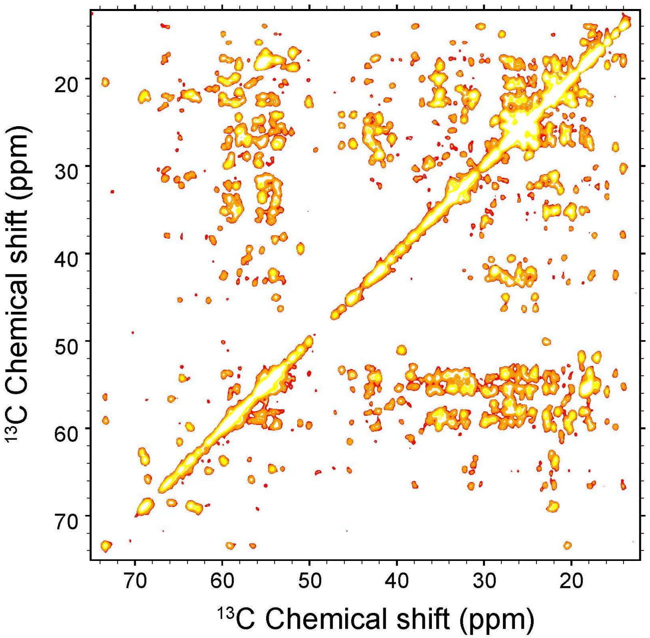
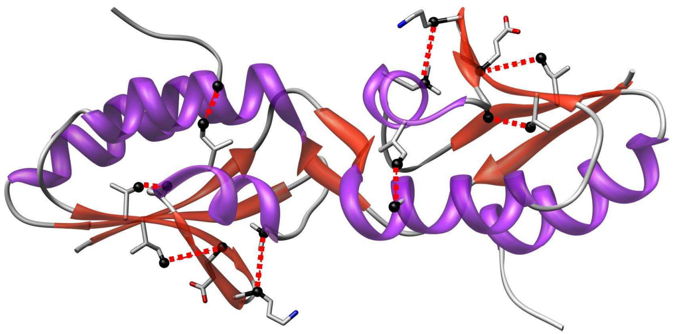
CMRR and TSAR Recoupling

Advanced Control for Structure Determination in Solid-State NMR





Crh 2x10.4 kDa - PAR 900 MHz

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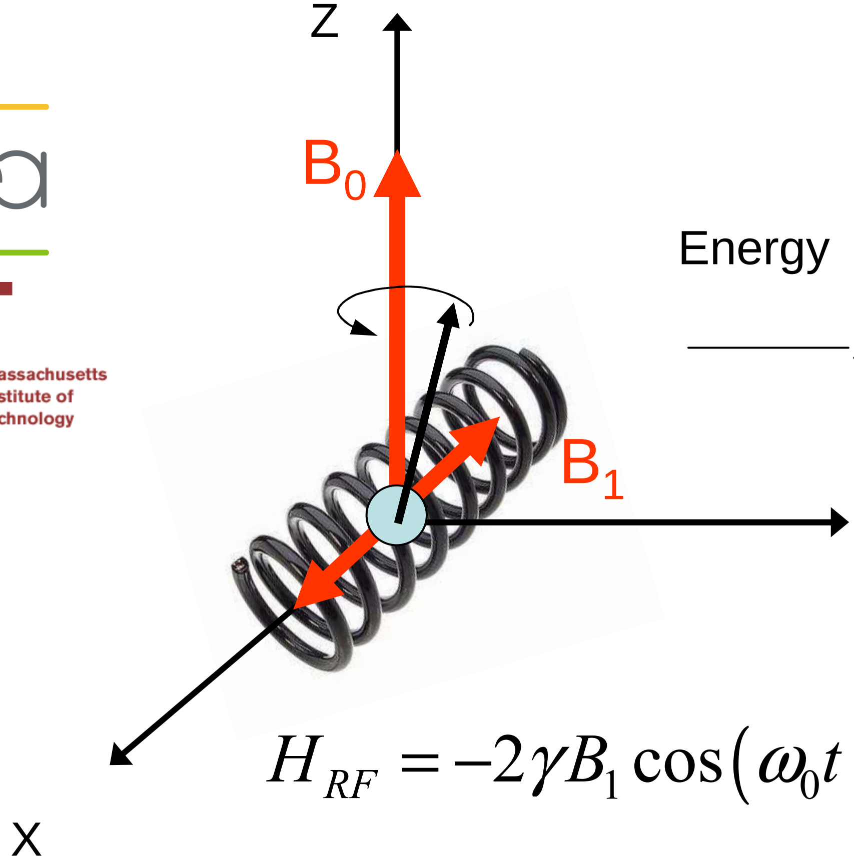
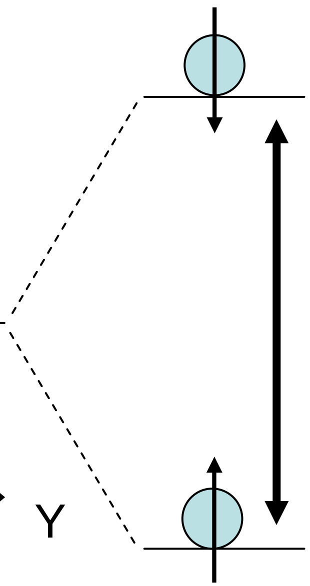
High Field Nuclear Magnetic Resonance





