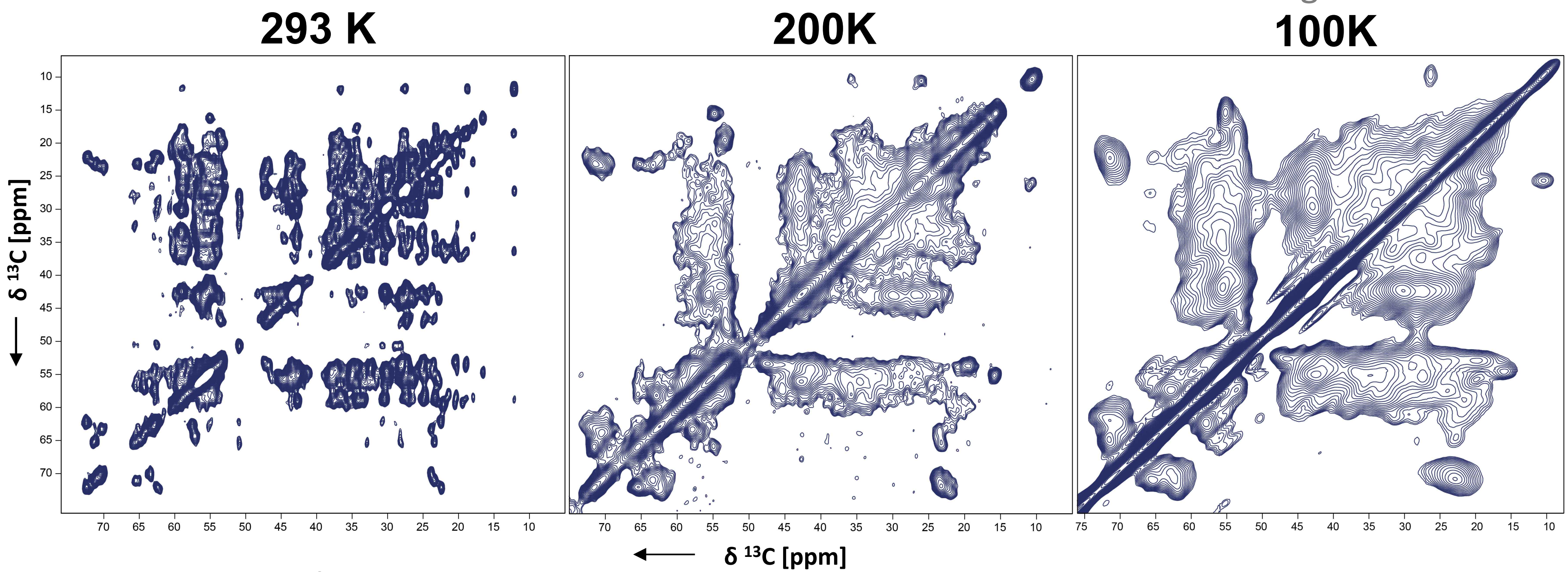


To be optimized:

- Microwave strength
- Radical type and concentration
- Measurement temperature
- Proton concentration, degree of deuteration
- Glass-forming properties of the matrix
- MAS rate

Towards a structure by DNP: better samples and higher temperature

- Hindered rotations of side chains and PRE effects contribute to line broadening



(No radical/no glycerol)

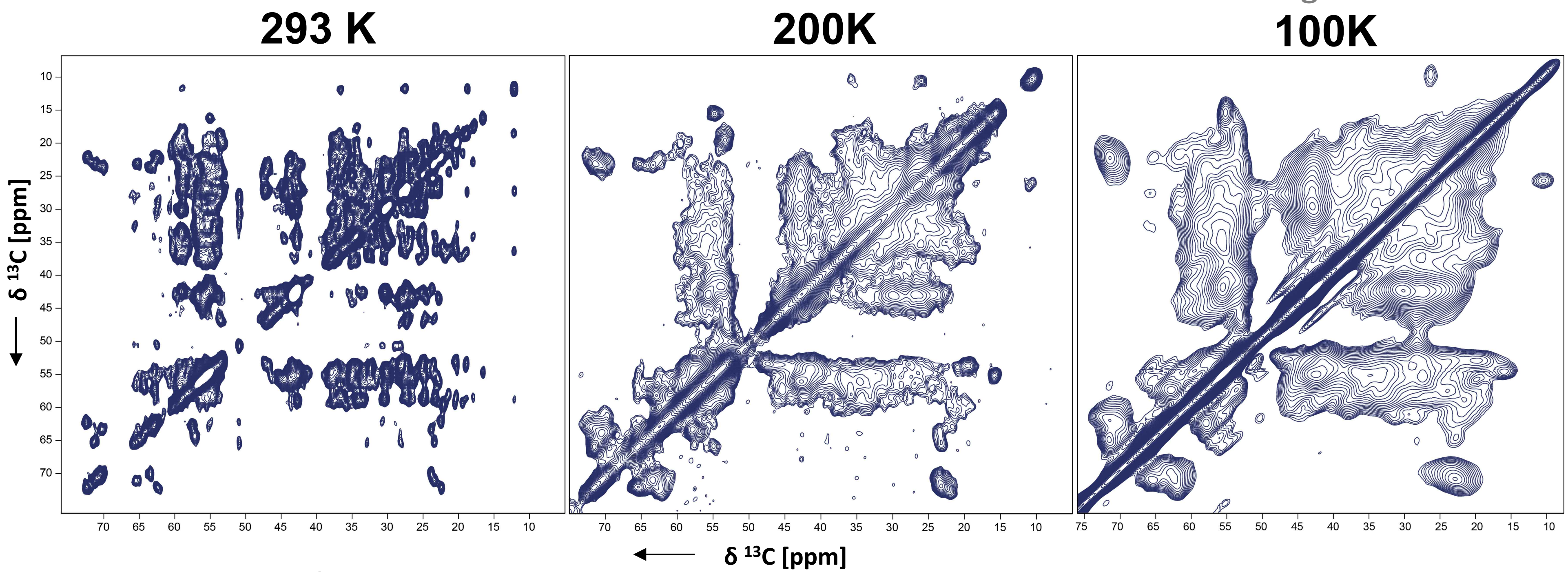
Boltzmann (x2)

Decrease of thermal noise

Enhancement

Towards a protein structure: better samples and higher temperature

- Hindered rotations of side chains and PRE effects contribute to line broadening



