Solid-State NMR

Anisotropic Interactions are not averaged due to tumbling of the molecule
- Dipolar Interaction
- Chemical Shift Anisotropy (CSA)
- Quadrupolar Interactions

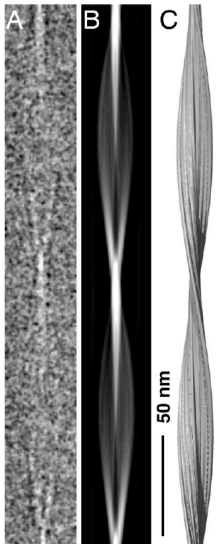
Consequences
- Broad Resonance Lines
- Low Sensitivity (X-Detection)
- A lot of Structural Information

Magic Angle Spinning (MAS):
The orientation of a molecule at any time ($t+\Delta t$) is known

Why Solid-State NMR ?

Misfolding Peptides and **Protein Amyloidosis**
is a common motif in many diseases

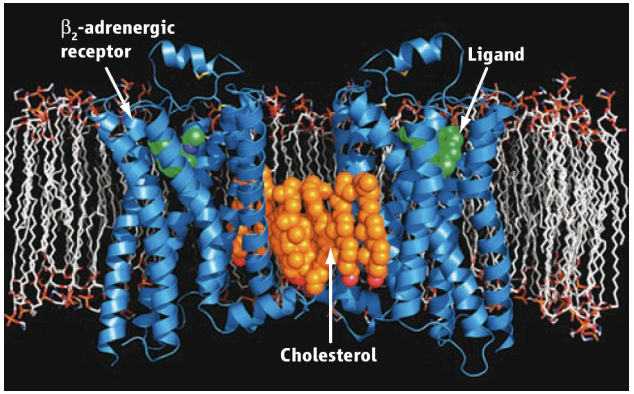
- β-Amyloid (Alzheimer´s Disease)
- α-synuclein (Parkinson´s Disease)
- Huntingtin
- Prion diseases
  (CJD, GSS, FFI, Kuru, BSE)



Grigorieff,
PNAS 2008

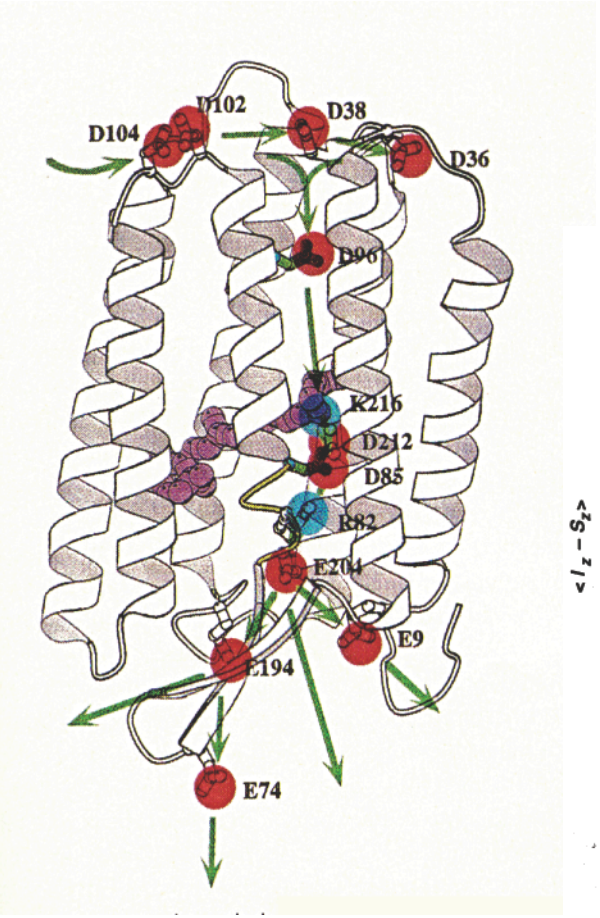
Membrane Proteins
GPCRs, Ion Channels, Transporters

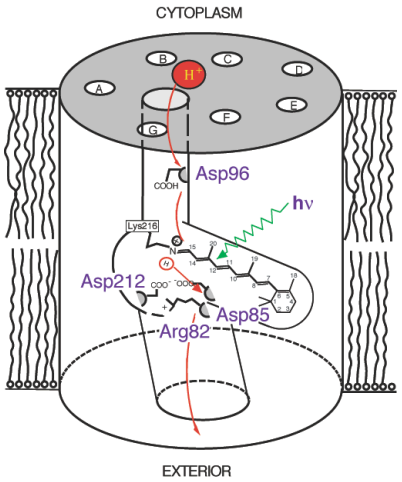
PDB Database: 160 / 51663 (July 3, 2008)
20-30% of open reading frames
60% of all drug targets

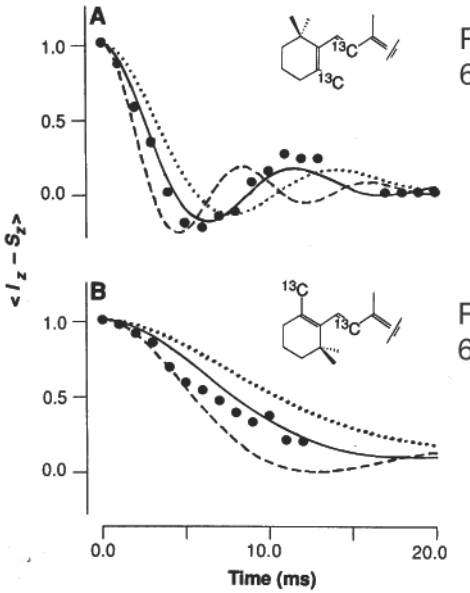


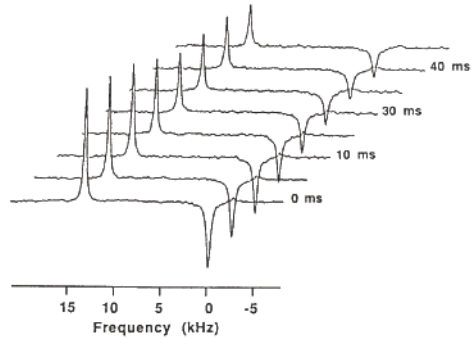
Kobilka, Stevens, Schertler, *Science* 2008

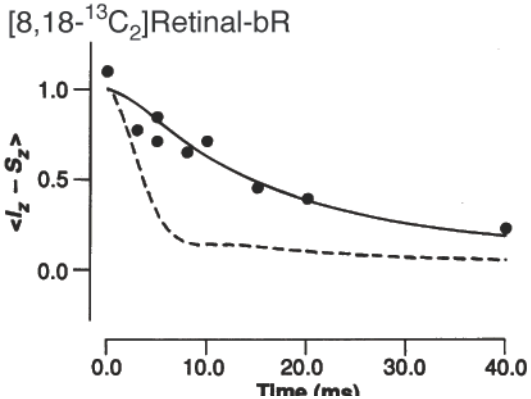
Bacteriorhodopsin





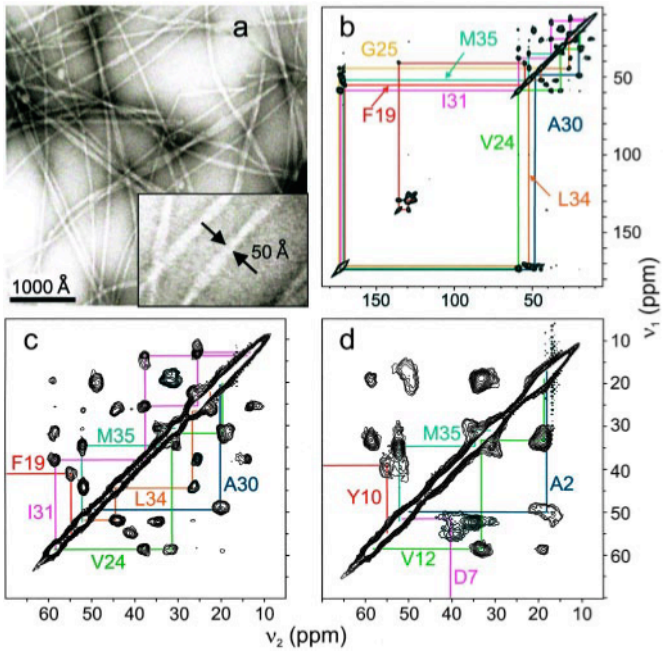


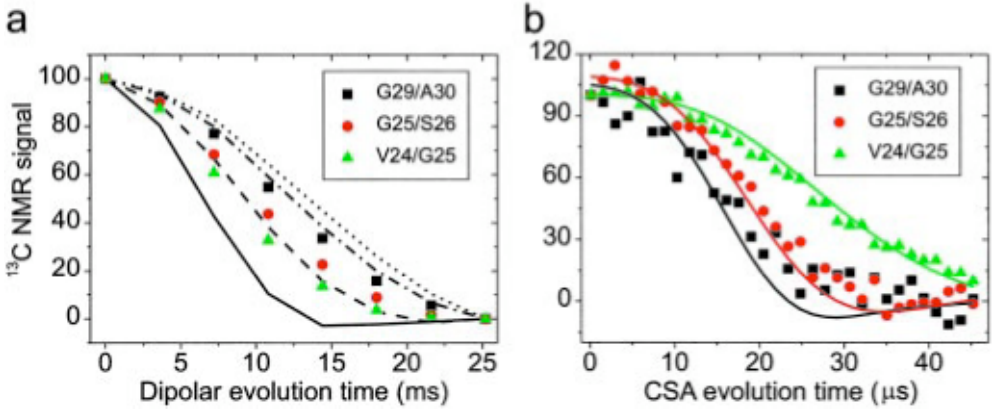




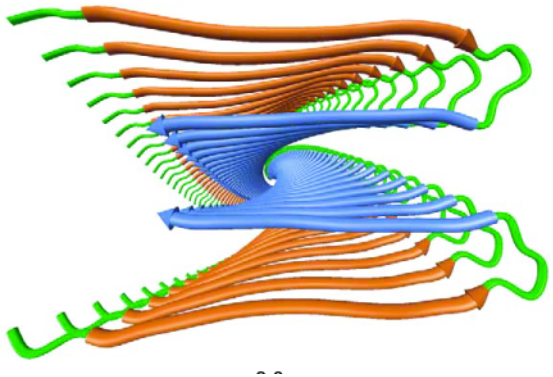
Griffin and co-worker, Determination of Membrane-Protein Structure by Rotational Resonance NMR – Bacteriorhodopsin, *Science* 251, 783-786 (1991)

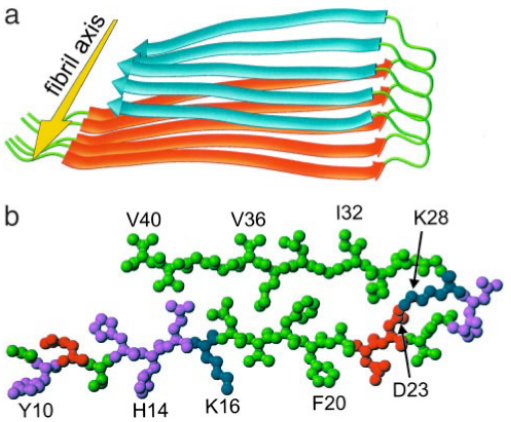
β-Amyloid and Alzheimer's disease





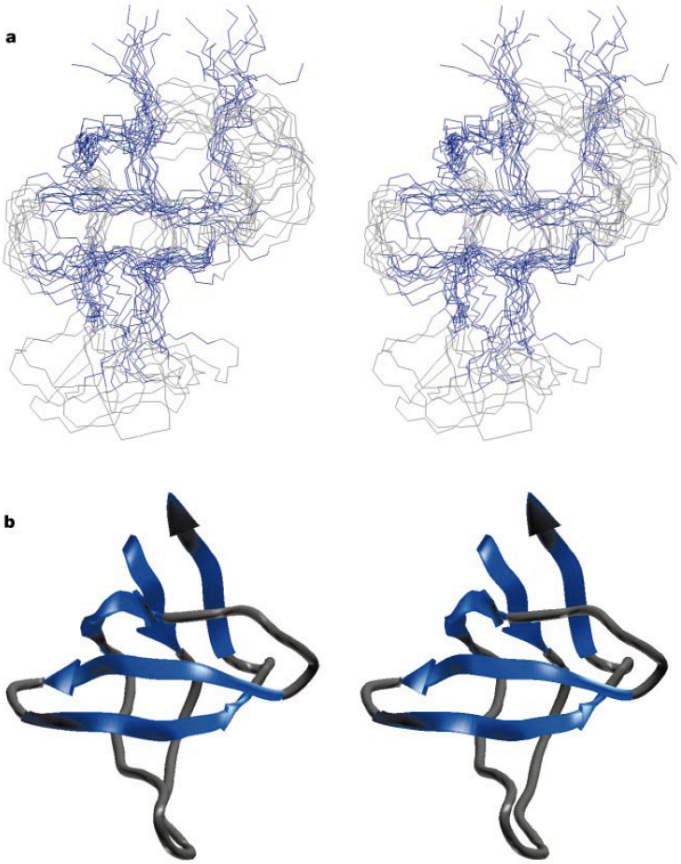
(a) fpRFDR-CT dephasing (b) DQCSA to characterize ϕ/ψ for CO selectively labeled $A\beta^{1-40}$

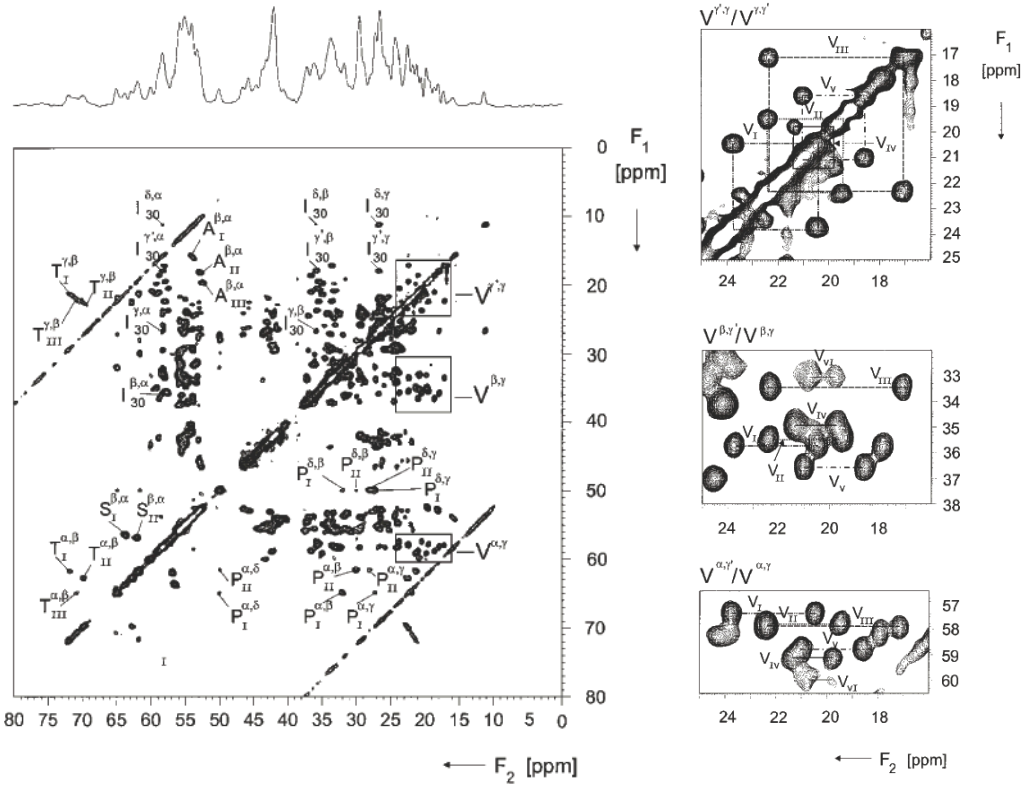




Tycko and co-worker (2002) *PNAS* 99, 16472-16747

Microcrystalline uniformly labeled proteins

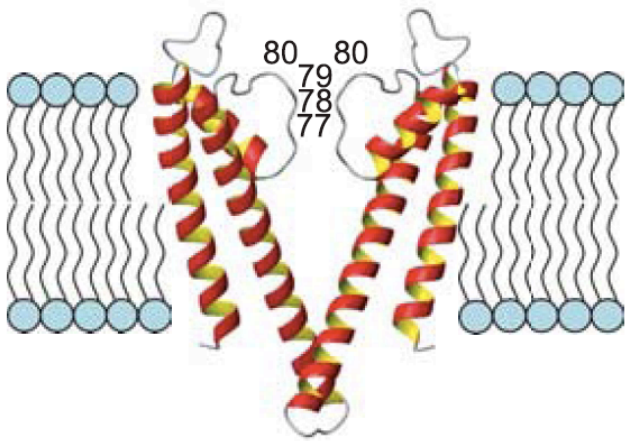




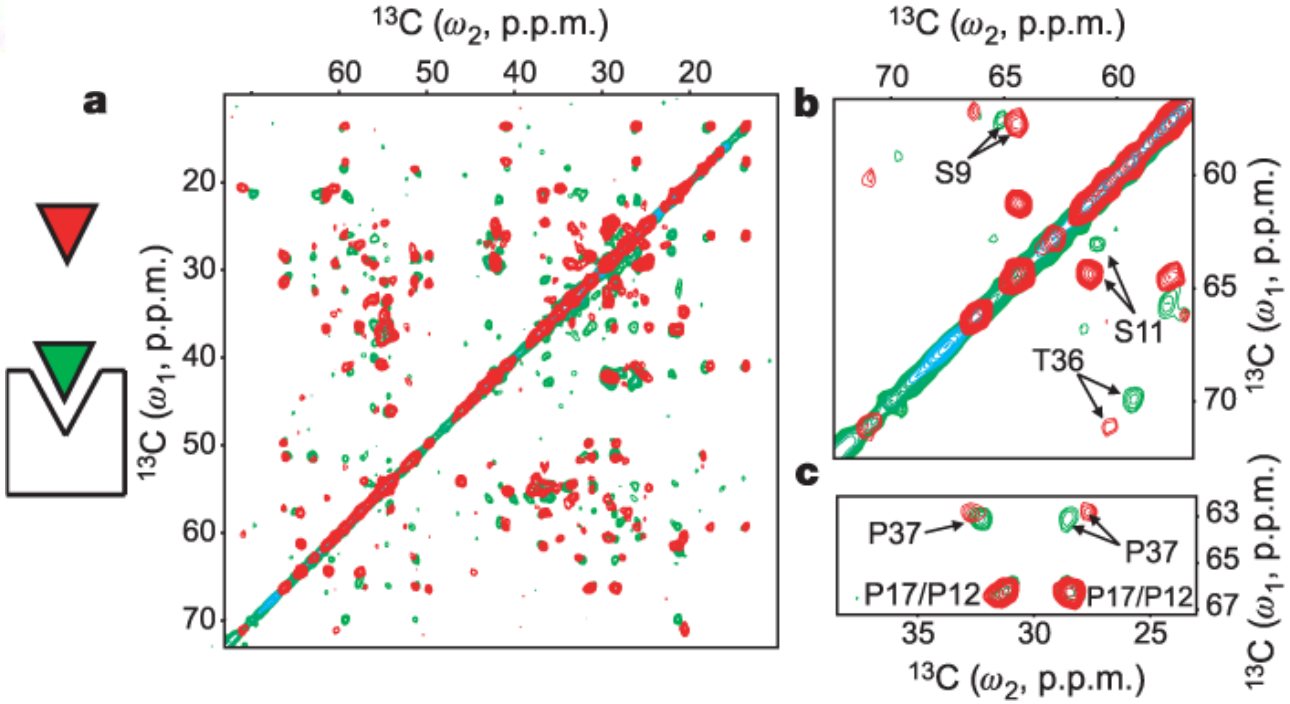
Resonance Assignment:
Oschkinat, Baldus, de Groot, SH3 (2000) *JMR* 143, 411-416
Zilm and McDermott, BPTI (2000) *JBNMR* 16 209-219

Structure Determination:
Oschkinat, α-spectrin SH3, *Nature* (2002)
Griffin + co-worker MLF, *PNAS* (2002)