For ^{1}H @ $B_0 = 11.74T$

$$\Delta E/\hbar = -\gamma B_0 = \omega_0$$

$\sim 500MHz$

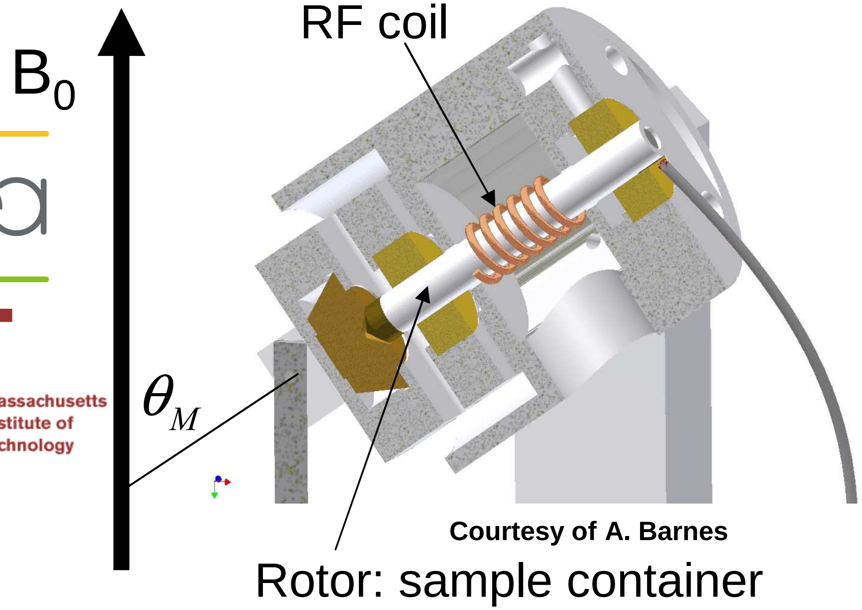
$$H_{RF} = -2\gamma B_1 \cos(\omega_0 t + \varphi) I_X = 2\omega_1 \cos(\omega_0 t + \varphi) I_X$$