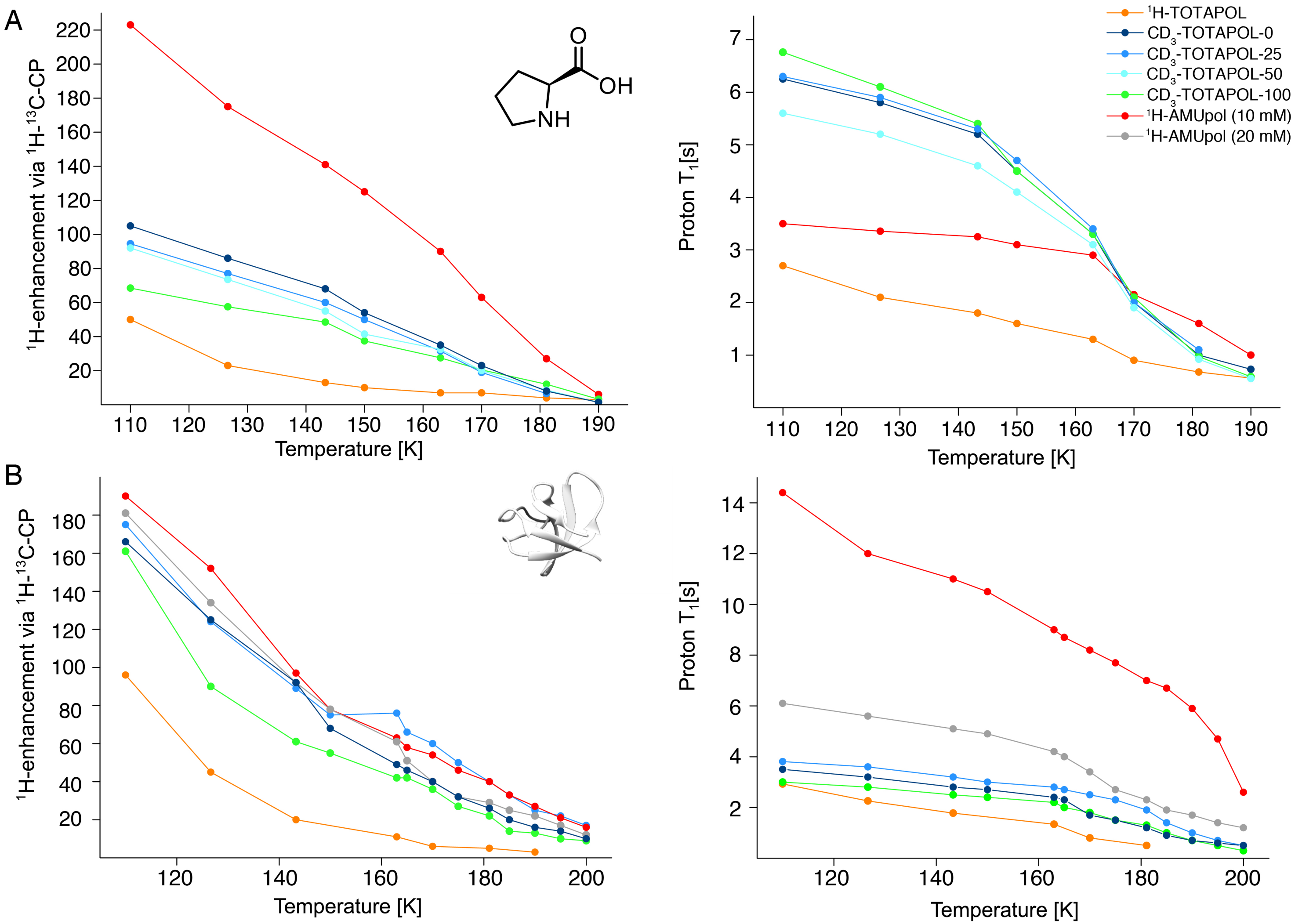
(No radical/no glycerol)