The KcsA Potassium Channel

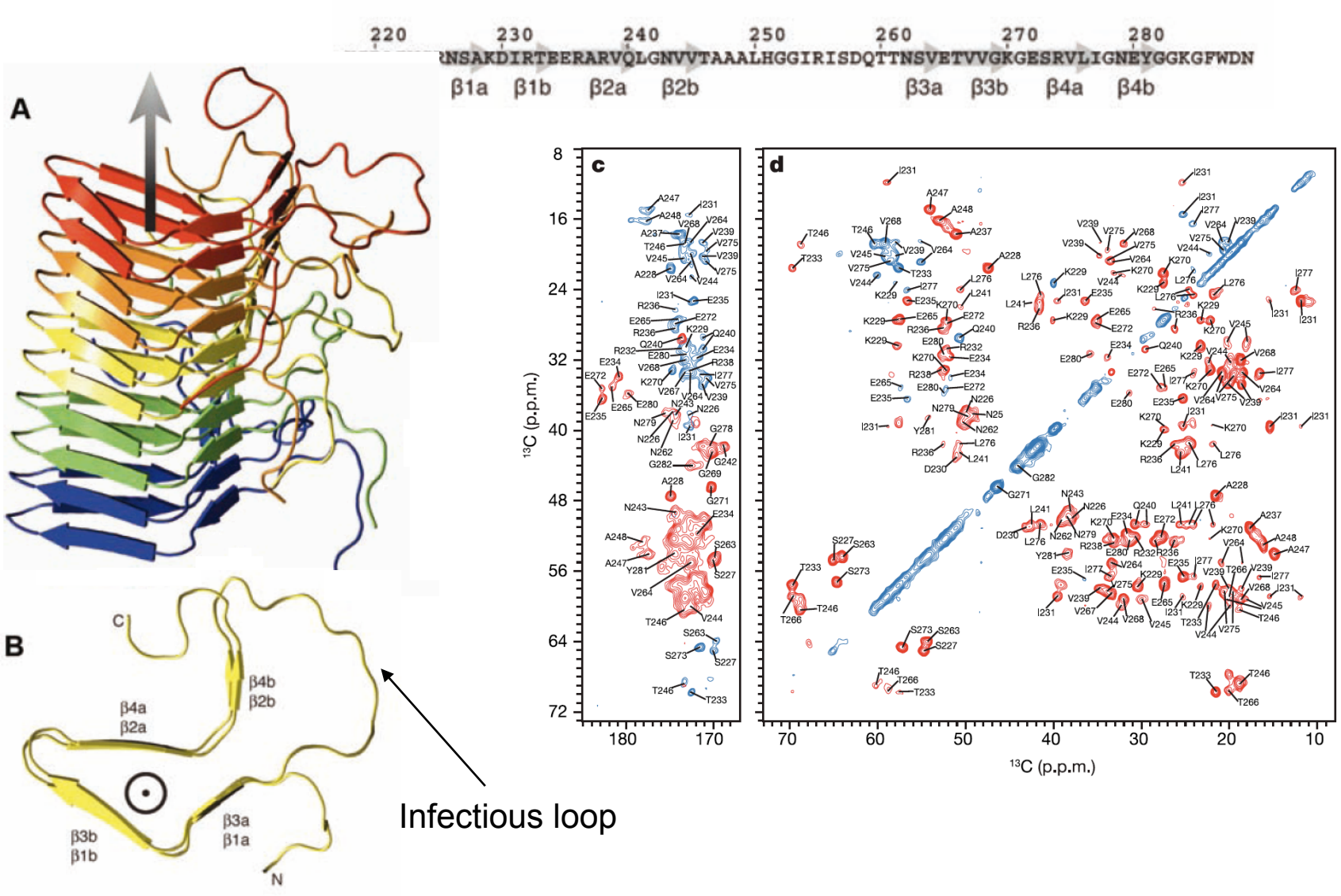


KcsA: Potassium (K+) channel
Kv1.3: Scorpion kaliotoxin



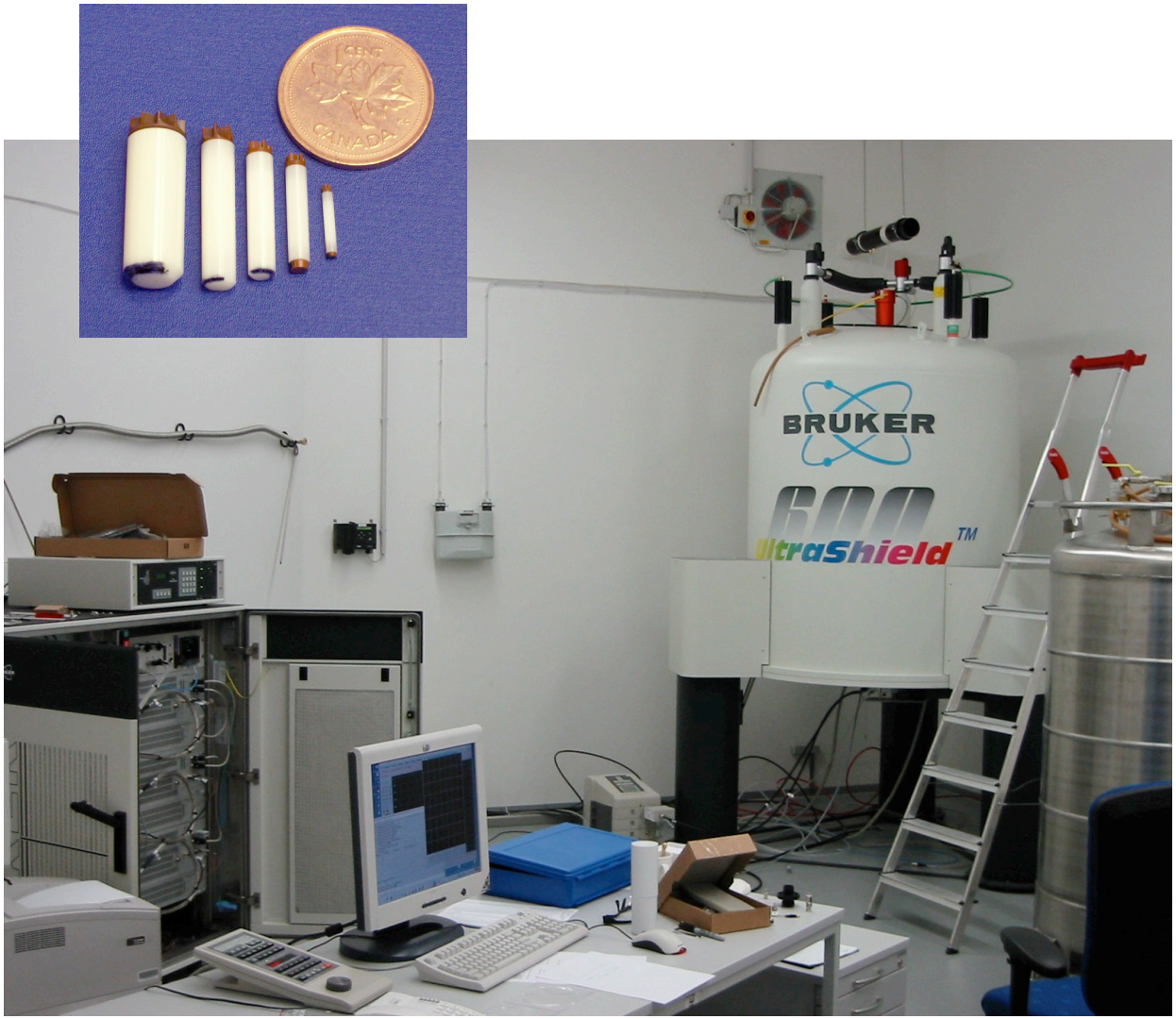
Baldus and co-worker (2006) *Nature* 440, 959-962

The prion domain of Het-s

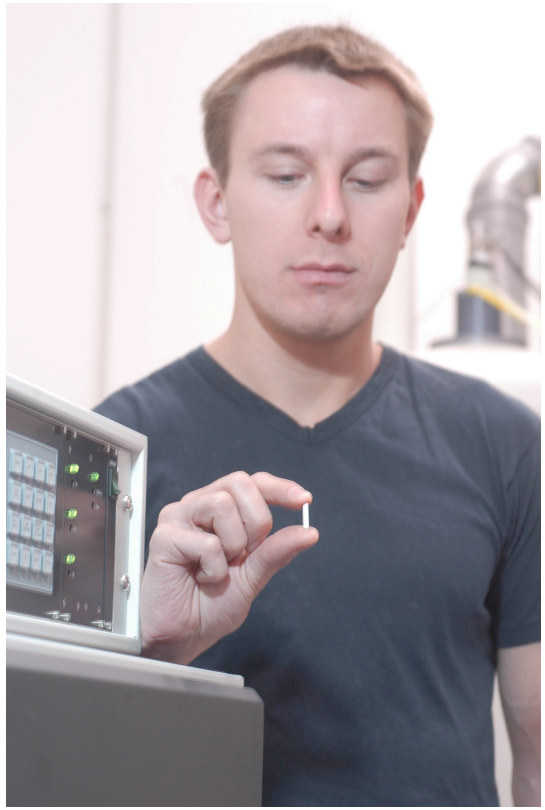


Meier and co-worker (2008) *Science* 319, 1523-1526

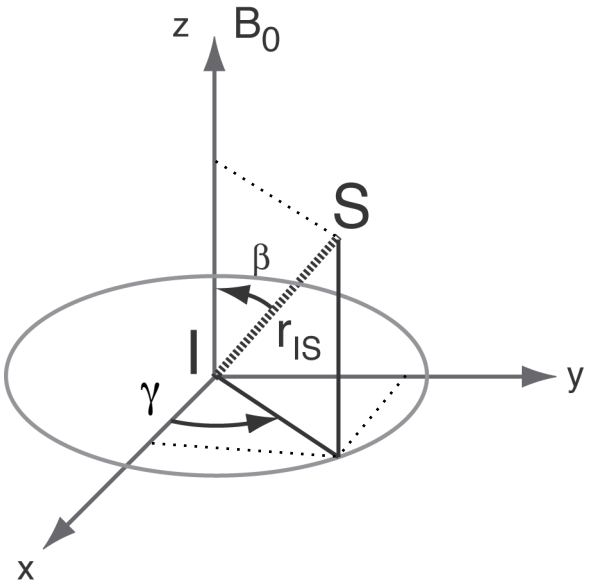
MAS solid-state NMR equipment



Diameter	MAS Frequency
7.0 mm	< 8 kHz
4.0 mm	< 18 kHz
3.2 mm	< 23 kHz
2.5 mm	< 35 kHz
1.3 mm	< 70 kHz



The Hamilton Operator of the Dipolar Interaction and the Dipolar Alphabet



The Hamilton Operator of the dipolar interaction for 2 particles with spin 1/2 is given by

$$H_{IS}^{DD} = b_{IS}\left\{\frac{3}{r_{IS}^2}\left(\vec{I}\vec{r}_{IS}\right)\left(\vec{S}\vec{r}_{IS}\right) - \vec{I}\vec{S}\right\}$$

with $$b_{IS} = -\mu_0 \frac{\gamma_I \gamma_S \hbar}{4\pi r_{IS}^3}$$

$\gamma \equiv$ gyromagnetic ratio of the nuclei

$\hbar \equiv$ Planck's constant

$\mu_0 \equiv$ magnetic permeability

$r_{IS} \equiv$ distance between the nuclei I and S.

$$I^+ = I_x + iI_y,\ I^- = I_x - iI_y$$

$$x = r\ \sin\beta\ \cos\gamma$$
$$y = r\ \sin\beta\ \sin\gamma$$
$$z = r\ \cos\beta$$

The Dipolar Alphabet

$$\hat{H}_{IS}^{DD} = b_{IS}\{A + B + C + D + E + F\}$$

$$A = I_z S_z \left(3\cos^2\beta - 1\right)$$

$$B = -\frac{1}{4}\left(I^+ S^- + I^- S^+\right)\left(3\cos^2\beta - 1\right)$$

$$C = +\frac{1}{4}\left(I^+ S_z + I_z S^+\right)\sin(2\beta)\exp(-i\gamma)$$

$$D = +\frac{1}{4}\left(I^- S_z + I_z S^-\right)\sin(2\beta)\exp(+i\gamma)$$

$$E = +\frac{1}{8}\left(I^+ S^+ + I^+ S^+\right)\sin^2(\beta)\exp(-2i\gamma)$$

$$F = +\frac{1}{8}\left(I^- S^- + I^- S^-\right)\sin^2(\beta)\exp(+2i\gamma)$$

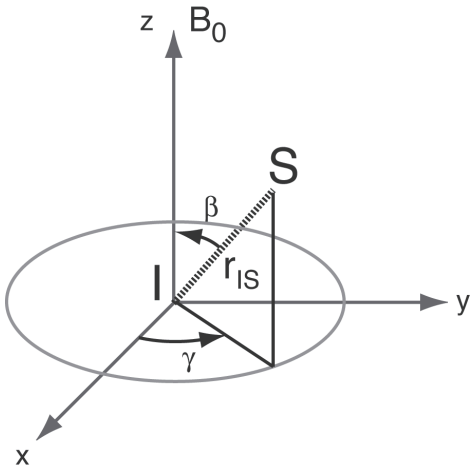
Secular approximation (only frequency independent terms)

$$\hat{H}_{IS}^{DD} = \mu_0 \frac{\gamma_I \gamma_S \hbar}{4\pi r_{IS}^3} \frac{3\cos^2\beta - 1}{2}\left(3I_z S_z - \mathbf{I}\cdot\mathbf{S}\right)$$

In the **heteronuclear case**:

$$\hat{H}_{IS}^{DD} = \mu_0 \frac{\gamma_I \gamma_S \hbar}{4\pi r_{IS}^3} \frac{3\cos^2\beta - 1}{2} 2I_z S_z$$

The Hamilton Operator of the Dipolar Interaction

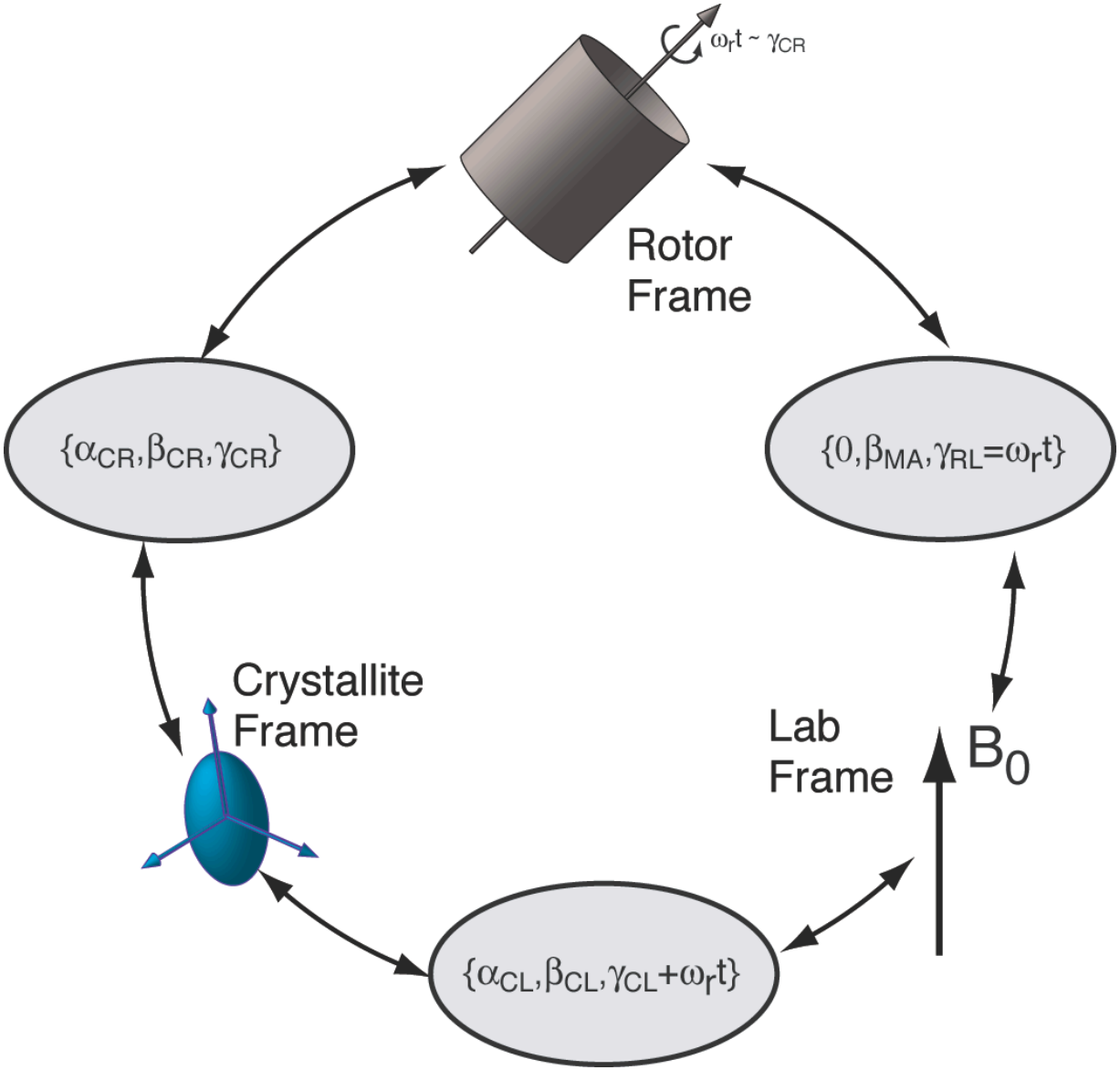


$$\hat{H}_{IS}^{dip} = b_{IS}\left\{\frac{3}{r_{IS}^2}\left(\vec{I}\mathbf{r}_{IS}\right)\left(\vec{S}\mathbf{r}_{IS}\right) - \vec{I}\vec{S}\right\} = \omega_{0,0}^{D} T_{20}^{IS}$$

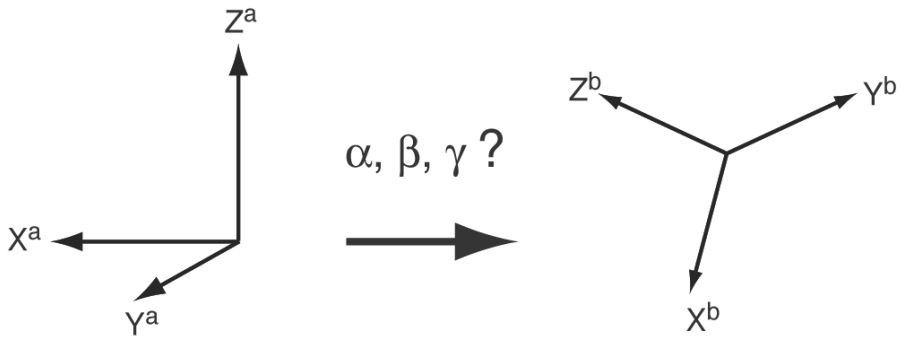
Spatial Components	Spin Operators (2nd rank tensor operators)
$\omega_{0,0} = b_{IS}\sqrt{\frac{3}{2}}\left(3\cos^2\beta - 1\right)$	$T_{20}^{IS} = \frac{1}{\sqrt{6}}\left(2I_zS_z - \frac{1}{2}I^+S^- - \frac{1}{2}I^-S^+\right)$
$\omega_{0,\pm1} = b_{IS}\,\frac{1}{2\sqrt{2}}\sin(2\beta)\exp(\mp i\gamma)$	$T_{2\pm1}^{IS} = \frac{1}{\sqrt{2}}\left(I^{\pm}S_z + I_zS^{\pm}\right)$
$\omega_{0,\pm2} = b_{IS}\,\frac{1}{4}\sin^2(\beta)\exp(\mp 2i\gamma)$	$T_{2\pm2}^{IS} = \frac{1}{2}I^{\pm}S^{\pm}$

$$b_{IS} = -\mu_0\frac{\gamma_I\gamma_S\hbar}{4\pi r_{IS}^3}$$

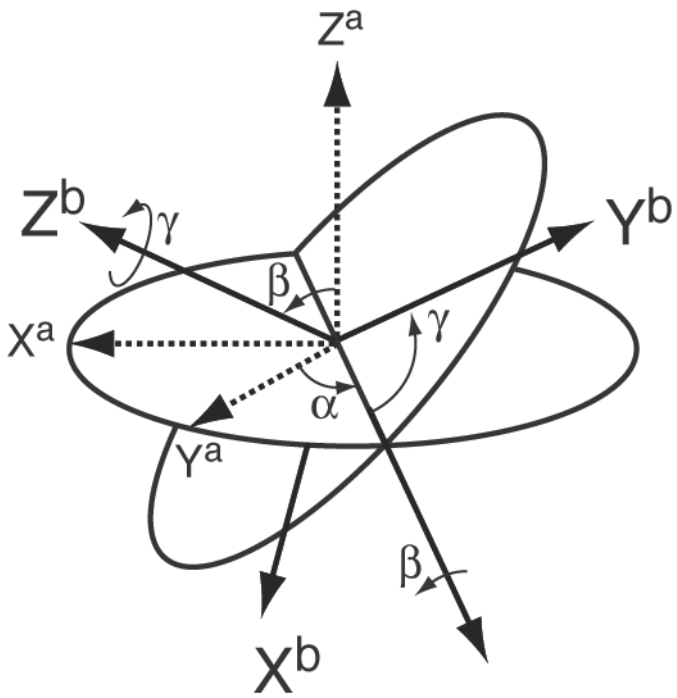
Conventions: Euler-Rotations, Coordinate System Transformations and Wigner-Rotation Matrices