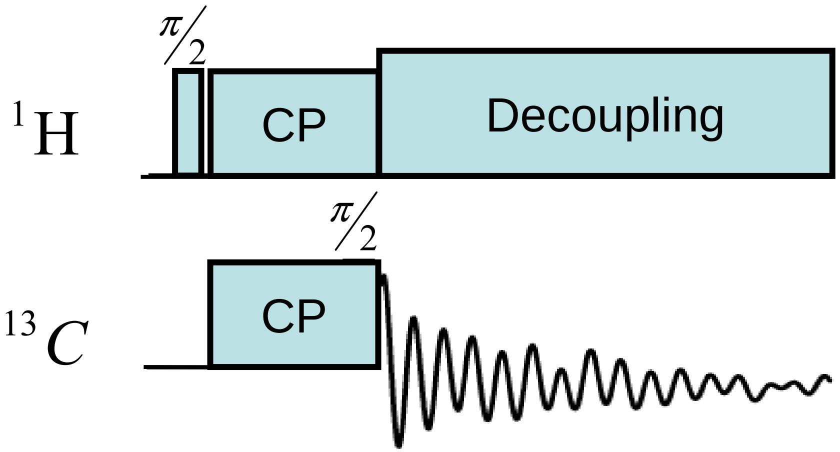
Amplitude, up to ~100 kHz

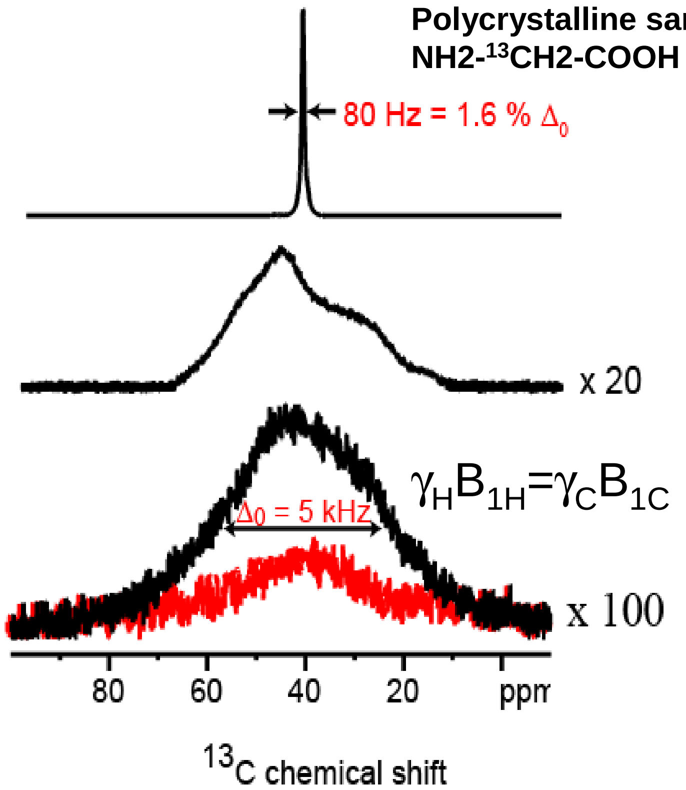
Phase

- **RF excitation of the nuclei at their Larmor Frequencies**

High Resolution Solid State NMR







Dipolar & Chemical Shift Anisotropy broadening

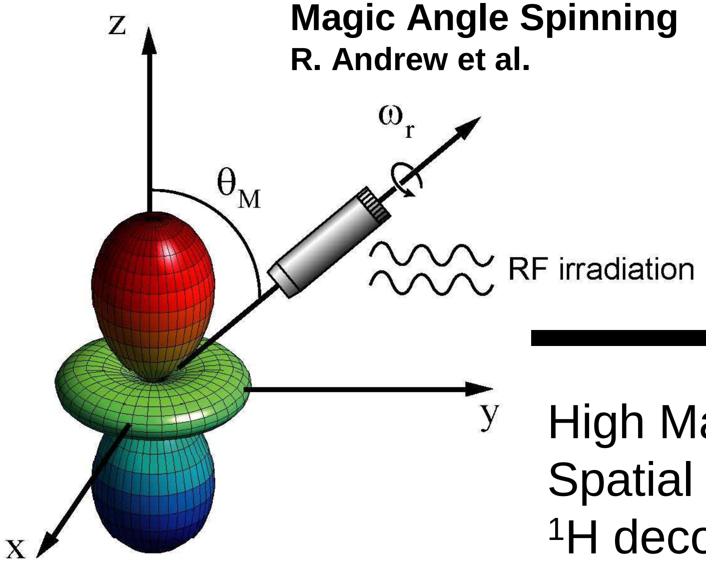
removed if $3\cos^2(\theta_M)-1=0$

$$\widehat{H}=\sum A_{l0}^{\lambda}\tau_{l0}^{\lambda}$$

- **Tremendous progress over the last 50 years..**

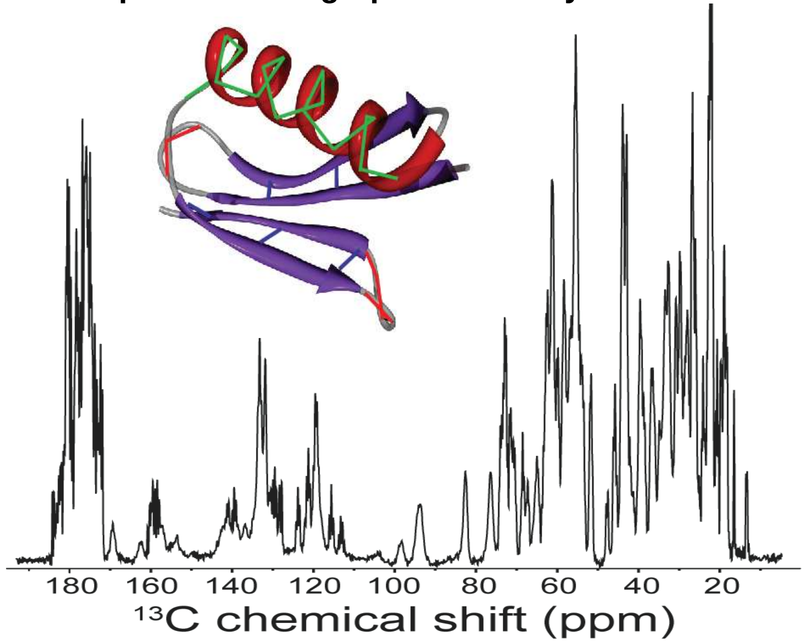
Key authors: Abragam, Andrew, Griffin, Hartmann, Hahn, Mehring, Pines, Schaefer, Waugh, et al.

Structure Determination in Solid State NMR



High Magnetic Field
Spatial Averaging