Decrease of line width ←

Decrease of T1 ←

Enhancement vs. T curves using proline and SH3 standards

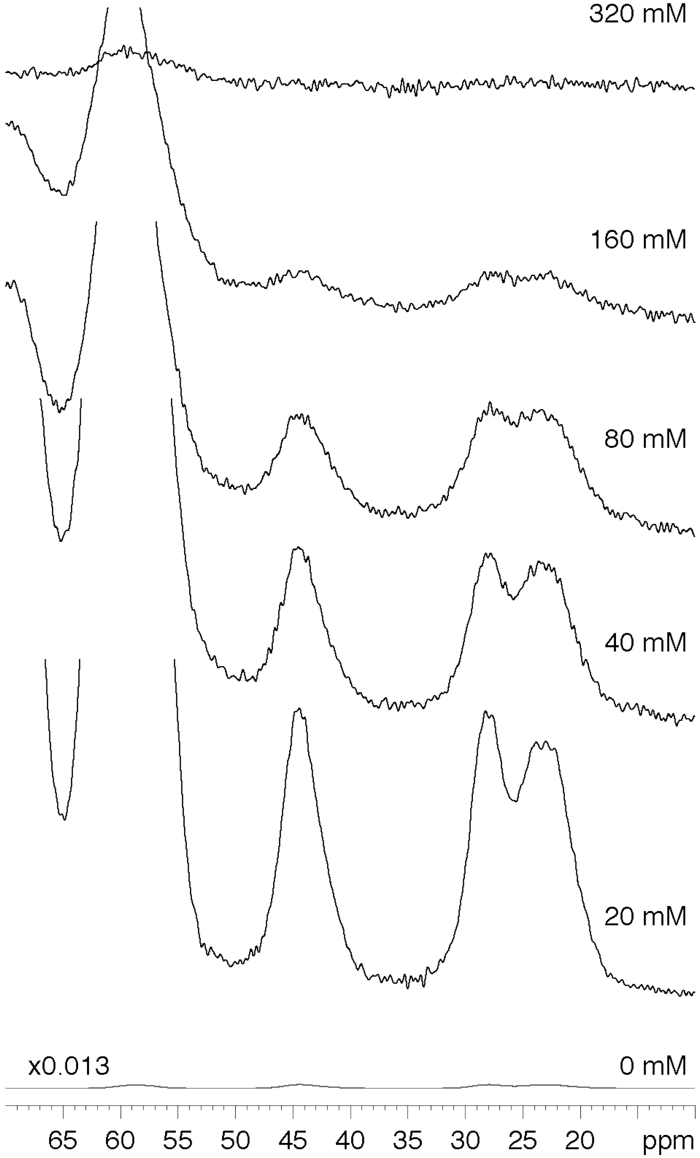
Enhancements up to 45 at 180 K



The influence of radical concentration

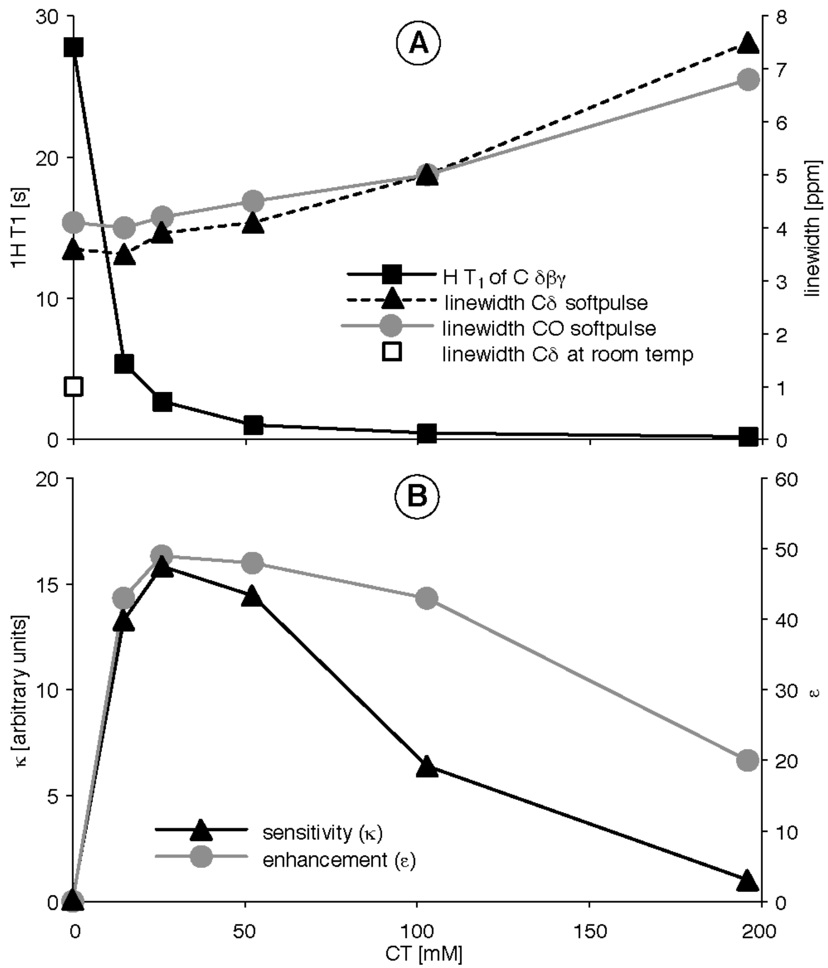
Effect of the biradical concentration on EFFECTIVE enhancement