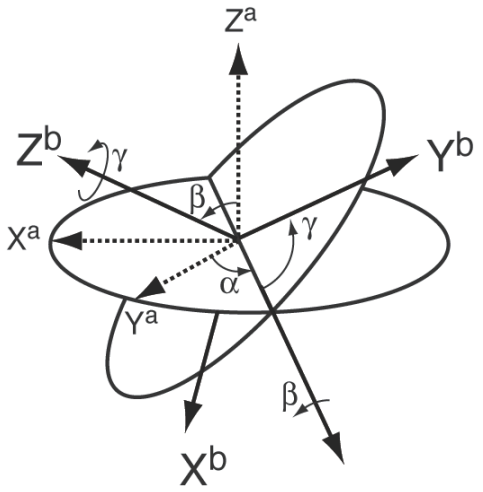
Conventions: Euler-Angles



Euler-Angles:



Conventions: Coordinate System Transformations



In general, a **rotation of any vector** can be described as:

$$R(\alpha,\beta,\gamma) = R_z(\gamma)R_y(\beta)R_z(\alpha) = \begin{pmatrix} c\gamma & s\gamma & 0 \\ -s\gamma & c\gamma & 0 \\ 0 & 0 & 1 \end{pmatrix}\begin{pmatrix} c\beta & 0 & -s\beta \\ 0 & 1 & 0 \\ s\beta & 0 & c\beta \end{pmatrix}\begin{pmatrix} c\alpha & s\alpha & 0 \\ -s\alpha & c\alpha & 0 \\ 0 & 0 & 1 \end{pmatrix}$$

$$R(\alpha,\beta,\gamma) = \begin{pmatrix} c\alpha\cdot c\beta\cdot c\gamma - s\alpha\cdot s\gamma & s\alpha\cdot c\beta\cdot c\gamma + c\alpha\cdot s\gamma & -s\beta\cdot c\gamma \\ -c\alpha\cdot c\beta\cdot s\gamma - s\alpha\cdot c\gamma & -s\alpha\cdot c\beta\cdot s\gamma + c\alpha\cdot c\gamma & s\beta\cdot s\gamma \\ c\alpha\cdot s\beta & s\alpha\cdot s\beta & c\beta \end{pmatrix}$$

where cα = cos α, sα = sin α etc.

Conventions: Wigner-Rotation Matrices

Rotation of a vector:

$$r'_i = \sum_{j=1}^{3} r_j \, R_{ij}(\alpha,\beta,\gamma)$$

Rotation of a Spin Operator (2nd rank tensor):
Application of a Wigner rotation matrix
(2nd rank tensor) ($-2 \le m \le +2$)

$$\hat{T}_{2,m}{}' = \sum_{m'=-2}^{+2} \hat{T}_{2,m'} D^2_{m',m}(\alpha,\beta,\gamma)$$

Definition of the **Wigner Rotation matrices**

$$D^2_{m',m}(\alpha,\beta,\gamma) = d^2_{m',m}(\beta)\ \exp(im'\alpha)\ \exp(im\gamma)$$

with the **reduced Wigner Rotation Matrices**

$$d^2_{m',m}(\beta) =$$

$$\begin{pmatrix}
\frac{1}{4}(1+\cos\beta)^2 & -\frac{1}{2}(1+\cos\beta)\sin\beta & \sqrt{\frac{3}{8}}\sin^2\beta & -\frac{1}{2}(1-\cos\beta)\sin\beta & \frac{1}{4}(1-\cos\beta)^2 \\
\frac{1}{2}(1+\cos\beta)\sin\beta & \frac{1}{2}(\cos\beta-1)+\cos^2\beta & -\sqrt{\frac{3}{8}}\sin 2\beta & \frac{1}{2}(1+\cos\beta)-\cos^2\beta & -\frac{1}{2}(1-\cos\beta)\sin\beta \\
\sqrt{\frac{3}{8}}\sin^2\beta & \sqrt{\frac{3}{8}}\sin 2\beta & \frac{1}{2}(3\cos^2\beta-1) & -\sqrt{\frac{3}{8}}\sin 2\beta & \sqrt{\frac{3}{8}}\sin^2\beta \\
\frac{1}{2}(1-\cos\beta)\sin\beta & \frac{1}{2}(1+\cos\beta)-\cos^2\beta & \sqrt{\frac{3}{8}}\sin 2\beta & \frac{1}{2}(\cos\beta-1)+\cos^2\beta & -\frac{1}{2}(1+\cos\beta)\sin\beta \\
\frac{1}{4}(1-\cos\beta)^2 & \frac{1}{2}(1-\cos\beta)\sin\beta & \sqrt{\frac{3}{8}}\sin^2\beta & \frac{1}{2}(1+\cos\beta)\sin\beta & \frac{1}{4}(1+\cos\beta)^2
\end{pmatrix}$$

Conventions: Wigner-Rotation Matrices

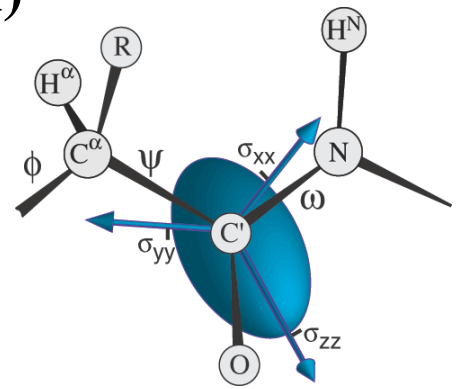
The elements d^2_{0x} are the time dependent spherical harmonics $\omega^{(m)}$ in the Hamilton operator of the dipolar interaction.

"**Addition theorem**" for Wigner rotation matrices:

$$D^2_{n',n}(\Omega_{AC}) = \sum_{m=-2}^{+2} D^2_{n',m}(\Omega_{AB}) D^2_{m,n}(\Omega_{BC})$$

The Chemical Shift Anisotropy (CSA)

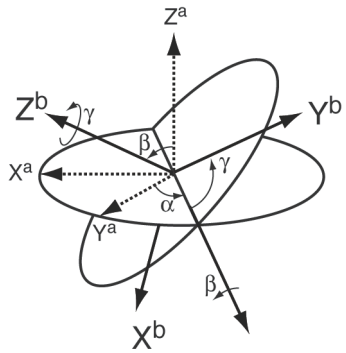
$$\hat{H}_I^{CS} = \gamma_I \vec{I}\ \tilde{\sigma}\ \vec{B}_0 = \gamma_I \left(I_x \sigma_{xx}^{PAS} + I_y \sigma_{yy}^{PAS} + I_z \sigma_{zz}^{PAS} \right) B_0$$



$\sigma^{PAS} \equiv$ **principal axis system** (molecule fixed CS).
Transformation of B_0 into molecule fixed CS:

$$\omega_I^{CS,PAS} = -\omega_0 \sum_\alpha \left(B_{0,\alpha}^{PAS} \right)^+ \sigma_{\alpha\alpha}^{PAS} B_{0,\alpha}^{PAS}$$

$$= -\omega_0 \left[\sigma_{xx}^{PAS} (\cos\gamma \sin\beta)^2 + \sigma_{yy} (\sin\gamma \sin\beta)^2 + \sigma_{zz} (\cos\beta)^2 \right]$$

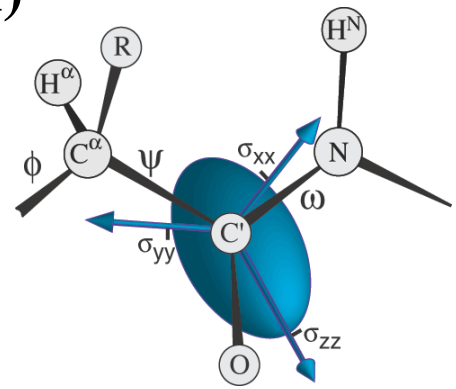


B_0 is a vector → [set α=0 in R(α,β,γ)]. With

$$R(\alpha,\beta,\gamma)\mathrm{B}_0 = \begin{pmatrix} c\alpha \cdot c\beta \cdot c\gamma - s\alpha \cdot s\gamma & s\alpha \cdot c\beta \cdot c\gamma + c\alpha \cdot s\gamma & -s\beta \cdot c\gamma \\ -c\alpha \cdot c\beta \cdot s\gamma - s\alpha \cdot c\gamma & -s\alpha \cdot c\beta \cdot s\gamma + c\alpha \cdot c\gamma & s\beta \cdot s\gamma \\ c\alpha \cdot s\beta & s\alpha \cdot s\beta & c\beta \end{pmatrix} \begin{pmatrix} 0 \\ 0 \\ 1 \end{pmatrix}$$

The Chemical Shift Anisotropy (CSA)

$$\hat{H}_I^{CS} = \gamma_I \vec{I}\ \tilde{\sigma}\ \vec{B}_0 = \gamma_I \left(I_x \sigma_{xx}^{PAS} + I_y \sigma_{yy}^{PAS} + I_z \sigma_{zz}^{PAS} \right) B_0$$



$\sigma^{PAS} \equiv$ **principal axis system** (molecule fixed CS).
Transformation of B_0 into molecule fixed CS:

$$\omega_I^{CS,PAS} = -\omega_0 \sum_\alpha \left(B_{0,\alpha}^{PAS} \right)^+ \sigma_{\alpha\alpha}^{PAS} B_{0,\alpha}^{PAS}$$

$$= -\omega_0 \left[\sigma_{xx}^{PAS} (\cos\gamma \sin\beta)^2 + \sigma_{yy} (\sin\gamma \sin\beta)^2 + \sigma_{zz} (\cos\beta)^2 \right]$$

B_0 is a vector → [set α=0 in R(α,β,γ)]. With

$$\sigma_{iso} \coloneqq \frac{1}{3} \left(\sigma_{xx}^{PAS} + \sigma_{yy}^{PAS} + \sigma_{zz}^{PAS} \right) \qquad \text{isotropic chemical shift}$$

$$\delta \coloneqq -\omega_0 \left(\sigma_{zz}^{PAS} - \sigma_{iso} \right) \qquad \text{anisotropy}$$

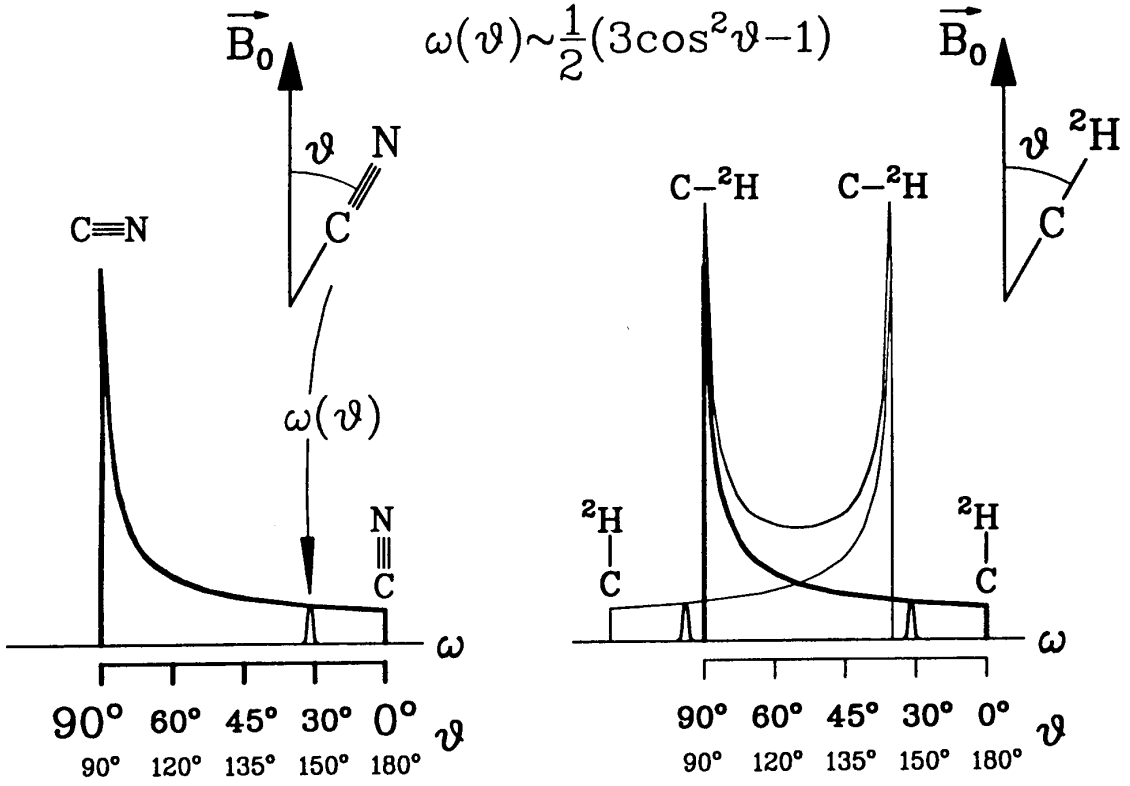
$$\eta \coloneqq \frac{\sigma_{yy}^{PAS} - \sigma_{xx}^{PAS}}{\sigma_{zz}^{PAS} - \sigma_{iso}} \qquad \text{asymmetry}$$

yields

$$\omega_I^{CS,PAS}(\beta,\gamma) = \frac{\delta}{2} \left[3\cos^2\beta - 1 - \eta \sin^2\beta \cos(2\gamma) \right]$$

Anisotropic chemical shift

The Powder Spectrum (Pake-Pattern), η=0



The Pake-Pattern for Quadrupole and CSA Interactions

S(ω): Spectral intensity

P(β): Probability to find a dipolar vector with a defined orientation β with respect to the external field B_0

$$S[\omega(\beta)]\,|d\omega| \;=\; P(\beta)\,|d\beta|$$

evaluation $d\omega/d\beta$ yields

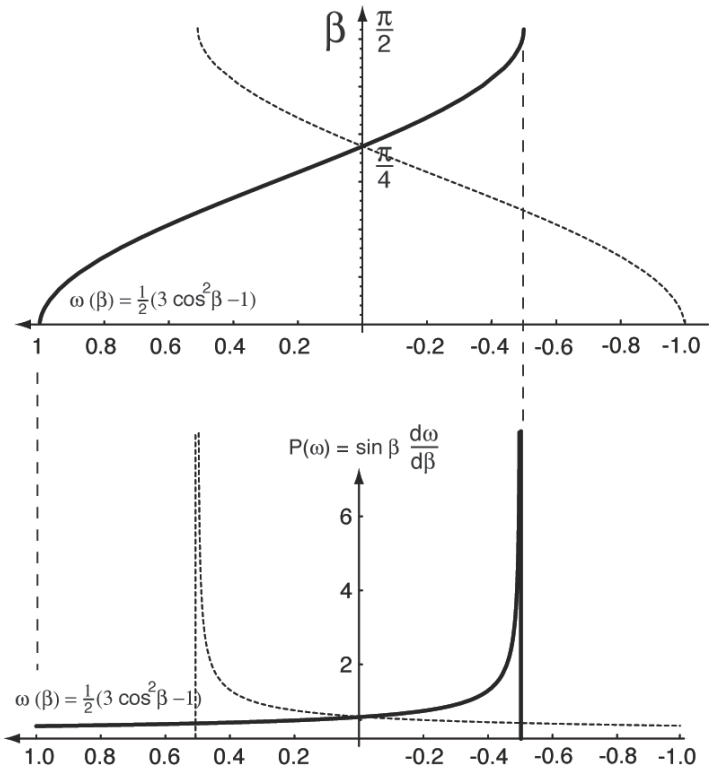
$$S[\omega(\beta)] \;=\; P(\beta)\;/\;|3\delta\cos\beta\sin\beta|$$

Since $P(\beta) = \sin\beta$ (again, by using $\omega(\beta) = \delta/2\,[3\cos^2\beta - 1]$)

$$S[\omega] \;=\; \frac{1}{\sqrt{6\delta}}\,\frac{1}{\sqrt{\omega + \frac{1}{2}\delta}}, \qquad \text{with} \qquad -\frac{\delta}{2} \le \omega \le \delta$$

Symmetry of α and β transition

→ spectrum is symmetric with respect $\omega(\beta) = 0$.

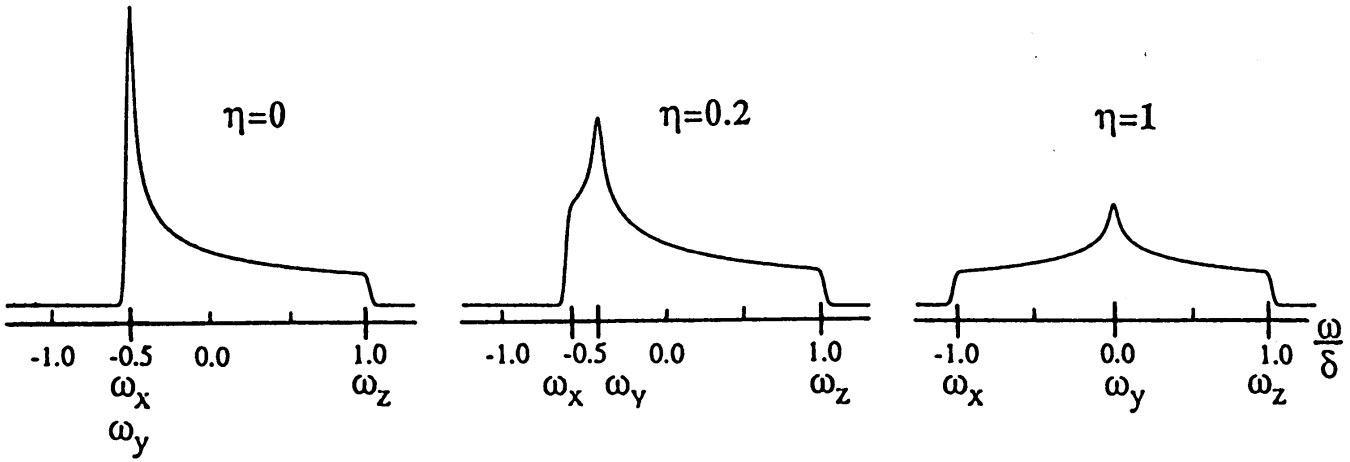


The CSA Pake-Pattern

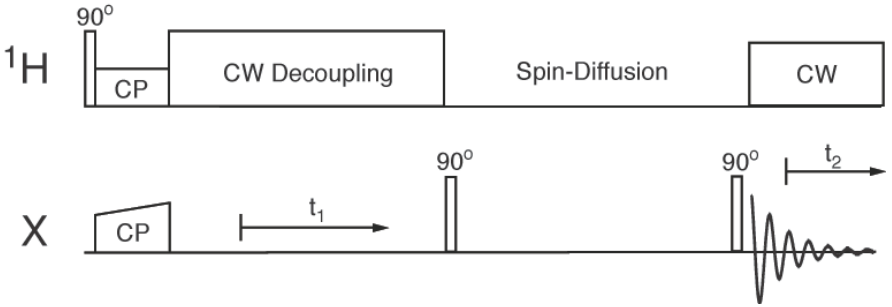
$$\omega_I^{CS,PAS}(\beta,\gamma) = \frac{\delta}{2}\left[3\cos^2\beta - 1 - \eta\sin^2\beta\cos(2\gamma)\right]$$

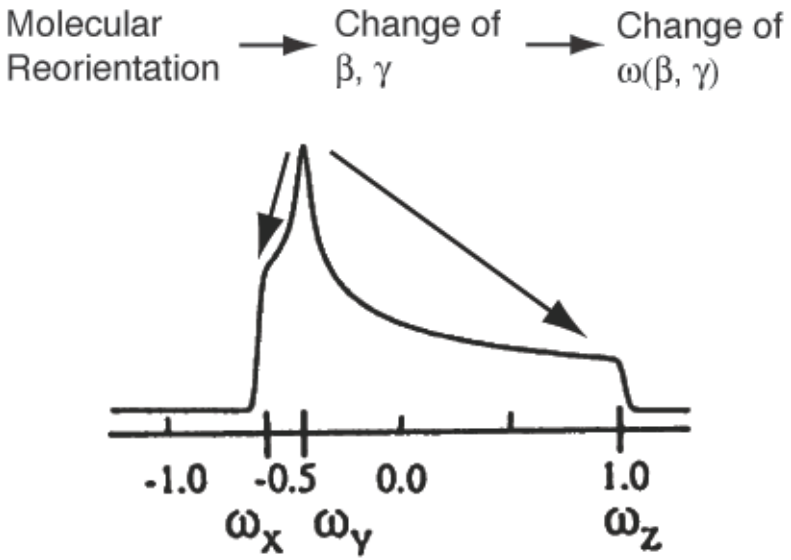
For $\eta=0$, Pake-pattern for CSA and dipole/quadrupole are equivalent

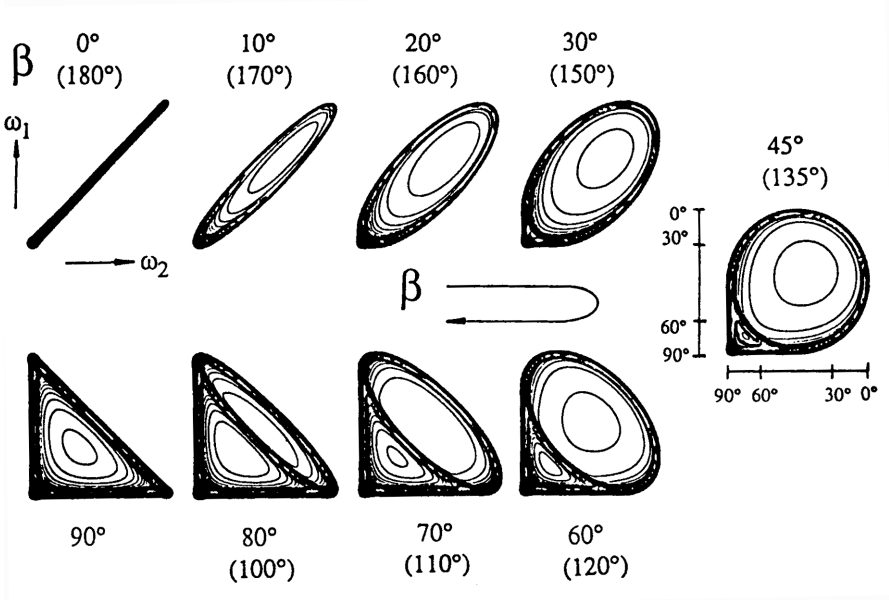
Otherwise:



Exchange Spectroscopy in Static Solids



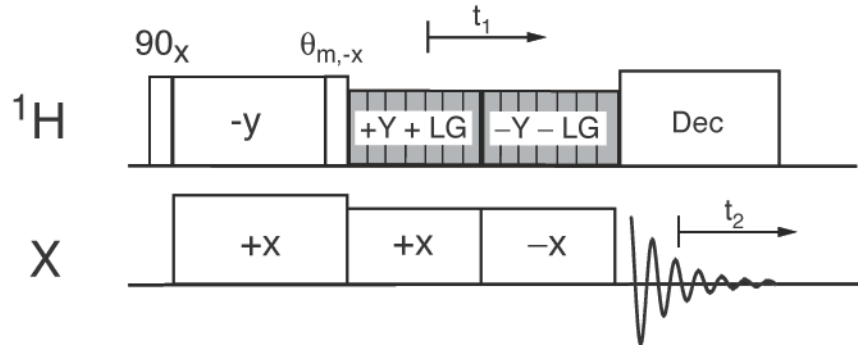




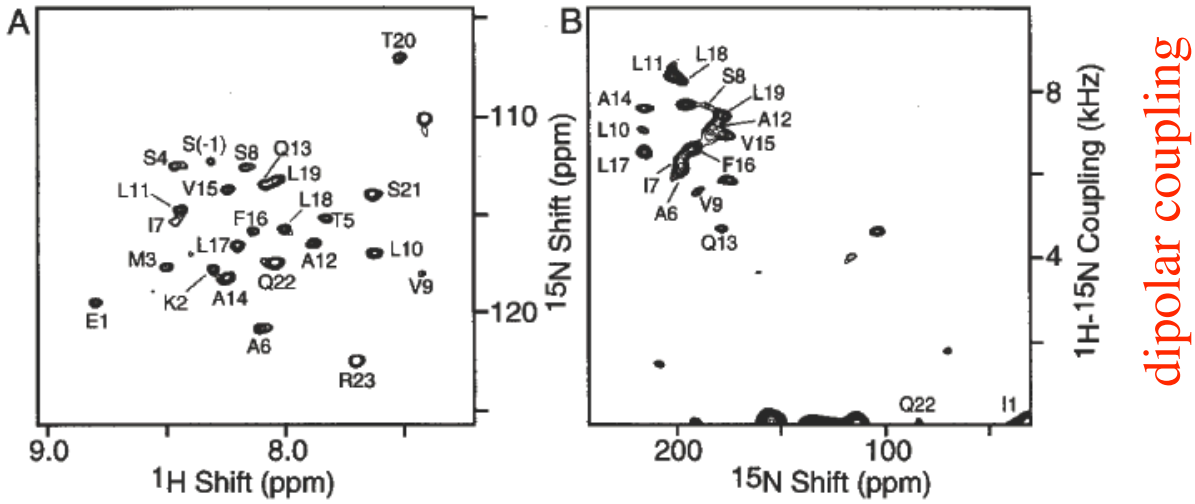
Simulation:

1. η=0
2. Exchange between different β-angles

PISEMA for Oriented Membrane Proteins (Glass Plates)

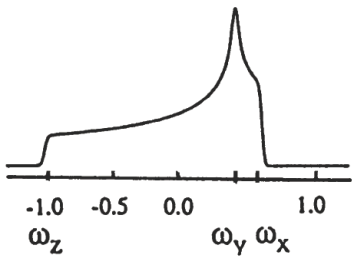


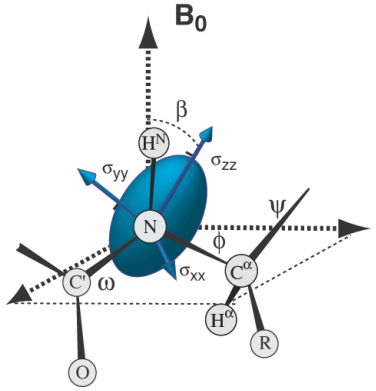
spectra for the M2 segment of the nicotinic acetylcholine receptor
in DPC micelles (A) and oriented DMPC bilayers (B)



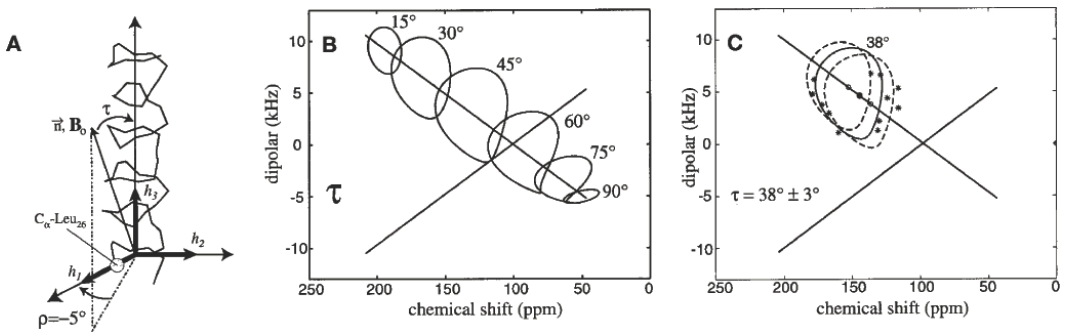
Wu, Ramamoorthy, Opella *JMR* **A109**, 270 (1994)

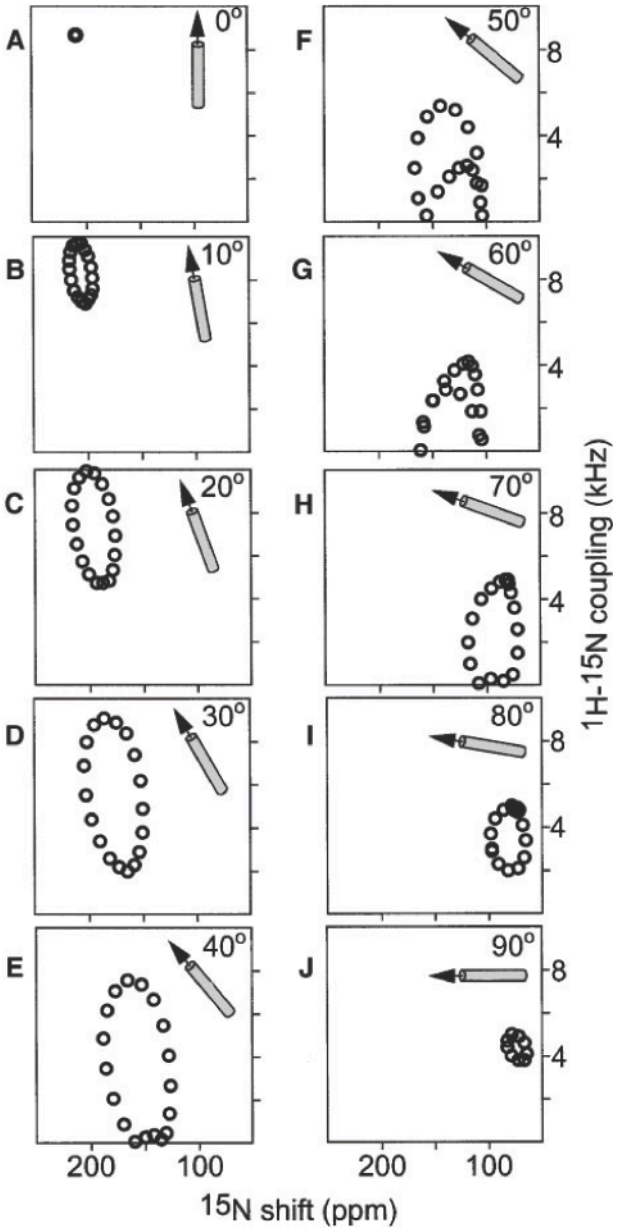
PISA-Wheels in Oriented Membrane Proteins





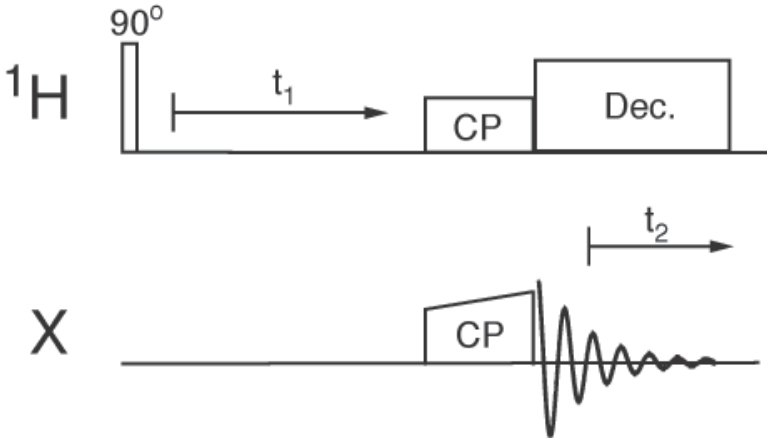
α-helix: orientation of a N-H bond ‖ helix-axis

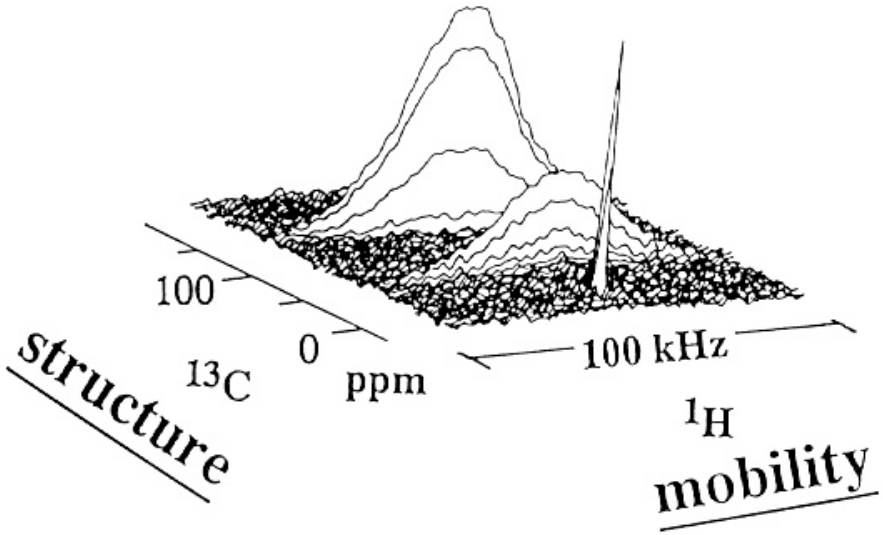




T. A. Cross and co-workers (2000) *JMR* **144**, 162
S. Opella and co-workers (1999), *NSB* **6**, 374.

Wideline Spectroscopy (WISE) in Static Solids yield information on Dynamics

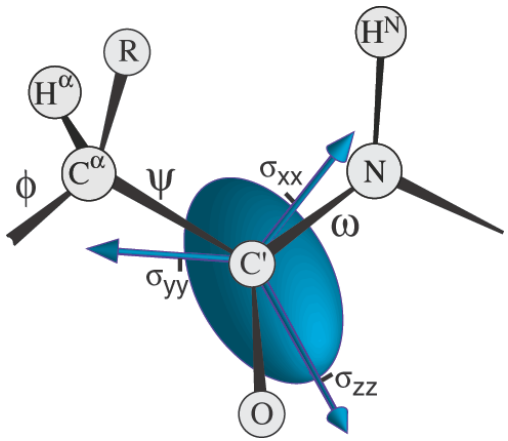


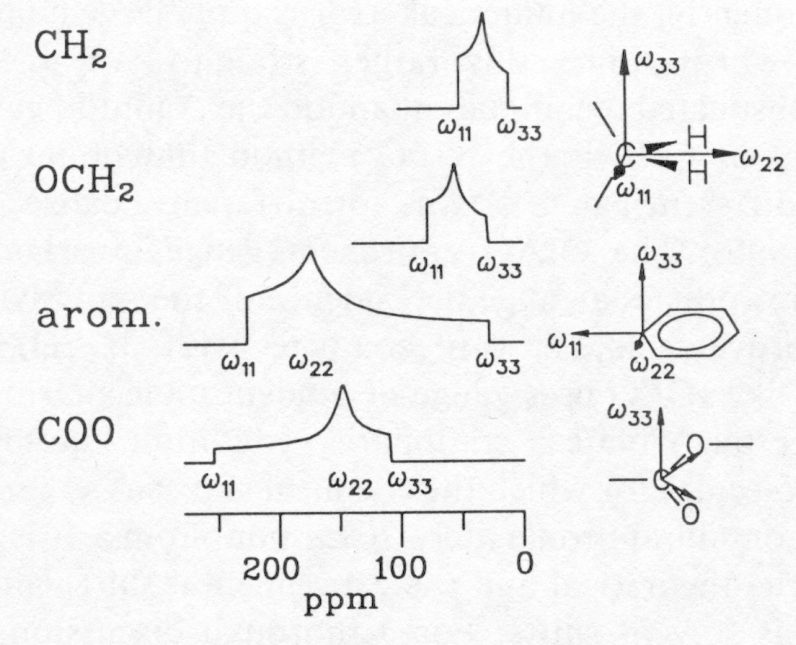


block copolymer PS-b-PDMS

Typical Interactions

^{13}C-^{1}H	Dipolar Interaction	ca. 20 kHz
^{15}N-^{1}H	Dipolar Interaction	ca. 10 kHz
^{15}N	CSA ($\Delta\sigma$ ~ 160 ppm)	ca. 13 kHz (@ 18.8 T)
^{13}C	Aliphat. CSA ($\Delta\sigma$ ~ 30 ppm)	ca. 6 kHz (@ 18.8 T≡800 MHz)
^{13}C	Aromat./Carbonyl CSA ($\Delta\sigma$ ~ 150 ppm)	ca. 30 kHz (@ 18.8 T)
^{1}H	Amide ^{1}H CSA ($\Delta\sigma$ ~ 12-14 ppm)	ca. 10.4 kHz (@ 18.8 T)





The Dipolar Coupling

$$D_{NH} = -\mu_0 \frac{\gamma_H \gamma_N \hbar}{r_{NH}^3} = 10.938 \text{ kHz}$$

$$\mu_0 = 10^{-7} \ \frac{\text{kg m}}{\text{s}^2\text{A}^2}$$

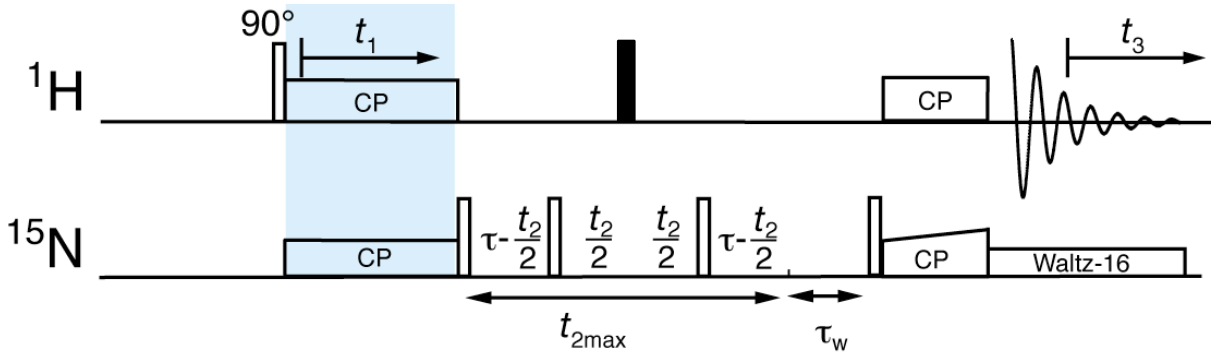
$$h = 1.055 * 10^{-34} \text{ Js}$$

$$\gamma_H = 26.75 * 10^7 \ \frac{1}{\text{s}^1\text{T}^1}$$

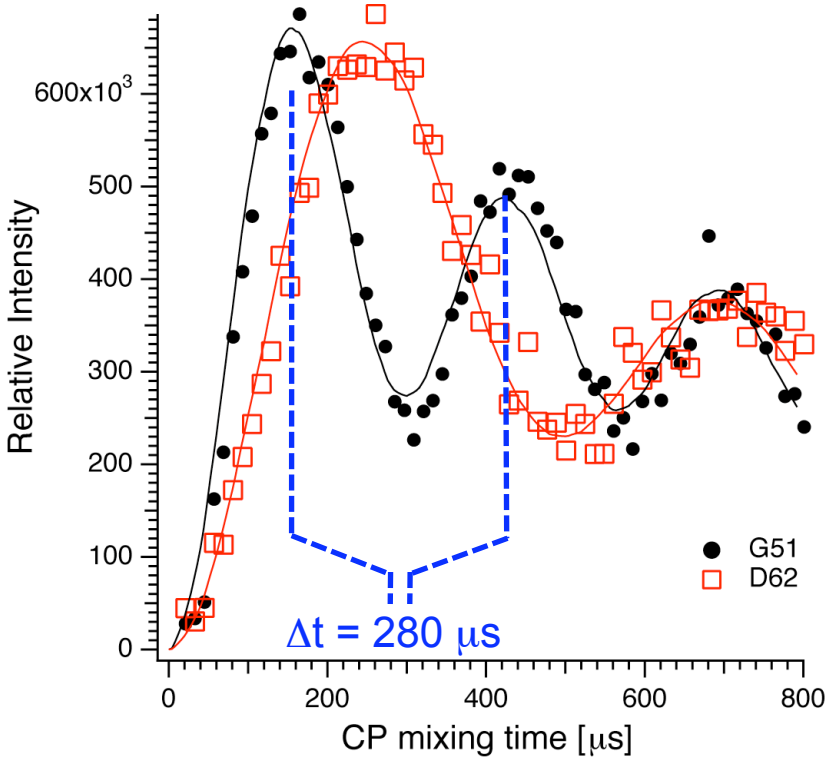
$$\gamma_N = -2.75 * 10^7 \ \frac{1}{\text{s}^1\text{T}^1}$$

$$r_{NH} = 1.04 * 10^{-10} \text{ m}$$

Motional effects are reflected in H-N dipolar couplings



→ Analysis of $S^2_F S^2_S$



$D_{NH} = \kappa / \Delta t$

$\kappa = 0.355$

→ $D_{NH} \approx 10.5$ kHz

Magic-Angle Spinning (MAS)

Consider the Hamilton operator of a heteronuclear dipolar interaction:

$$\hat{H}_{IS}^{DD} = \mu_0 \frac{\gamma_I \gamma_S \hbar}{4\pi r_{IS}^3} \frac{3\cos^2\beta - 1}{2} 2I_z S_z$$

Intuitive Explanation:

In a vector model, the average orientation of a dipolar vector is along rotor axis.