SH3 domain carbon spectra, methyl region

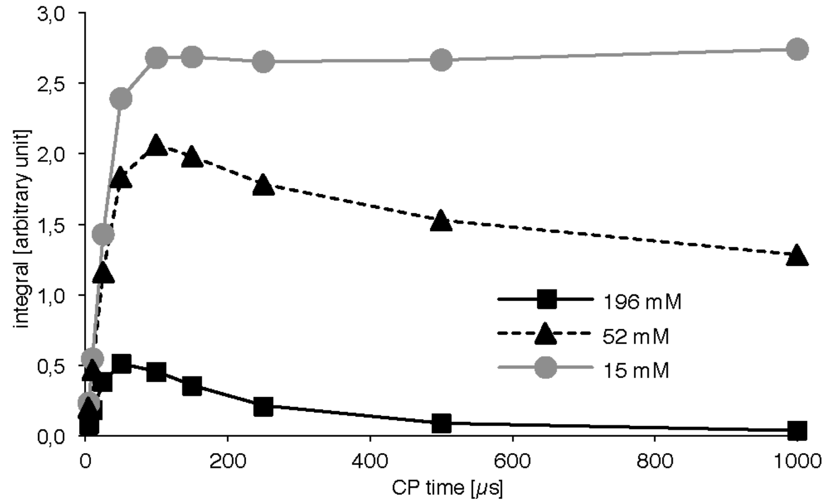


Effect of the biradical concentration, Proline

T_1, linewidth, sensitivity

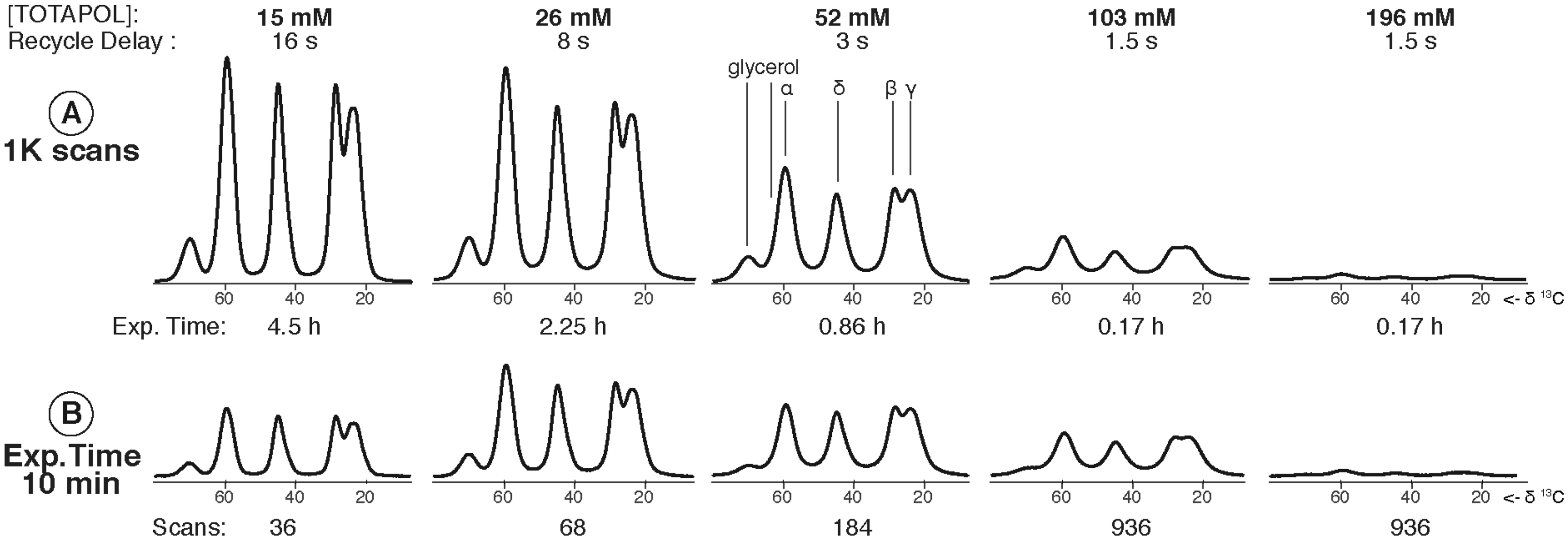


CP efficiency

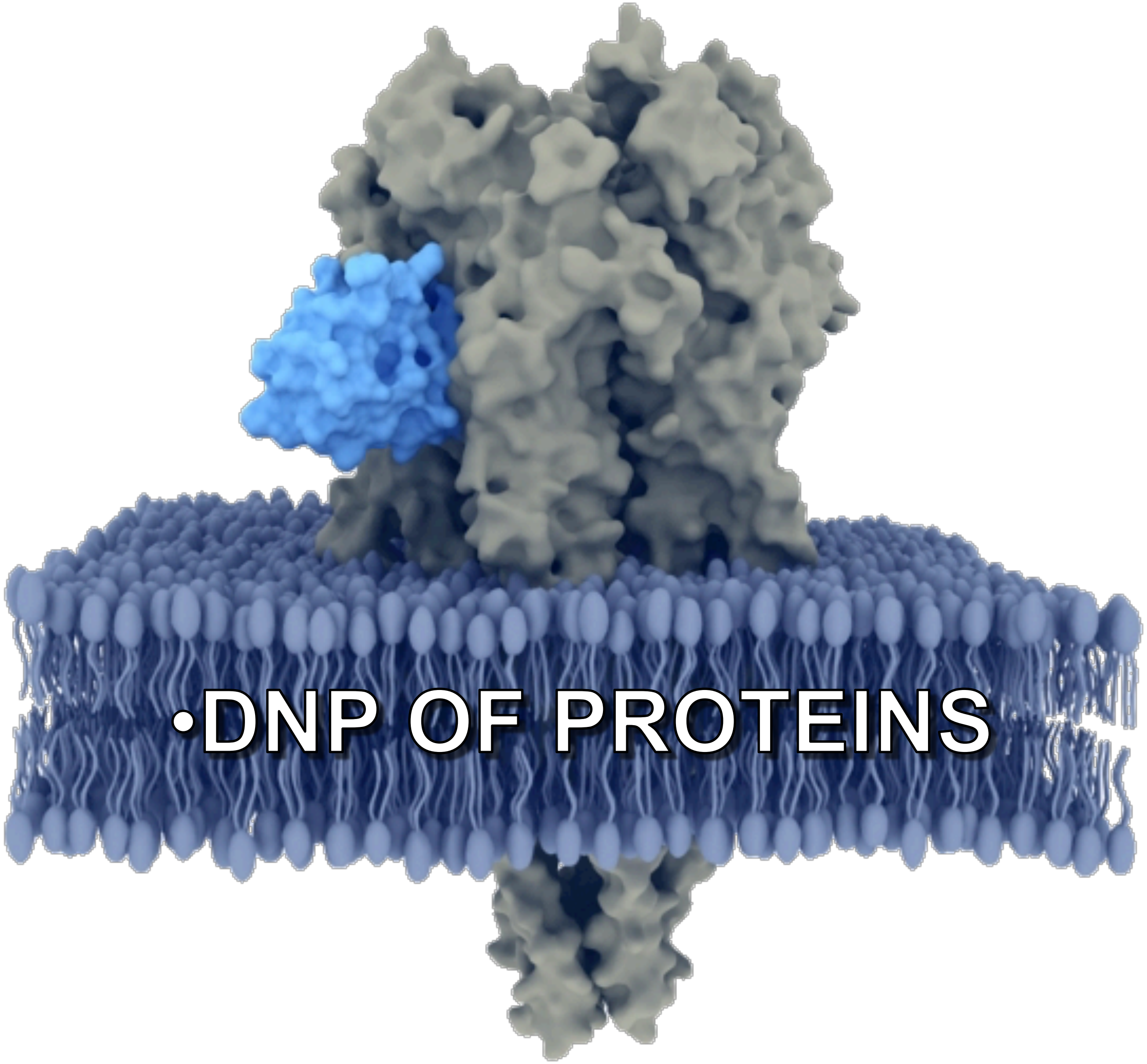


Effect of the biradical concentration, proline

high concentrations broaden signals and lower their intensities



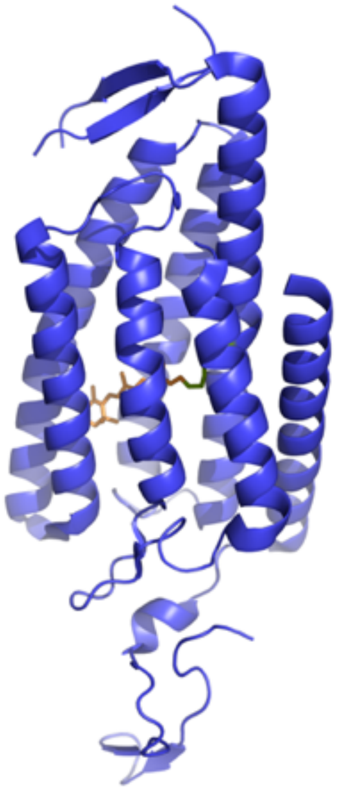
Dynamic Nuclear Polarisation – solid state – NMR



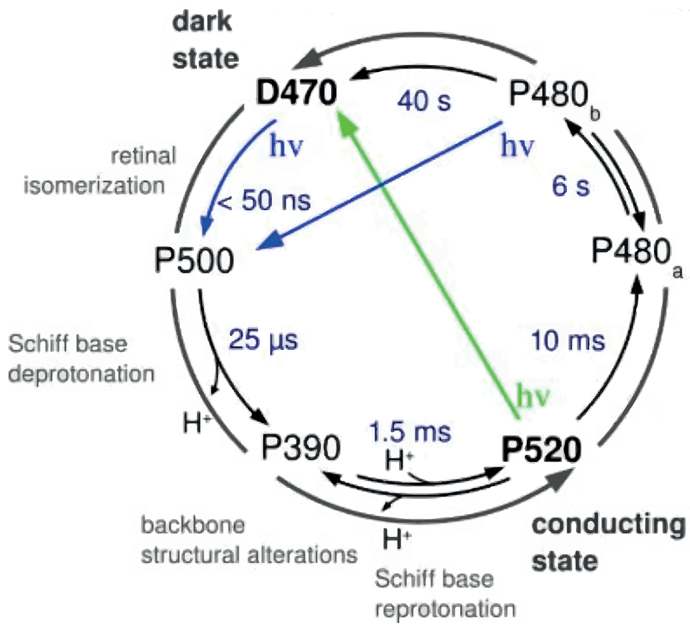
technique – nACh-Receptor

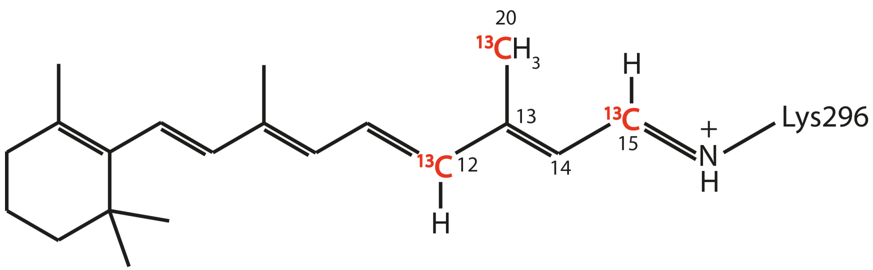
Channelrhodopsin: chromophor configuration all-trans?