$\rightarrow \; \frac{1}{2}\left(3\cos^2\beta^{MA} - 1\right) = 0.$

Magic-Angle Spinning (MAS)

Hamilton operator of a heteronuclear dipolar interaction

$$\hat{H}_{IS}^{DD} = \mu_0 \frac{\gamma_I \gamma_S \hbar}{4\pi r_{IS}^3} \frac{3\cos^2\beta - 1}{2} 2I_z S_z$$

$$\hat{H}_{IS}^{DD} = \sum_{m=-2}^{+2} \omega_{0,m}^{IS}(\Omega) \exp(im\omega_r t) T_{20}^{IS}$$

(In the rotor fixed frame, under MAS)

1. In the rotor fixed frame, terms $m \neq 0$ are averaged to zero.

Under MAS:

2. For $m=0$, $\omega_{0,m}$ has to be represented in the laboratory coordinate system. **Application** of the "**addition theorem**" for a Wigner rotation matrix yield

$$\omega_{0,m}^{IS} = -2b_{IS} D_{0,-m}^2(\Omega_{CR}) d_{-m,0}^2(\beta_{RL}) \exp(im\omega_r t)$$ and

$$\omega_{0,m}^{IS} = -2b_{IS}\, d_{0,-m}^2(\beta_{CR}) \exp[im(\gamma_{CR} + \omega_r t)]\, d_{-m,0}^2(\beta_{RL})$$

For $m=0$, this expression is exactly zero if $\beta_{RL} = 54.735°$ (= $\arctan^{-1}\sqrt{2}$).

($\alpha=0$ for symmetric interactions).

Magic-Angle Spinning (MAS)

with the **reduced Wigner Rotation Matrices**

$$d^2_{m',m}(\beta) =$$

$$\begin{pmatrix}
\frac{1}{4}(1+\cos\beta)^2 & -\frac{1}{2}(1+\cos\beta)\sin\beta & \sqrt{\frac{3}{8}}\sin^2\beta & -\frac{1}{2}(1-\cos\beta)\sin\beta & \frac{1}{4}(1-\cos\beta)^2 \\
\frac{1}{2}(1+\cos\beta)\sin\beta & \frac{1}{2}(\cos\beta-1)+\cos^2\beta & -\sqrt{\frac{3}{8}}\sin 2\beta & \frac{1}{2}(1+\cos\beta)-\cos^2\beta & -\frac{1}{2}(1-\cos\beta)\sin\beta \\
\sqrt{\frac{3}{8}}\sin^2\beta & \sqrt{\frac{3}{8}}\sin 2\beta & \frac{1}{2}(3\cos^2\beta-1) & -\sqrt{\frac{3}{8}}\sin 2\beta & \sqrt{\frac{3}{8}}\sin^2\beta \\
\frac{1}{2}(1-\cos\beta)\sin\beta & \frac{1}{2}(1+\cos\beta)-\cos^2\beta & \sqrt{\frac{3}{8}}\sin 2\beta & \frac{1}{2}(\cos\beta-1)+\cos^2\beta & -\frac{1}{2}(1+\cos\beta)\sin\beta \\
\frac{1}{4}(1-\cos\beta)^2 & \frac{1}{2}(1-\cos\beta)\sin\beta & \sqrt{\frac{3}{8}}\sin^2\beta & \frac{1}{2}(1+\cos\beta)\sin\beta & \frac{1}{4}(1+\cos\beta)^2
\end{pmatrix}$$

Under MAS:

2. For m=0, $\omega_{0,m}$ has to be represented in the laboratory coordinate system. **Application** of the "**addition theorem**" for a Wigner rotation matrix yield

$$\omega^{IS}_{0,m} = -2b_{IS} D^2_{0,-m}(\Omega_{CR}) d^2_{-m,0}(\beta_{RL}) \exp(im\omega_r t) \quad \text{and}$$

$$\omega^{IS}_{0,m} = -2b_{IS}\, d^2_{0,-m}(\beta_{CR})\, \exp\left[im(\gamma_{CR}+\omega_r t)\right] d^2_{-m,0}(\beta_{RL})$$

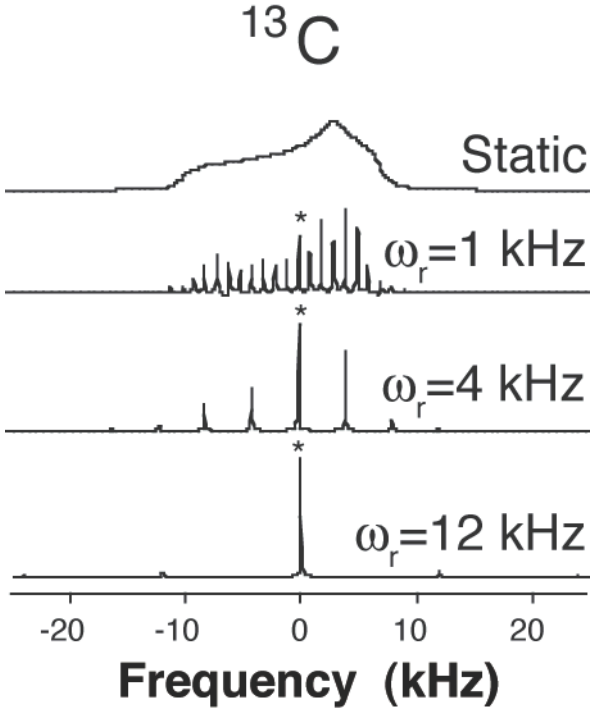
For m=0, this expression is exactly zero if β_{RL}**= 54.735°** (= $\arctan^{-1}\sqrt{2}$).

(α=0 for symmetric interactions).

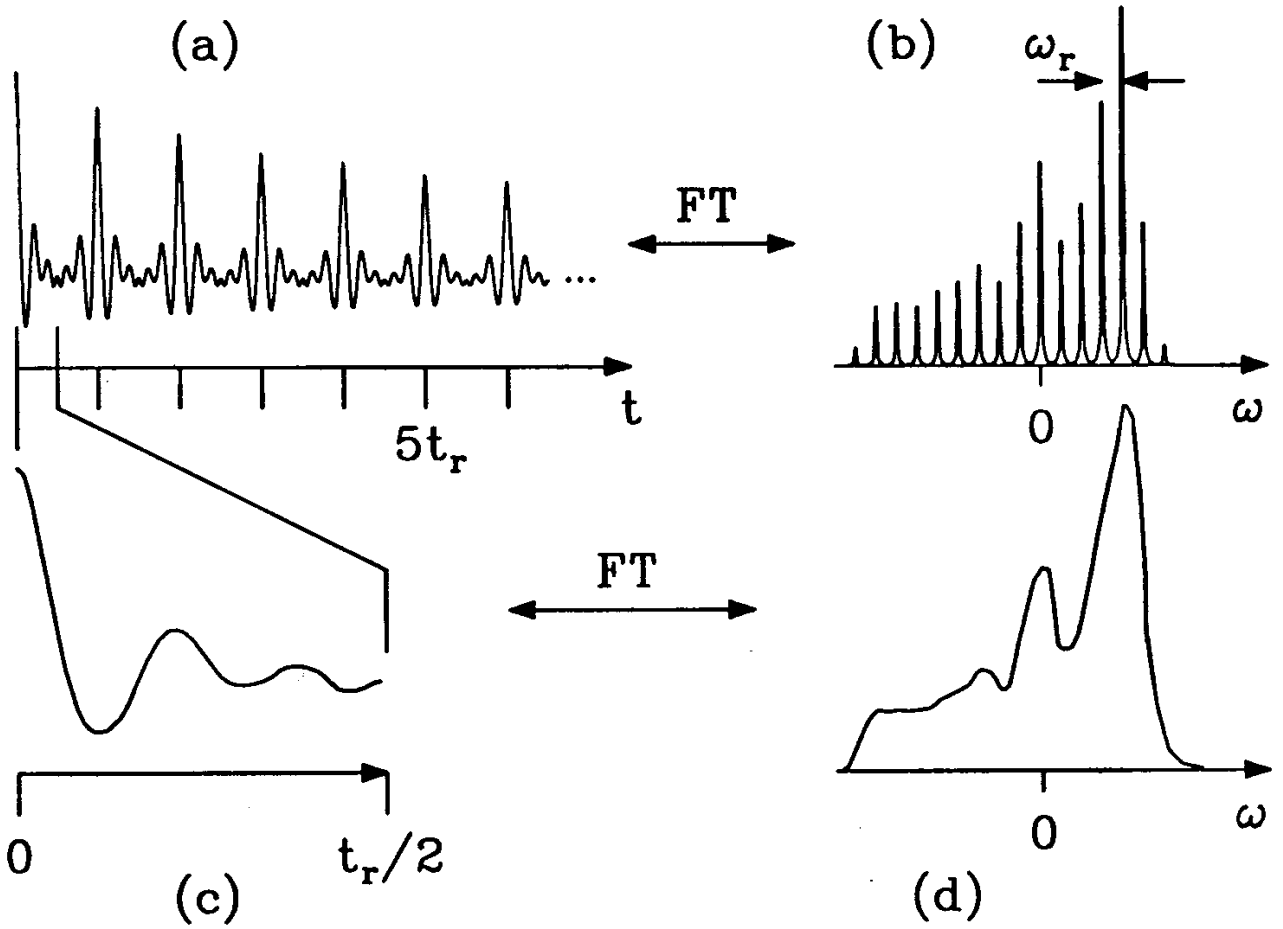
Magic-Angle Spinning (MAS)

Rotational frequency $<$ Size of the interaction

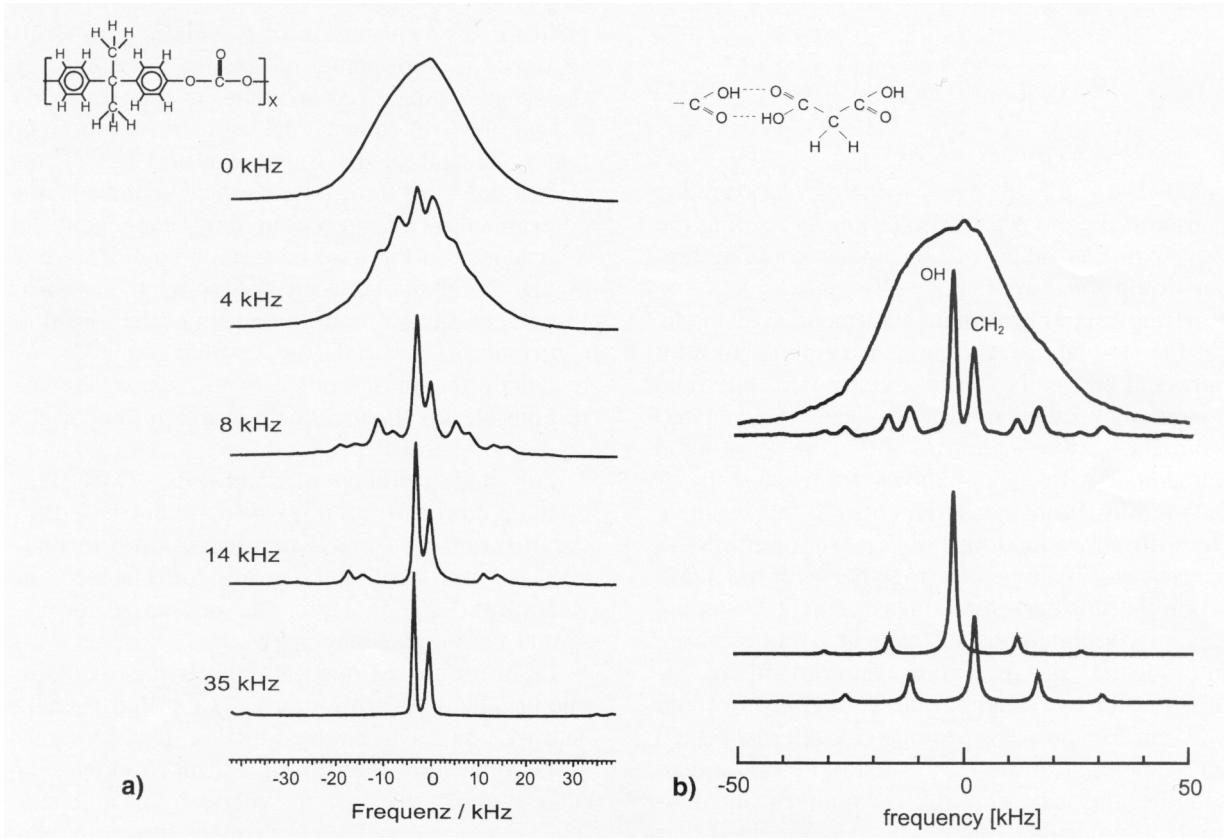
$\rightarrow$ Powder spectrum is split into rotational sidebands.



Note on MAS spinning sidebands



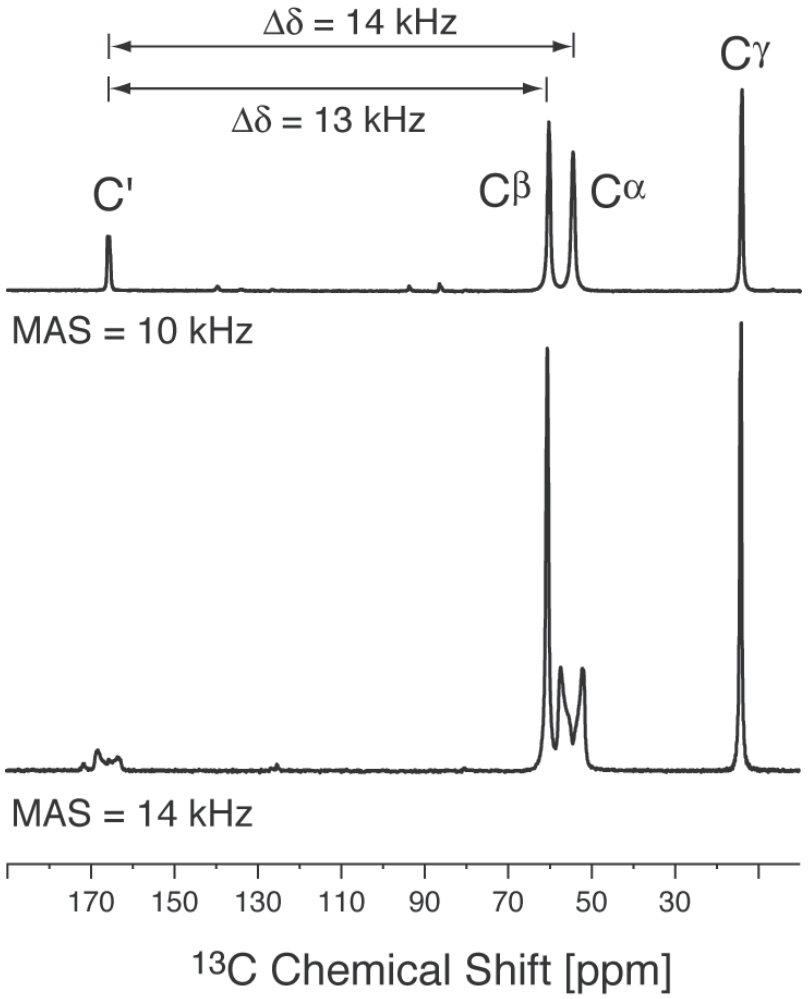
Note on MAS spinning sidebands



Rotational Resonance (R^2): Recoupling under MAS (1)

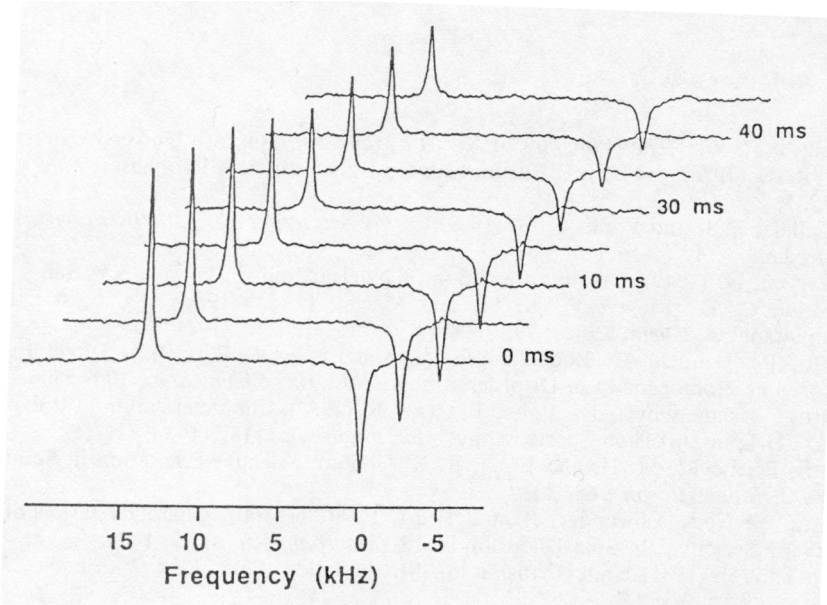
$$\Delta\omega = n\omega_r$$

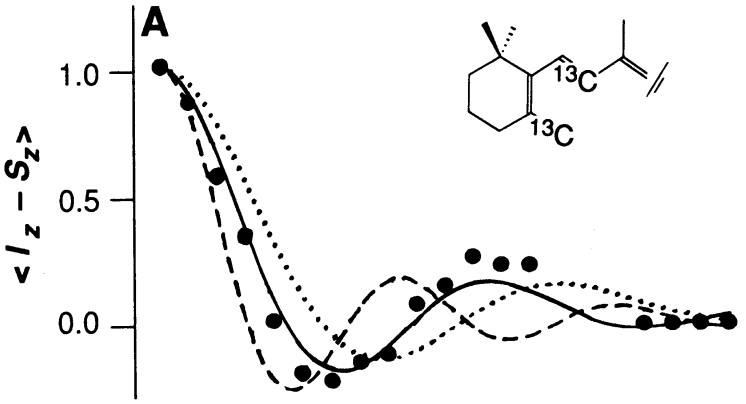
u-^{13}C,^{15}N-Thr



Levitt, Raleigh, Creuzet, Griffin, *J. Chem. Phys.* **92**, 6347-6364 (1990).

Rotational Resonance (R^2): Recoupling under MAS (1)

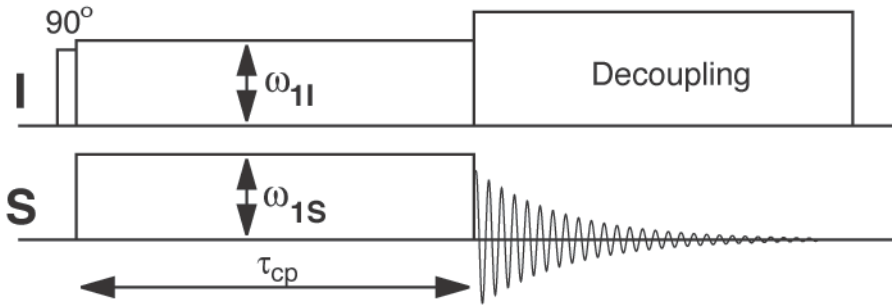




Creuzet, McDermott, Gebhard, Vanderhoef, Spijkerassink, Herzfeld, Lugtenburg, Levitt, Griffin, Determination of Membrane-Protein Structure by Rotational Resonance NMR – Bacteriorhodopsin, *Science* **251**, 783-786 (1991)

Exercise: Derivation of the R^2 condition

Cross Polarization (CP): Recoupling under MAS (2)



$$S/N = I(I+1)N\gamma^{3}$$

I: spin-quantum number
N: number of nuclei in the sample
γ: Gyromagnetic Ratio

$$\frac{S}{N} = I(I+1)N\gamma^{5/2}$$

since noise contributes $\gamma^{1/2}$.

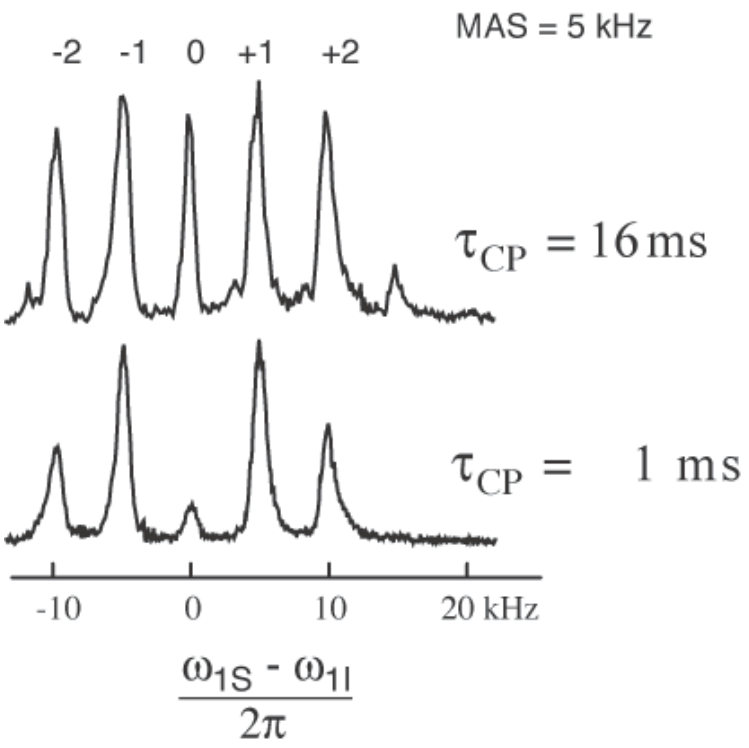
→ Inverse Detection

Hartmann-Hahn condition:

$$\omega_{1I} = \omega_{1S}$$
$$\gamma_I B_{1I} = \gamma_S B_{1S}$$

Cross Polarization (CP) under MAS

CP transfer as a function of $\Delta\omega_1$



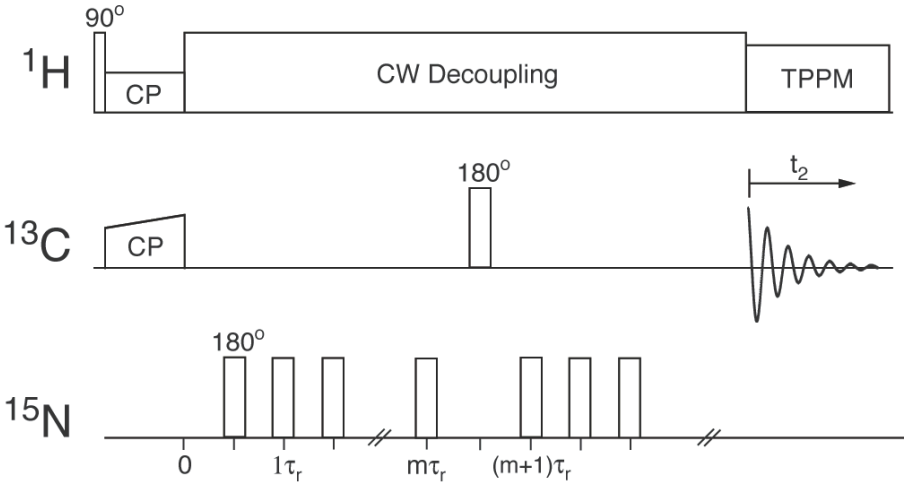
Hartmann-Hahn matching under MAS:

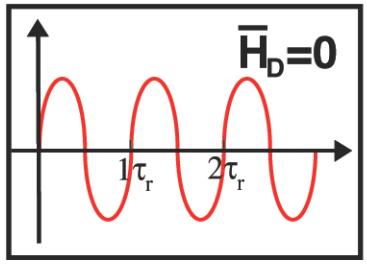
$$\omega_{1I} = \omega_{1S} + n\omega_r$$

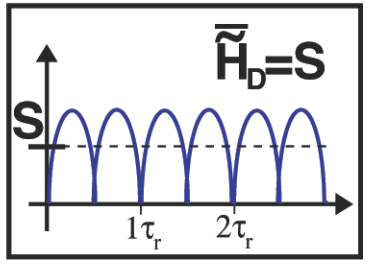
with $-2 \leq n \leq +2$

Exercise: Derivation of the Hartmann-Hahn match condition under MAS

REDOR: Recoupling in MAS NMR (3)







Heteronuclear dipolar interaction (under MAS)

$$\hat{H} = \omega_{IS}^{D}(t) I_z S_z$$

where

$$\omega_{IS}^{D}(t) = \sum_{m=-2}^{+2} \omega_{0,m}^{IS} \exp\left(im\omega_r t\right)$$

$$= \frac{b_{IS}}{2} \left\{ -\sqrt{2}\sin(2\beta)\cos\left(\gamma + \omega_r t\right) + \sin^2\beta \cos\left[2\left(\gamma + \omega_r t\right)\right] \right\}$$

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

Gullion & Schaefer (1989) *JMR* **81**, 196-200

Exercise: Calculate the Average Hamiltonian for REDOR

The Average-Hamiltonian over one rotor period yields

$$\bar{\hat{H}}_D = \int_0^{\tau_r} dt \;\; \hat{\mathrm{H}}^D(t) = \Phi \;\; 2I_z S_z$$

The effective dipolar coupling is given then as Φ

$$\Phi = \frac{2\pi}{\tau_r}\left\{\int_0^{\tau_r/2} dt \;\; \omega_{IS}^D(t) - \int_{\tau_r/2}^{\tau_r} dt \;\; \omega_{IS}^D(t)\right\}$$

$$= \frac{2\pi}{\tau_r}\sqrt{2}\sin(2\beta)\left\{\int_0^{\tau_r/2} dt \;\; \cos\left(\gamma+\omega_r t\right) \; - \int_{\tau_r/2}^{\tau_r} dt \;\; \cos\left(\gamma+\omega_r t\right)\right\}$$

$$+ \frac{2\pi}{\tau_r} \;\; \sin^2\beta \;\; \left\{\int_0^{\tau_r/2} dt \cos\left[2\left(\gamma+\omega_r t\right)\right] \; - \int_{\tau_r/2}^{\tau_r} dt \cos\left[2\left(\gamma+\omega_r t\right)\right]\right\}$$

$$= \frac{2\pi}{\tau_r}\sqrt{2}\sin(2\beta)\left\{\frac{1}{\omega_r}\left[\sin\left(\gamma+\omega_r t\right)\right]_0^{\tau_r/2} - \frac{1}{\omega_r}\left[\sin\left(\gamma+\omega_r t\right)\right]_{\tau_r/2}^{\tau_r}\right\}$$

$$+ \quad \frac{2\pi}{\tau_r}\sin^2\beta \;\; \left\{\frac{1}{2\omega_r}\sin\left[2\left(\gamma+\omega_r t\right)\right]_0^{\tau_r/2} \; - \frac{1}{2\omega_r}\sin\left[2\left(\gamma+\omega_r t\right)\right]_{\tau_r/2}^{\tau_r}\right\}$$

$$= \frac{2\pi}{\tau_r}\sqrt{2}\sin(2\beta)\left\{\frac{1}{\omega_r}\left[\sin(\gamma+\pi) - \sin(\gamma) - \left(\sin(\gamma+2\pi) - \sin(\gamma+\pi)\right)\right]\right\}$$

$$= b_{IS}\sqrt{2}\sin 2\beta \;\; \sin\gamma$$

Recoupling in MAS NMR: REDOR

The REDOR *dephasing* signal can be represented as

$$S_{dephasiert}[t] = \int_0^{2\pi} d\gamma \int_0^{\pi} d\beta \;\; \sin\beta \;\; \cos(\Phi\, t)$$

$$\Phi = b_{IS}\sqrt{2}\sin 2\beta \;\sin\gamma$$

(Powder average)

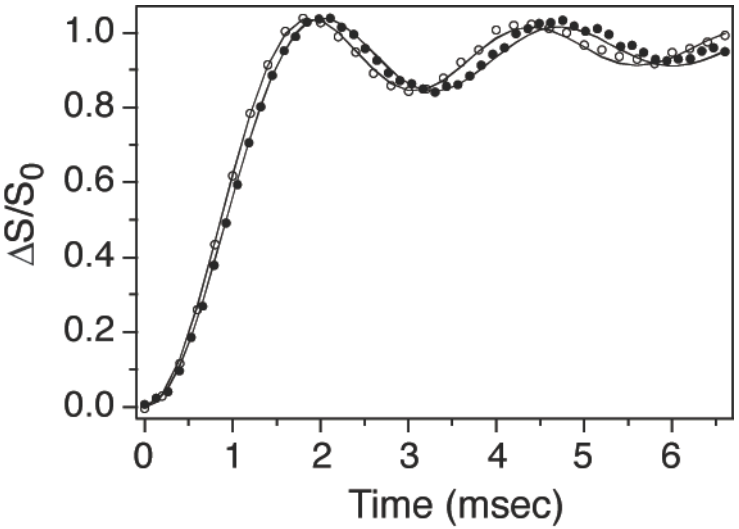
Experimentally, a dephasing and a reference experiment are recorded.

(Reference experiment: simultaneous 180° pulse on ^{15}N and ^{13}C in the center.

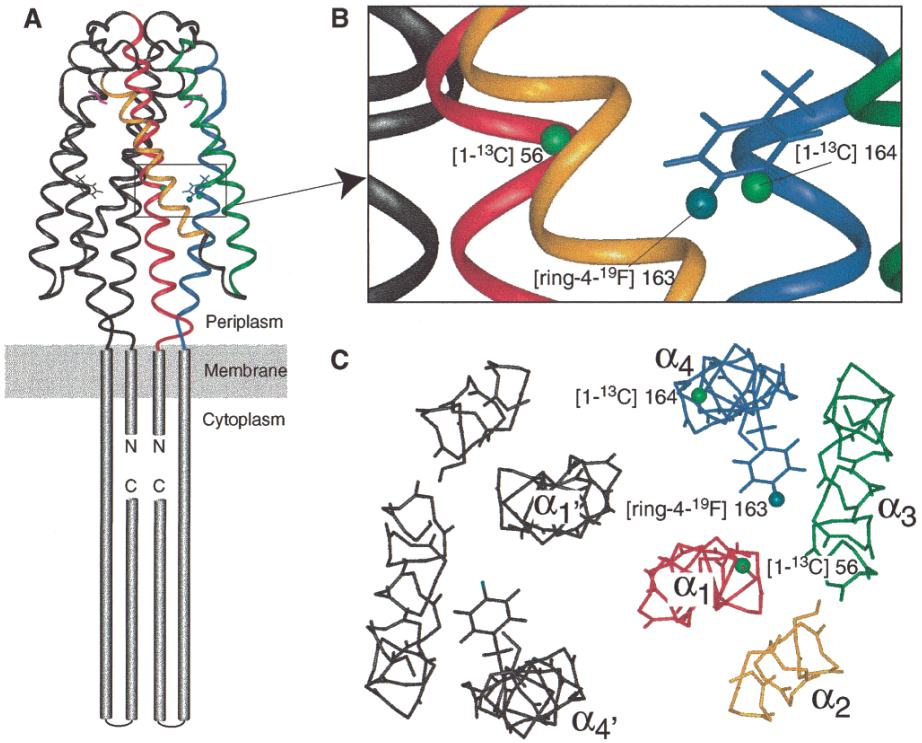
To eliminate relaxation effects, the following expression is used

$$\frac{\Delta S}{S_0} = \frac{S_0 - S_{dephasiert}}{S_0}$$

$^{15}N,^{13}C^{\alpha}$-Glycine



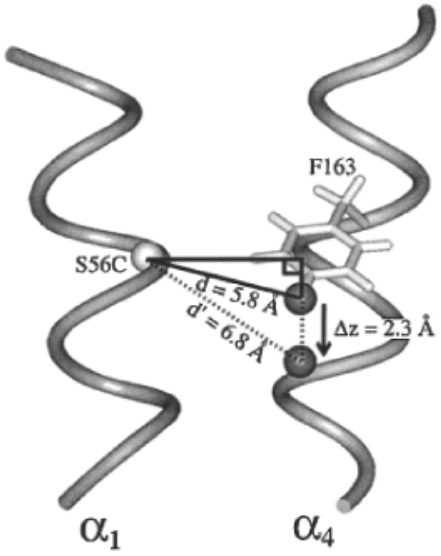
REDOR-Example 1: Study of the signalling mechanism in a bacterial chemoreceptor (aspartate receptor)

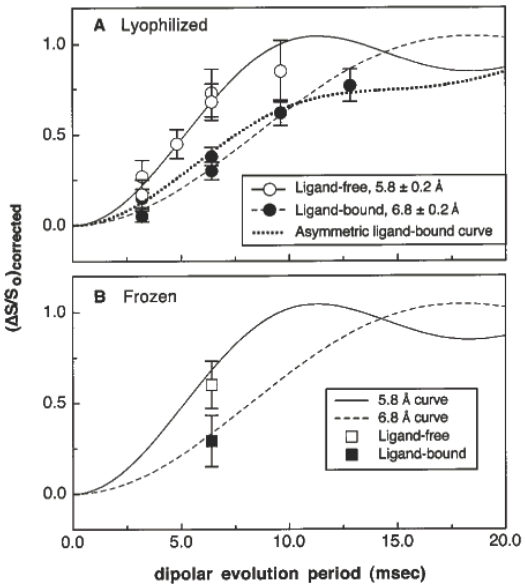


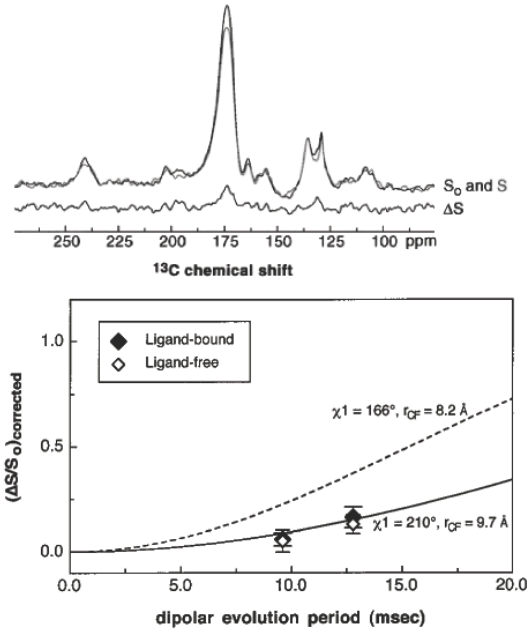
L.K. Thompson and co-workers, *Biochem.* (2001)

1. Ligand induced changes are propagated through the membrane and control the phosphorylation of a histidine-kinase (CheA).
2. X-ray structure available from the periplasmic domain.
3. Proposed mechanism:
   (a) 4˚ pivot motion between monomer subunits or
   (b) 1.6 Å piston motion of the C-terminal helix (α4) relative to the other helices within one subunit.
4. Labeling: ^{19}F in Leu163Phe and ^{13}CO in Ser56Cys.

REDOR-Example 1: Study of the signalling mechanism in a bacterial chemoreceptor (aspartate receptor)

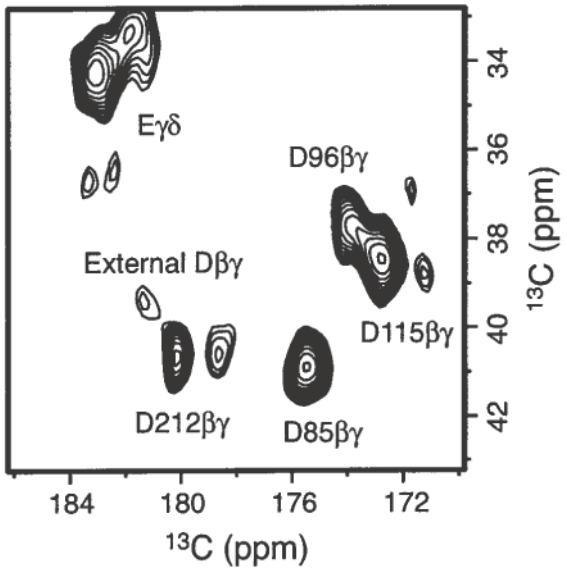


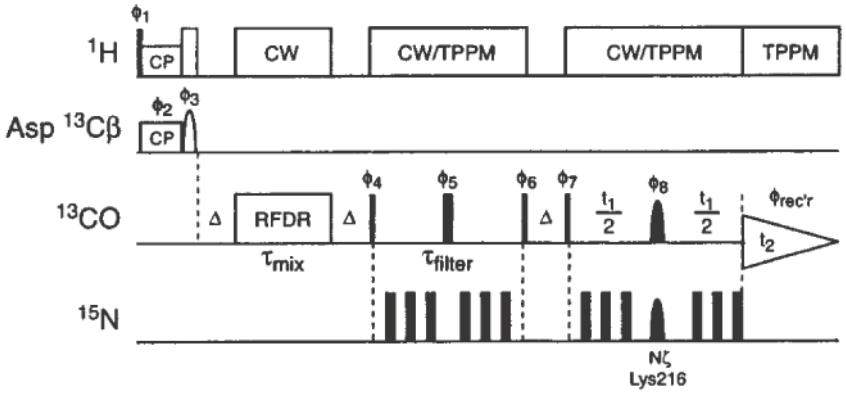




REDOR: Ligand-induced conformational change lead to a change of (1.0±0.3) Å in the distance between helices α1 and α4.