•Mixture of 13cis,15syn and all-trans expected



Channelrhodopsin (ChR)
384 aa, (pdb: 3UG9)



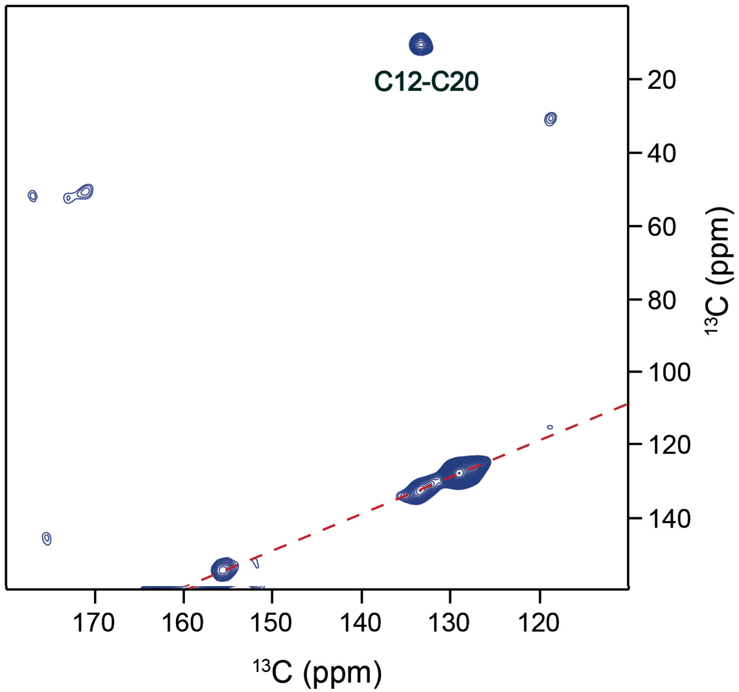


12-15-20-^{13}C-retinal
all *trans* isomer

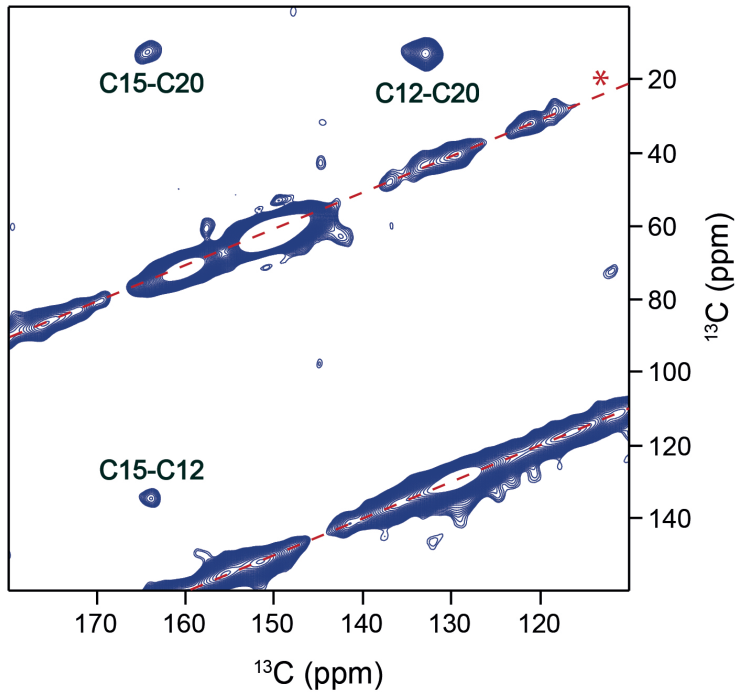
Solid-state NMR works only with DNP!

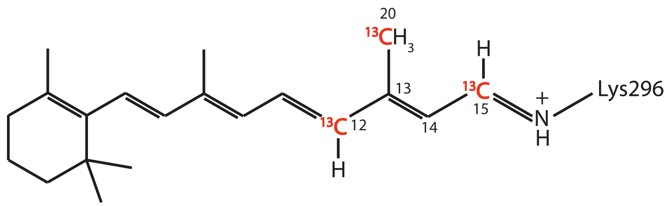
- Mixture of 13cis,15syn and all-trans expected

standard solid-state MAS NMR
(DARR, 265 K, ~ 10 days)



DNP solid-state MAS NMR
(DARR, 100 K, ε = 9, ~ 10 hours)