REDOR-Example 2: Frequency Selective REDOR in Uniformly Labeled Samples (Bacteriorhodopsin)

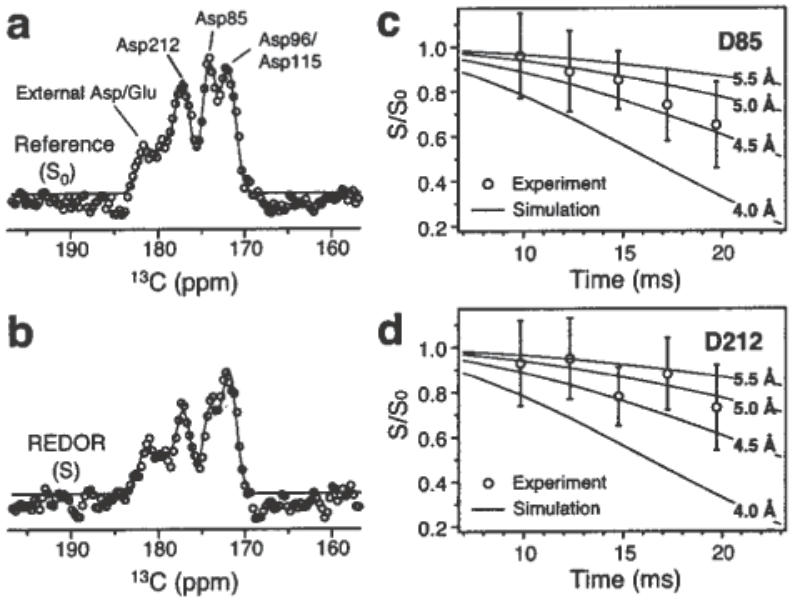




R.G. Griffin and co-workers

1. Only Asp: REDOR filter to suppress all C=O which are bonded to ^{15}N (Asn).
2. Measurement of distances between the Schiff-base **^{15}N** (K216) to Asp **^{13}C**OOH

REDOR-Example 2: Frequency Selective REDOR in Uniformly Labeled Samples (Bacteriorhodopsin)



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1. Only Asp: REDOR filter to suppress all C=O which are bonded to ^{15}N (Asn).
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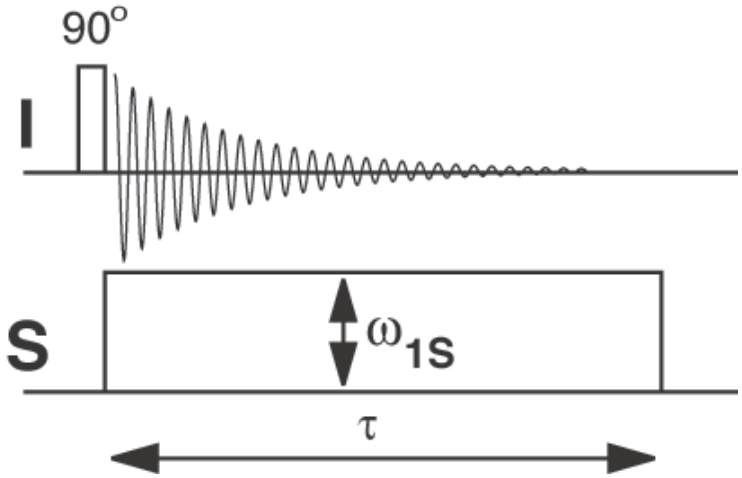
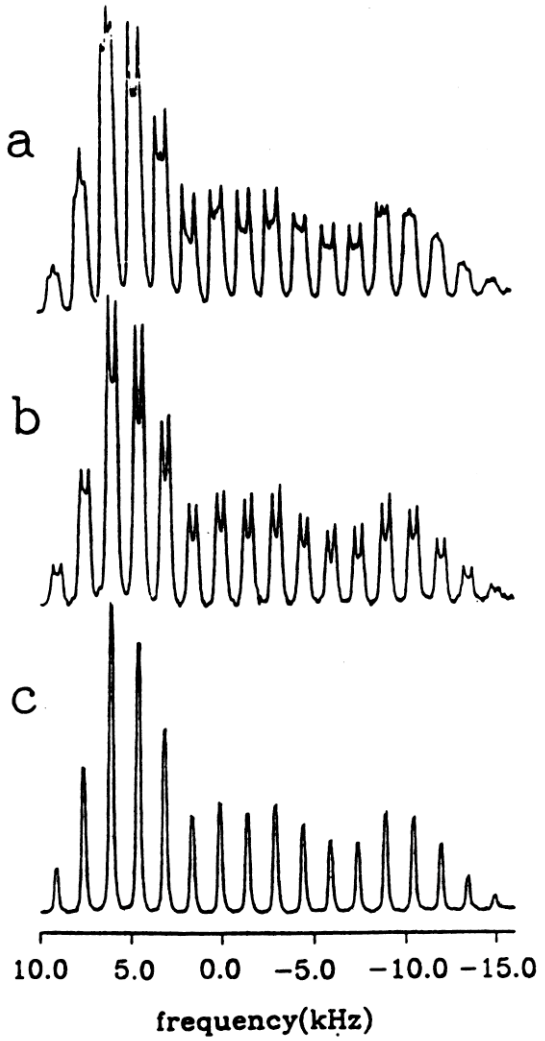
Rotary Resonance Recoupling (R3)



^{15}N-labeled N-methyldiphenyl-phosphoamidate

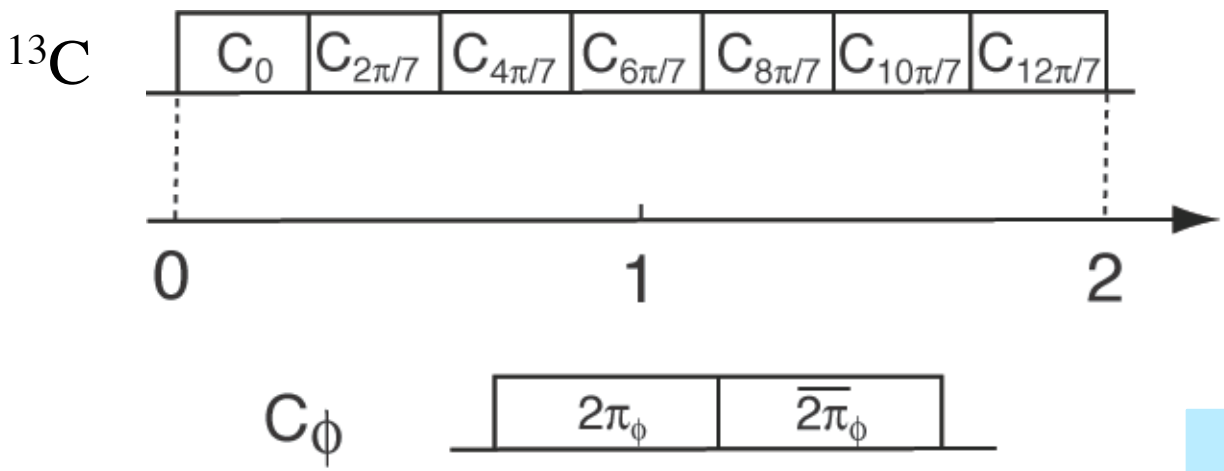
R3 spectra at n = ±1 (a), ±2 (b) and ±0 (c)

[$\omega_{RF} = 2\omega_r$], ω_{RF} on ^{15}N



^{31}P

C7: Recoupling under MAS



$$\tau_c = \frac{2\tau_r}{7} = \frac{4\pi}{7\omega_r}$$

1 rotation in spin space
2 rotations in real space

In C7, 2π pulses reintroduce ^{13}C,^{13}C dipolar couplings

In addition, 2π pulses are phase shifted synchronously to the rotor revolution

Levitt and co-workers, *Chem. Phys. Lett.* **242**, 304-309 (1995)

C7: Recoupling under MAS

spatial spin

Static Hamiltonian: $$H_D^{IS}(t) = \omega^{(0)} T_{20}^{IS} = \mu_0 \frac{\gamma_I \gamma_S \hbar}{4\pi r_{IS}^3} \frac{3\cos^2\beta - 1}{2} I_z S_z$$

Under MAS: $$\omega_D^{IS}(t) = \sum_{m=-2}^{+2} \omega^{(m)}\left(\Omega_{PR}\right) \exp\left(-im\omega_r t\right)$$

Application of RF Pulses:

$$\overline{H}_D(t) = \frac{1}{\tau_c}\int_0^{\tau_c} dt\ \exp(+i\omega_{rf} t\, I_x)\, T_{20}^{IS} \exp(-i\omega_{rf} t\, I_x)$$

"Average Hamiltonian"

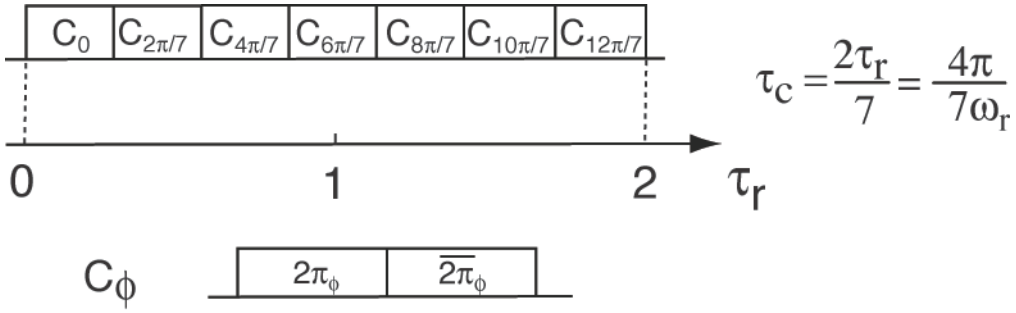
$$= \frac{1}{\tau_c}\int_0^{\tau_c} dt \sum_{\mu=-2}^{+2} d_{\mu,0}^{(2)}\left(\omega_{rf} t\right) T_{2,\mu}^{IS}$$

$$= \frac{1}{\tau_c}\int_0^{\tau_c} dt\ \left[T_{2,0}^{IS}\frac{1}{2}\left(3\cos^2\omega_{rf} t - 1\right) - i\sqrt{\frac{3}{8}}\left(T_{2,+1}^{IS} + T_{2,-1}^{IS}\right)\sin 2\omega_{rf} t - \right.$$

$$\left. - \sqrt{\frac{3}{8}}\left(T_{2,+2}^{IS} + T_{2,-2}^{IS}\right)\sin^2\omega_{rf} t\right]$$

$$\propto T_{2,0}^{IS} + \frac{1}{2}\sqrt{\frac{3}{8}}\left(T_{2,+2}^{IS} + T_{2,-2}^{IS}\right)$$

C7: Recoupling under MAS

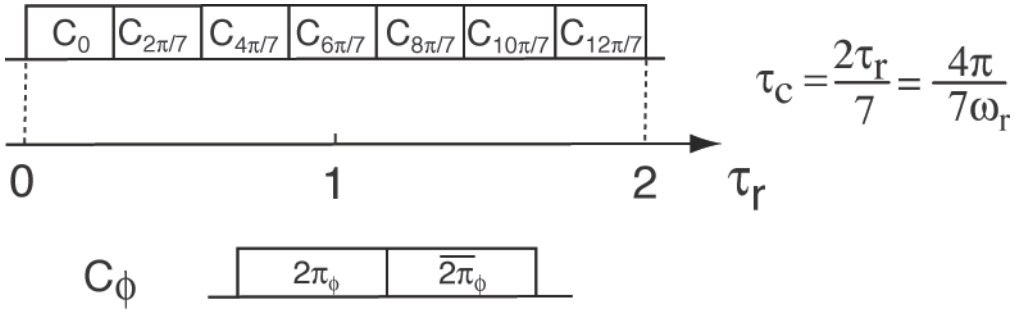


$$\tau_c = \frac{2\tau_r}{7} = \frac{4\pi}{7\omega_r}$$

1 rotation in spin space
2 rotations in real space

$$H_D^{IS}(t) = \sum_{m=-2}^{+2} \sum_{\mu=-2}^{+2} \omega^{(m)}\left(\Omega_{PR}\right) \exp\left(im\omega_r t\right) \times d_{\mu 0}^{(2)}\left(\omega_{rf} t\right) T_{2\mu}^{IS}$$

C7: Recoupling under MAS



$$\tau_C = \frac{2\tau_r}{7} = \frac{4\pi}{7\omega_r}$$

1 rotation in spin space
2 rotations in real space

In C7, rf elements are phase shifted synchronously with the rotor revolution:

$$H_D^{IS}(t) = \sum_{m=-2}^{+2}\sum_{\mu=-2}^{+2} \omega^{(m)}(\Omega_{PR}) \exp(im\omega_r t) \times d_{\mu 0}^{(2)}(\omega_{rf} t)\, T_{2\mu}^{IS} \exp(-i\mu\phi_p)$$

$$\overline{H}_D^{IS}(t) \propto \exp(im\omega_r t) \times \exp(-i\mu\phi_p)$$

$$= \exp\left[i2\pi p\left(\frac{2m}{7} - \frac{\mu}{7}\right)\right] \qquad p = [0,6]$$

$$\frac{2m-\mu}{7} = q \quad ; \text{q=integer}$$

Since μ=±2
→ Recouling of m= ± 1

$$\longrightarrow \quad \omega^{(\pm 1)} = \mp\frac{3}{2}\sqrt{3}\frac{\gamma_I\gamma_S}{r^3}\sin(2\beta_{PR})\exp(\pm i\gamma_{PR})$$

C7: Recoupling under MAS

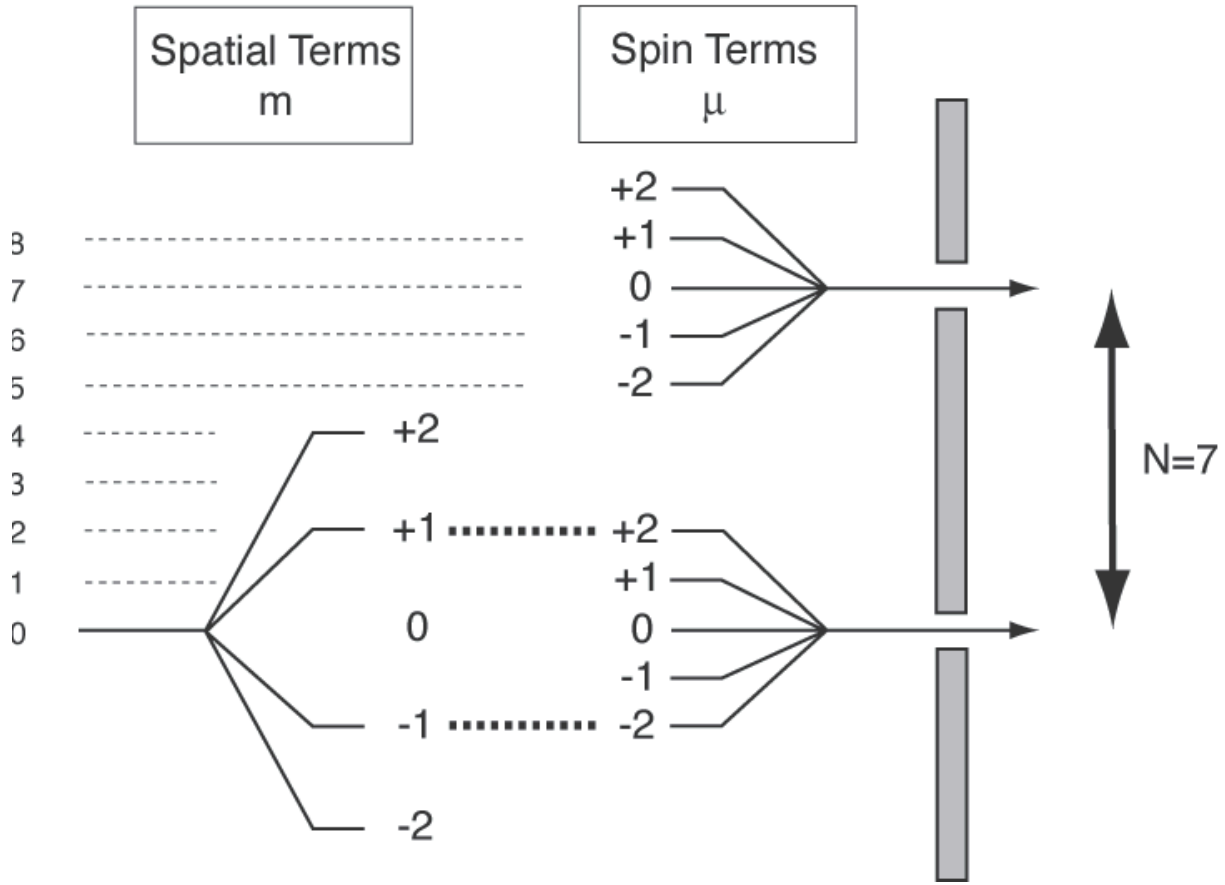
$CN^{\nu}_{n} \longrightarrow C7^{1}_{2}$

$mn - \mu = qN$

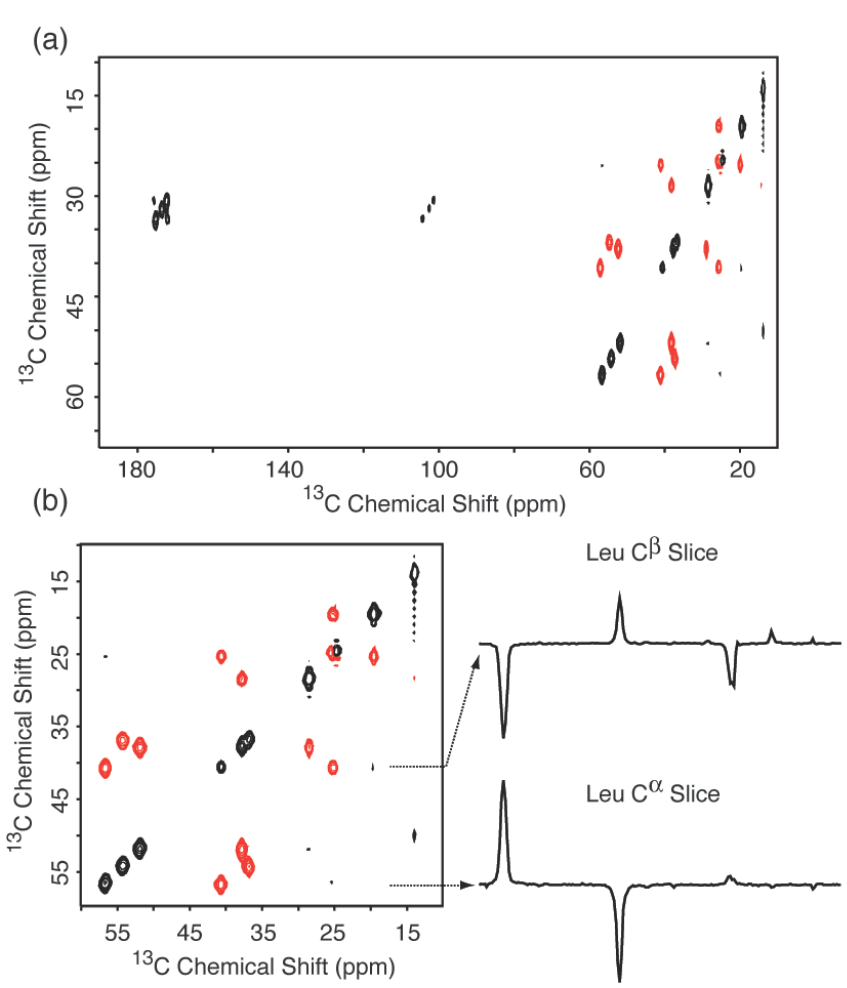
q = integer

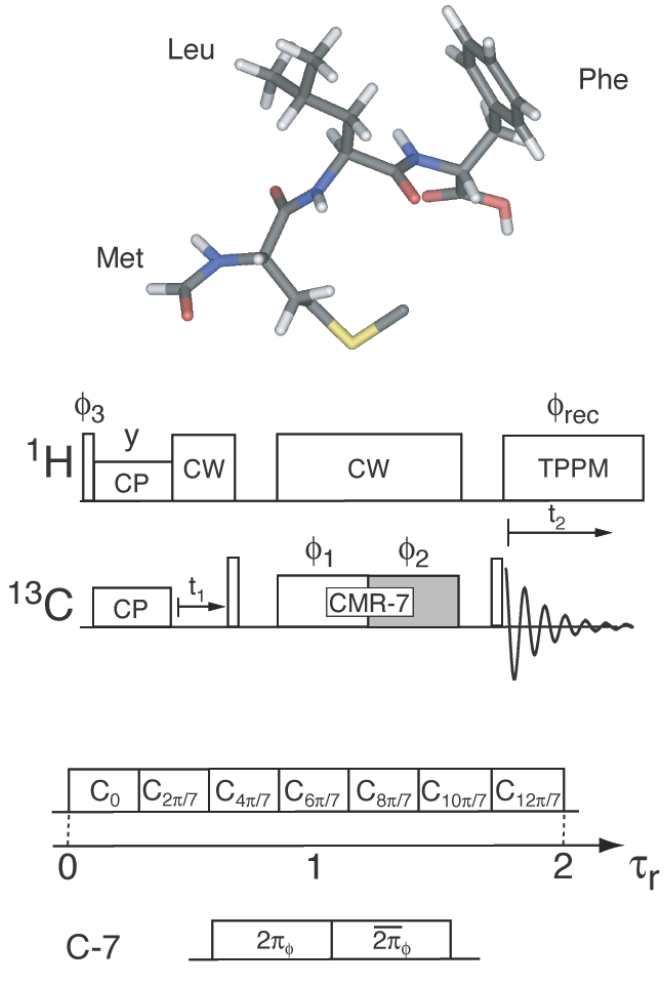
$$\frac{2m - \mu}{7} = q$$

N RF elements in
n rotor periods

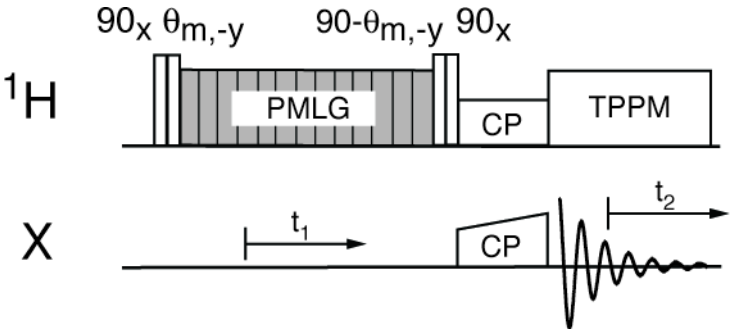


C7: Recoupling under MAS
^{13}C-^{13}C Correlations in N-formyl-Met-Leu-Phe

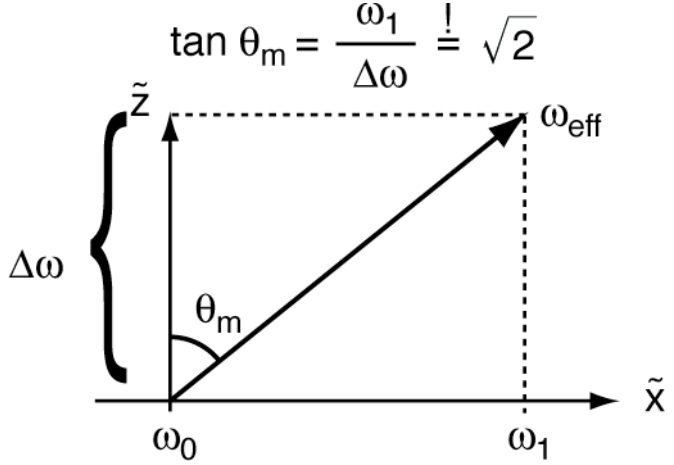


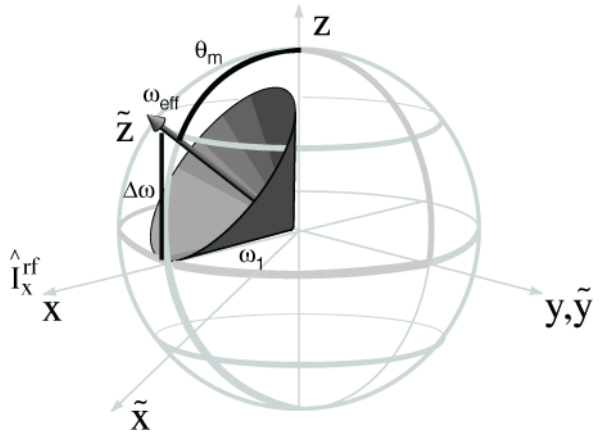


Frequency Switched Lee-Goldburg (FSLG) and Phase Modulated Lee-Goldburg (PMLG) Experiments for homonuclear ^{1}H,^{1}H Decoupling