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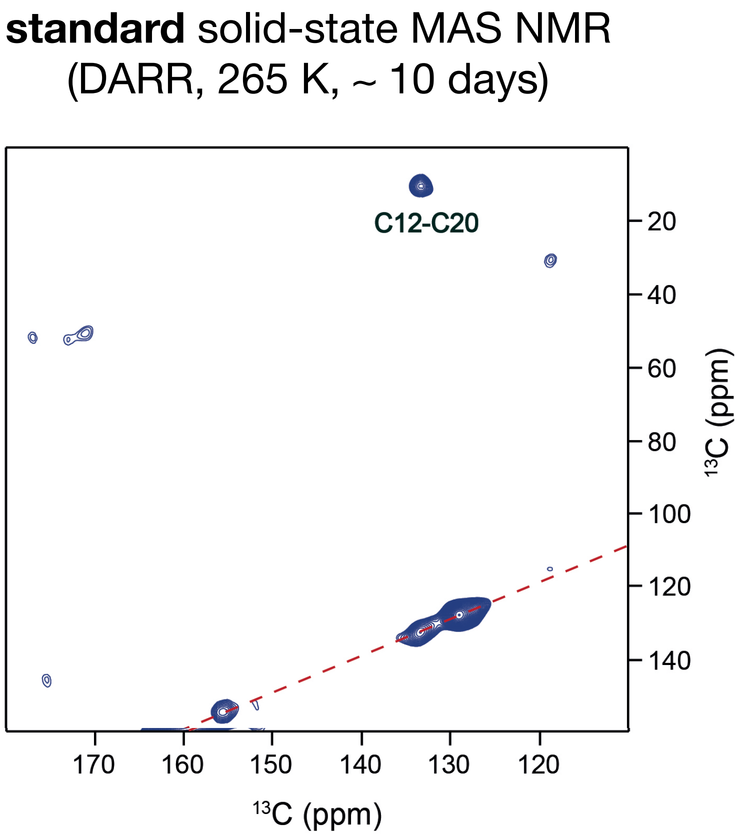
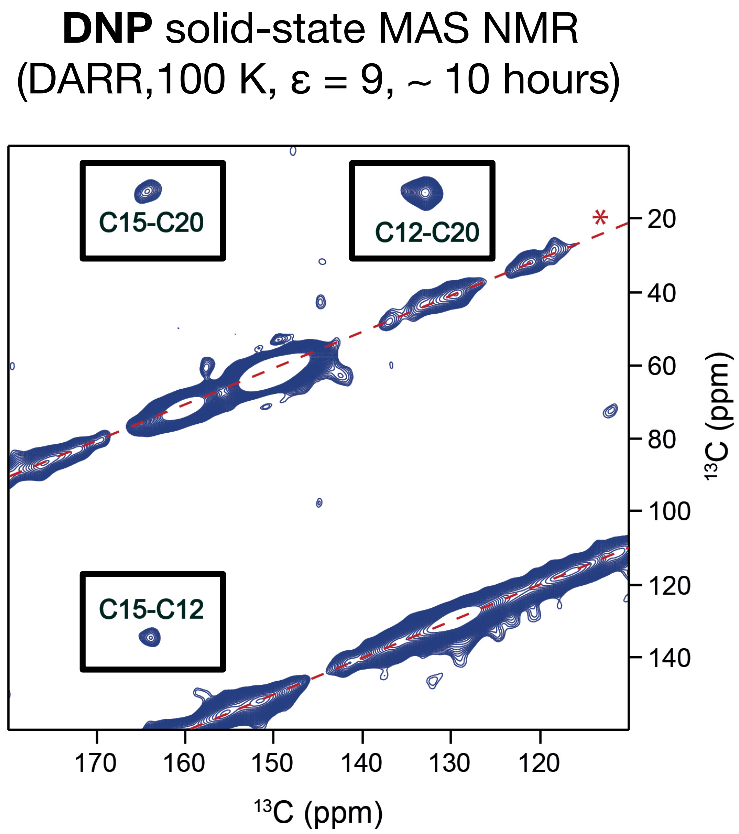
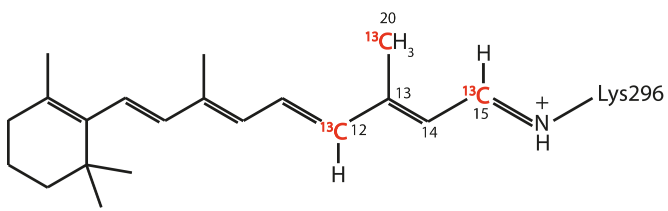
Solid-state NMR works only with DNP!

- Mixture of 13cis,15syn and all-trans expected



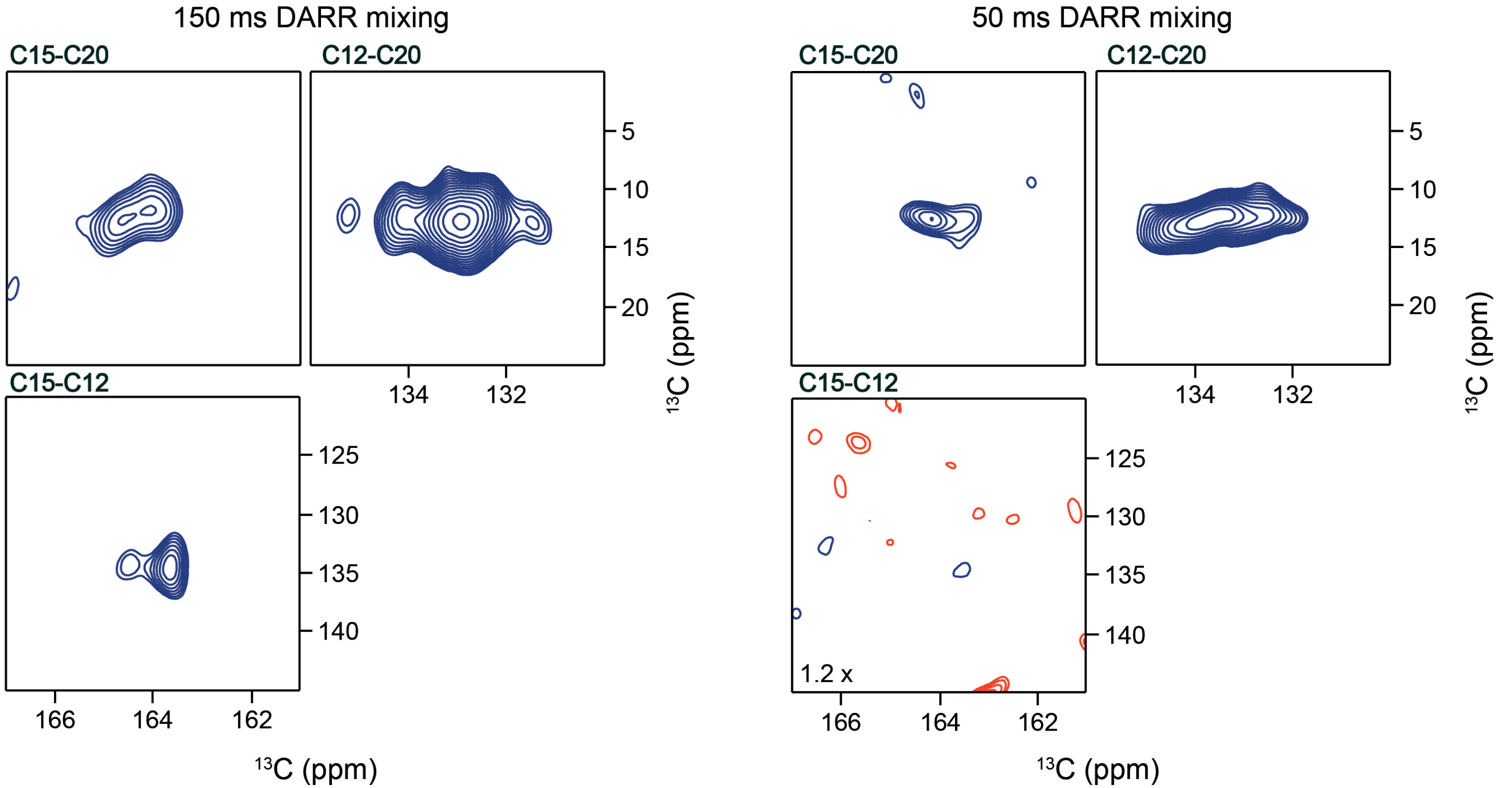


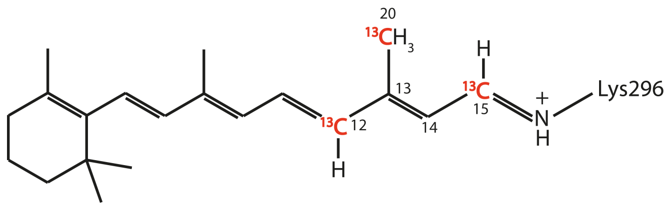


12-15-20-^{13}C-retinal
all *trans* isomer

The fully dark-adapted state of ChR is only all-trans

- Mixture of 13cis,15syn and all-trans expected, however, only all-trans observed





12-15-20-^{13}C-retinal
all *trans* isomer

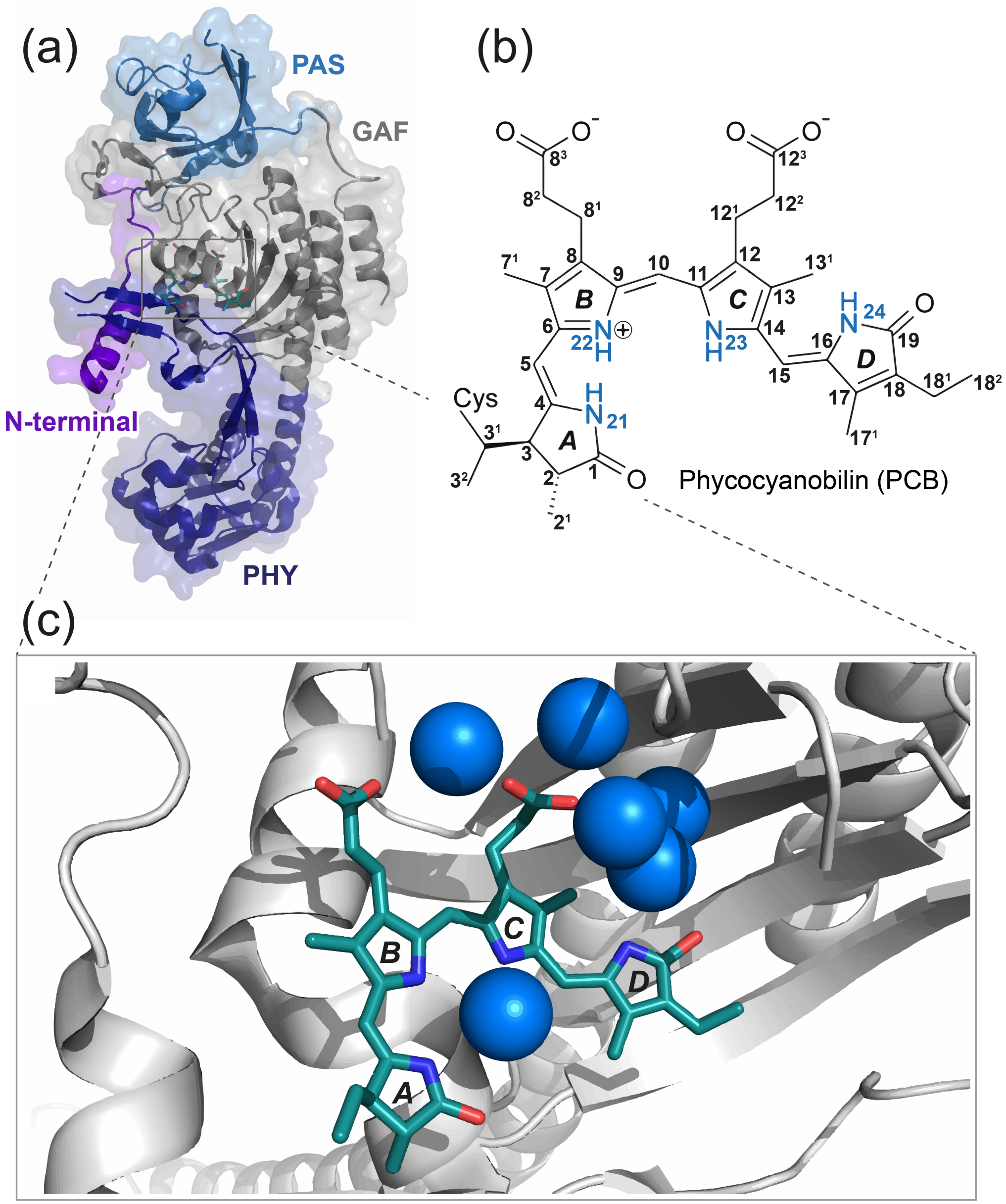
Phytochrome Cph1: assignment of PCB with the help of DNP

Where is the positive charge?

Phytochromes switch 25 % of all genes (in plants)

Cph1delta2 has 515 aa

A solution of Cph1 was investigated in a mixture of 60% d8-glycerol, 30% D2O, 10 %H2O at 110 K