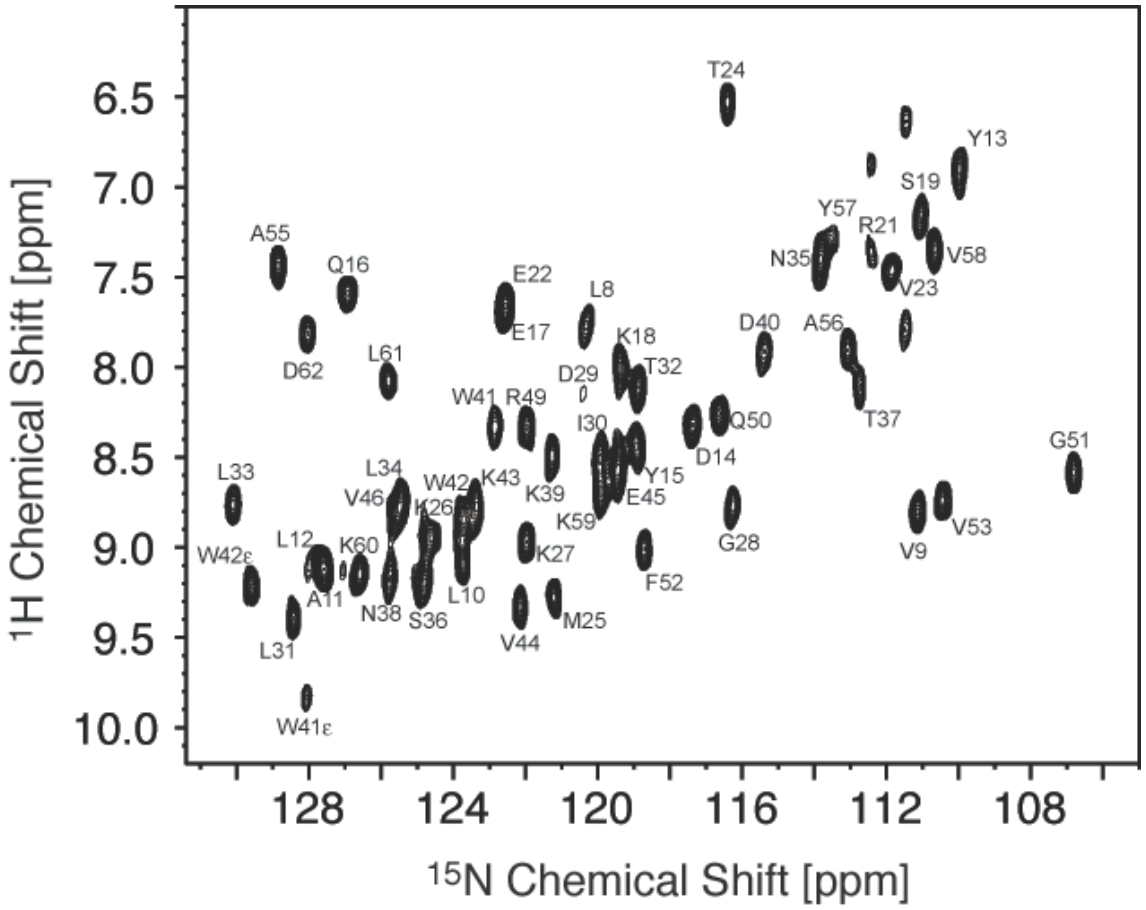


To obtain decoupling, magnetization has to be rotated around the *magic angle* in spin space:





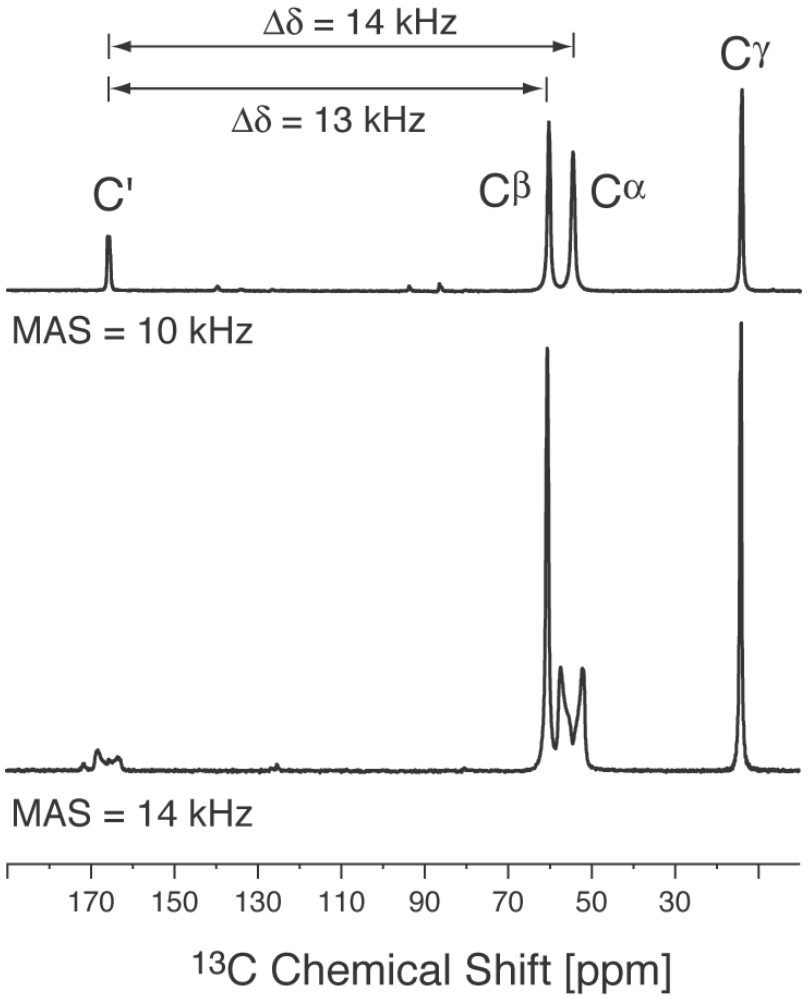
PMLG spectrum of a 15N labeled sample of a SH3 domain



Rotational Resonance (R^2): Recoupling under MAS (1)

$$\Delta\omega = n\omega_r$$

u-^{13}C,^{15}N-Thr



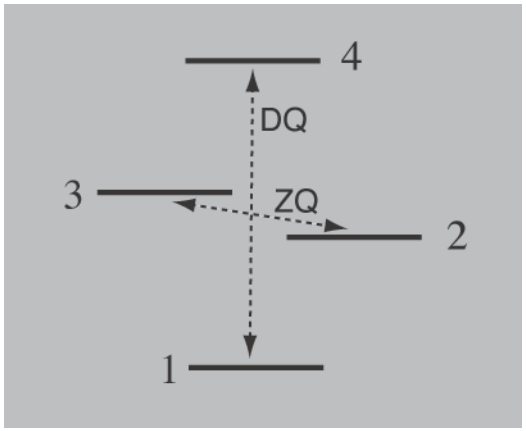
Levitt, Raleigh, Creuzet, Griffin, *J. Chem. Phys.* **92**, 6347-6364 (1990).

Exercise: Derivation of the R^2 condition

Hamilton Operator

$$H = \Omega_I I_z + \Omega_S S_z + \omega_{IS}^D(t)\, T_{20}^{IS}$$

Basis Set of Single-Transition operators



$$I_x^{(2,3)} = \frac{1}{2}\left[I^+S^- + I^-S^+\right] \qquad I_x^{(1,4)} = \frac{1}{2}\left[I^+S^+ + I^-S^-\right]$$

$$I_y^{(2,3)} = -\frac{i}{2}\left[I^+S^- - I^-S^+\right] \qquad I_y^{(1,4)} = -\frac{i}{2}\left[I^+S^+ - I^-S^-\right]$$

$$I_z^{(2,3)} = \frac{1}{2}\left[I_z - S_z\right] \qquad I_z^{(1,4)} = \frac{1}{2}\left[I_z + S_z\right]$$

Hamilton-Operator in the Basis Set of Single Transition Operators

$$\begin{aligned} H &= (\Omega_I + \Omega_S) I_z^{(1,4)} + (\Omega_I - \Omega_S) I_z^{(2,3)} - \omega_{IS}^D(t) I_x^{(2,3)} \\ &= \Omega^\Sigma\, I_z^{(1,4)} + \left(\Omega^\Delta I_z^{(2,3)} - \omega_{IS}^D(t) I_x^{(2,3)}\right) \end{aligned}$$

Exercise: Derivation of the R^2 condition

We used: $$\hat{T}_{20}^{IS} = \frac{1}{2}\left[3\hat{I}_{1z}\hat{I}_{2z} - \hat{\mathbf{1}}_1\hat{\mathbf{1}}_1\right] = 2\hat{I}_{1z}\hat{I}_{2z} - \frac{1}{2}\left(\hat{I}_1^+\hat{I}_2^- + \hat{I}_1^-\hat{I}_2^+\right)$$

1. The term $\boldsymbol{I_z S_z}$ can be neglected, since it corresponds to the unity operator in the Basis Set of Single Transition Operators

$$2I_{1z}I_{2z} = \left(I_z^{(1,4)}\right)^2 - \left(I_z^{(2,3)}\right)^2 = \frac{1}{4}\left(\mathbf{1}^{(1,4)} - \mathbf{1}^{(2,3)}\right)$$

2. $\boldsymbol{I_z^{(1,4)}}$ is a Constant of Motion and commutes

$\rightarrow$ only $\quad \Omega^{\Delta} I_z^{(2,3)} - \omega_{IS}^{D}(t) I_x^{(2,3)}$

has to be considered

Exercise: Derivation of the R^2 condition

Example: Propagator for free evolution applied to a Spin I_x

$$U^{+} I_{1x} U =$$

$$\exp\left(-i\Omega_1 I_{1z} t\right) I_{1x} \exp\left(+i\Omega_1 I_{1z} t\right) \rightarrow I_{1x} \cos(\Omega_1 t) + I_{1y} \sin(\Omega_1 t)$$

Transformation into a Time independent Frame

$$U = \exp\left(-i\Omega^{\Delta}(t) I_z^{(2,3)}\right)$$

$$\Omega^{\Delta}(t) = \Omega_{\Delta}^{iso} t + \int_0^t dt' \sum_{\substack{k=-2 \\ k\neq 0}}^{+2} \Omega_{\Delta}^{(k)} \exp\left(ik\omega_r t'\right)$$

Exercise: Derivation of the R^2 condition

The Hamilton Operator in the time independent Frame

$$\hat{H}^{(2,3)}(t) = \omega_{IS}^{D}(t) \quad \hat{U}^{+}\hat{I}_{x}^{(2,3)}\hat{U}$$

$$= \omega_{IS}^{D}(t) \quad \hat{U}^{+}\left[\frac{1}{2}\left(\hat{I}_{(2,3)}^{+} + \hat{I}_{(2,3)}^{-}\right)\right]\hat{U}$$

$$= \frac{\omega_{IS}^{D}(t)}{2} \quad \left[\hat{I}_{x}^{(2,3)}\cos\Omega^{\Delta}(t) + \hat{I}_{y}^{(2,3)}\sin\Omega^{\Delta}(t)\right]$$

$$= \frac{\omega_{IS}^{D}(t)}{2} \quad \left[\hat{I}_{(2,3)}^{+}\exp(-i\Omega^{\Delta}) + \hat{I}_{(2,3)}^{-}\exp(+i\Omega^{\Delta})\right]$$

Since $$\omega_{IS}^{D}(t) = \sum_{m=-2}^{+2}\omega_{0,m}^{IS}\exp\left(im\omega_{r}t\right)$$

Exercise: Derivation of the R^2 condition

Finally, the Hamilton Operator can be re-written as

$$\hat{H}^{(2,3)}(t) = \sum_{m=-2}^{+2} \omega_{0,m}^{IS} e^{im\omega_r t} \left\{ I_{(2,3)}^{+} \exp\left[-i\left(\Omega_{\Delta}^{iso} t + \int_0^t dt' \sum_{k\neq 0} \Omega_{\Delta}^{(k)} e^{ik\omega_r t'} \right) \right] + \right.$$

$$\left. + I_{(2,3)}^{-} \exp\left[+i\left(\Omega_{\Delta}^{iso} t + \int_0^t dt' \sum_{k\neq 0} \Omega_{\Delta}^{(k)} e^{ik\omega_r t'} \right) \right] \right\}$$

For vanishing anisotropy and asymmetry, one obtains

$$\hat{H}^{(2,3)}(t) = \sum_{m=-2}^{+2} \omega_{0,m}^{IS} \left\{ \exp\left[i(m\omega_r - \Omega_{iso}^{\Delta}) t \right] I_{(2,3)}^{+} + \exp\left[i(m\omega_r + \Omega_{iso}^{\Delta}) t \right] I_{(2,3)}^{-} \right\}$$

Rotational Resonance Condition:

$$\Delta\omega = n\omega_r$$

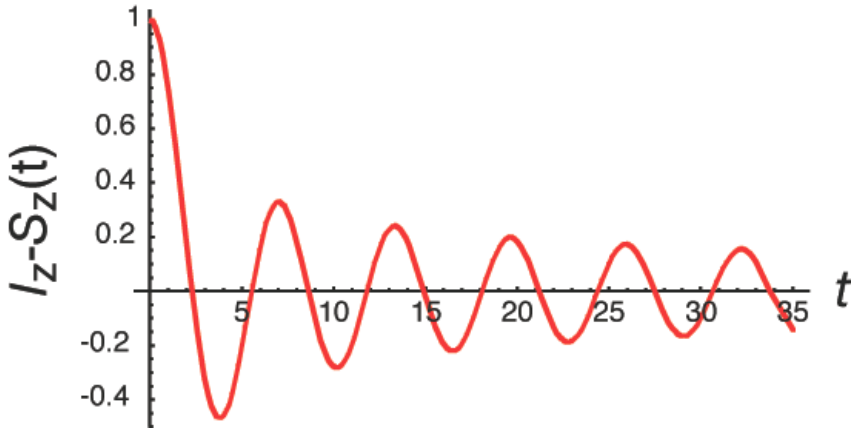
Exercise: Derivation of the R^2 condition

This means that e.g. the n=1 spatial component is recoupled

$$\omega_{0,\pm1}^{IS} = \mu_0 \frac{\gamma_I \gamma_S}{4\pi r_{IS}^3} \hbar \sqrt{\tfrac{3}{8}} \sin(2\beta) \exp(i\gamma)$$

The mixing time dependence can therefore be analytically written as

$$\tfrac{1}{2}\left(I_{1z} - I_{2z}\right)[t] = I_z^{(2,3)}[t] = \int_0^{2\pi} d\gamma \int_0^{\pi} d\beta \ \sin\beta \ \cos\left(\omega_{0,\pm1}^{IS} t\right)$$



Exercise: Derivation of the Hartmann-Hahn match condition under MAS

Hamilton-Operator in the Doubly Rotating Frame (rf along x-axis):

$$H = \omega_{1I} I_z + \omega_{1S} S_z + 2b_{IS}^{D}(t) I_x S_x$$

This can be rewritten

$$H^{T}(t) = \frac{\omega^{D}(t)}{2}\left(I^{+} + I^{-}\right)\left(S^{+} + S^{-}\right) \quad + \quad \omega_{1I} I_z + \omega_{1S} S_z$$

$$= H_1^{T}(t) + H_0^{T}$$

Exercise: Derivation of the Hartmann-Hahn match condition under MAS

Eliminate the oscillatory rf field contribution

$$\tilde{H}(t) = \tilde{U}^{+}\ H\ (t)\ \tilde{U}$$

$$\tilde{U} = \hat{T}\exp\left\{-i\int_{0}^{\tau_C} dt'\ H_0^T(t')\right\} = \exp\left\{-i\omega_{1I}I_z t\right\}\exp\left\{-i\omega_{1S}S_z t\right\}$$

Exercise: Derivation of the Hartmann-Hahn match condition under MAS

$$\tilde{H}(t) = \exp\{+i\omega_{1S}S_z t\}\exp\{+i\omega_{1I}I_z t\}\left[\frac{\omega^D}{2}\left(S^+ + S^-\right)\left(I^+ + I^-\right)\right]\exp\{-i\omega_{1I}I_z t\}\exp\{-i\omega_{1S}S_z t\}$$

$$= \frac{1}{2}\sum_{n=-2}^{+2}\omega_D^{(n)}\exp\{-in\omega_r t\}\left[S^+\exp\{-i\omega_{1S}t\} + S^-\exp\{+i\omega_{1S}t\}\right]\left[I^+\exp\{-i\omega_{1I}t\} + I^-\exp\{+i\omega_{1I}t\}\right]$$

$$= \frac{1}{2}\sum_{n=-2}^{+2}\omega_D^{(n)}\exp\{-in\omega_r t\}$$

$$\left[S^+I^+\exp\{-i(\omega_{1S}+\omega_{1I})t\}\right] +$$

$$\left[S^-I^-\exp\{+i(\omega_{1S}+\omega_{1I})t\}\right] +$$

$$\left[S^+I^-\exp\{-i(\omega_{1S}-\omega_{1I})t\}\right] +$$

$$\left[S^-I^+\exp\{+i(\omega_{1S}-\omega_{1I})t\}\right]$$

Average Hamiltonian ≠ 0 for

$$n\omega_r = \omega_{1S} + \omega_{1I}$$

and

$$n\omega_r = \omega_{1S} - \omega_{1I}$$

Remember:

$$\hat{H}_{IS}^{DD} = \sum_{m=-2}^{+2}\omega_{0,m}^{IS}(\Omega)\exp(im\omega_r t)\,T_{20}^{IS}$$