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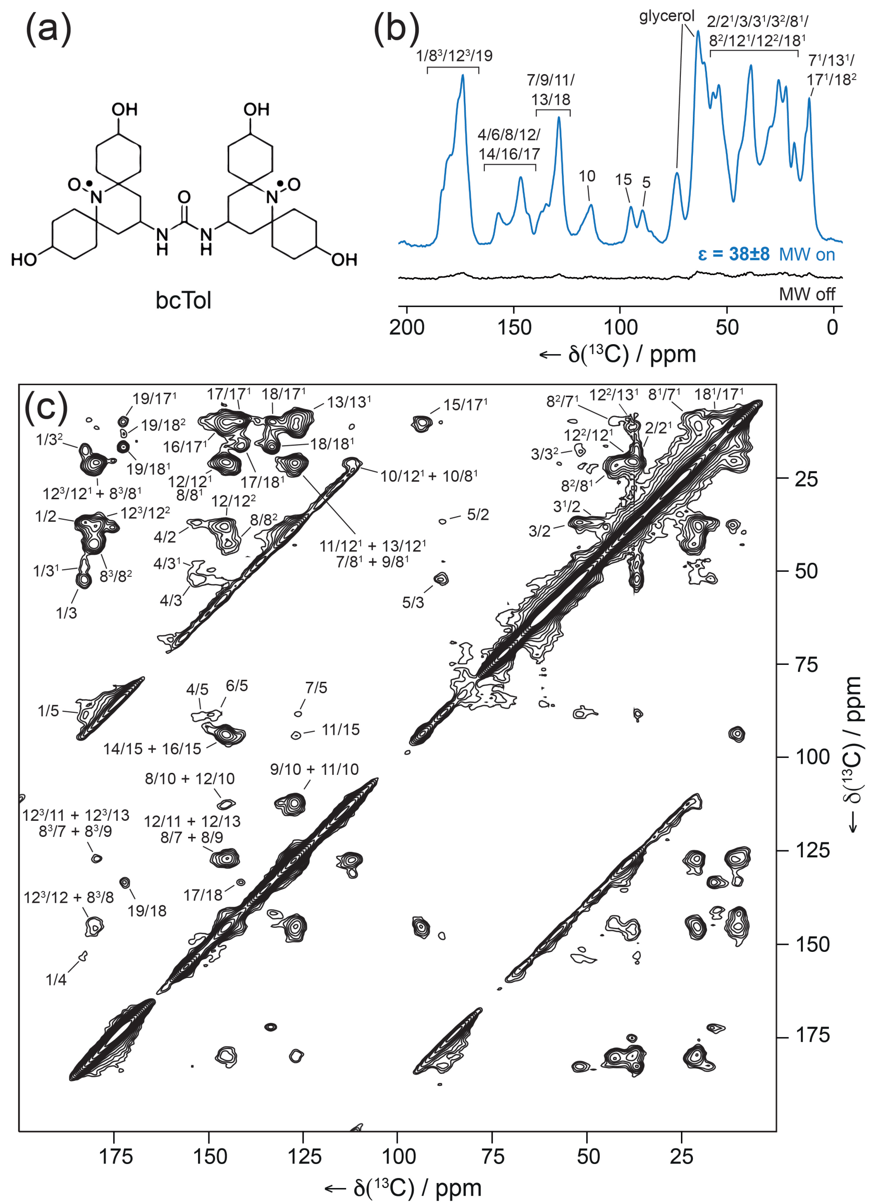
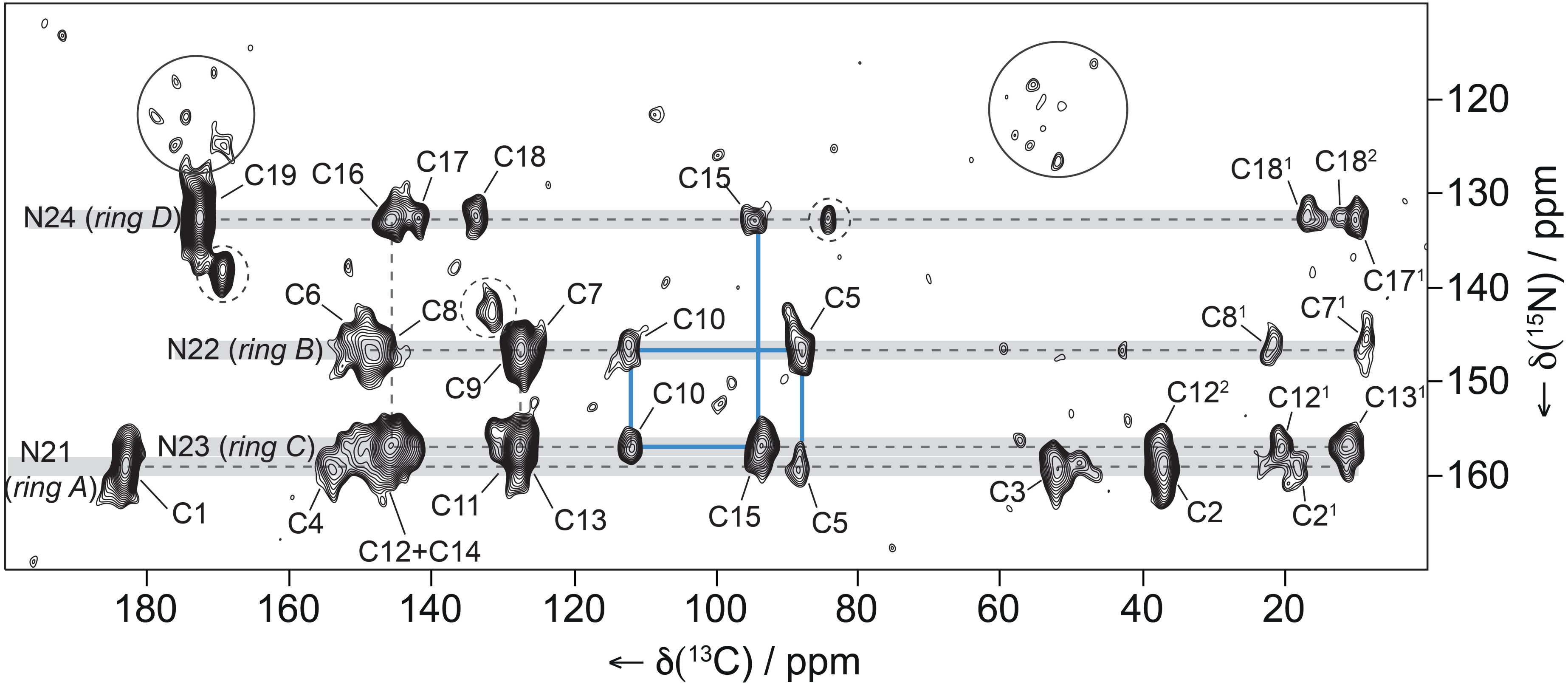
bcTOL was used as a radical

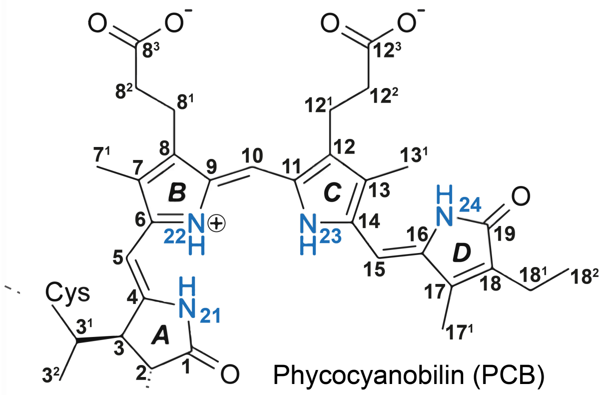
Ɛ was 38

Surprising resolution!



Phytochrome Cph1: assignment of PCB with the help of DNP





Correlating nitrogens with bridging methine carbons yields the assignment

Chemical shift of the Ring B nirogen suggests positive charge