^{1}H decoupling

1D spectrum = fingerprint of the system!



- **MAS provides high resolution:** $H_{\text{detection}} = H_{\text{CS}} + \cancel{H_{\text{CSA}}} + \cancel{H_{\text{DIP}}}$
- **In order to recover the distance information, we need to design RF pulses that can interfere with MAS averaging:**

$$H_{\text{mixing}} = \cancel{H_{\text{CS}}} + \cancel{H_{\text{CSA}}} + H_{\text{DIP}} \longrightarrow$$

Recoupling sequences in multi-dimensional experiments

Key authors: Schaefer, Griffin, Levitt, Tycko, Nielsen, Khaneja, Baldus etc.

New methods for structure determination

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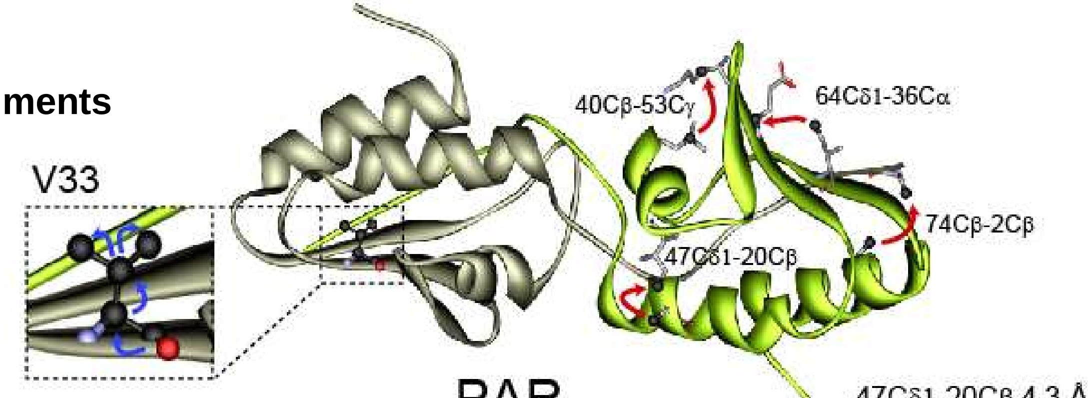
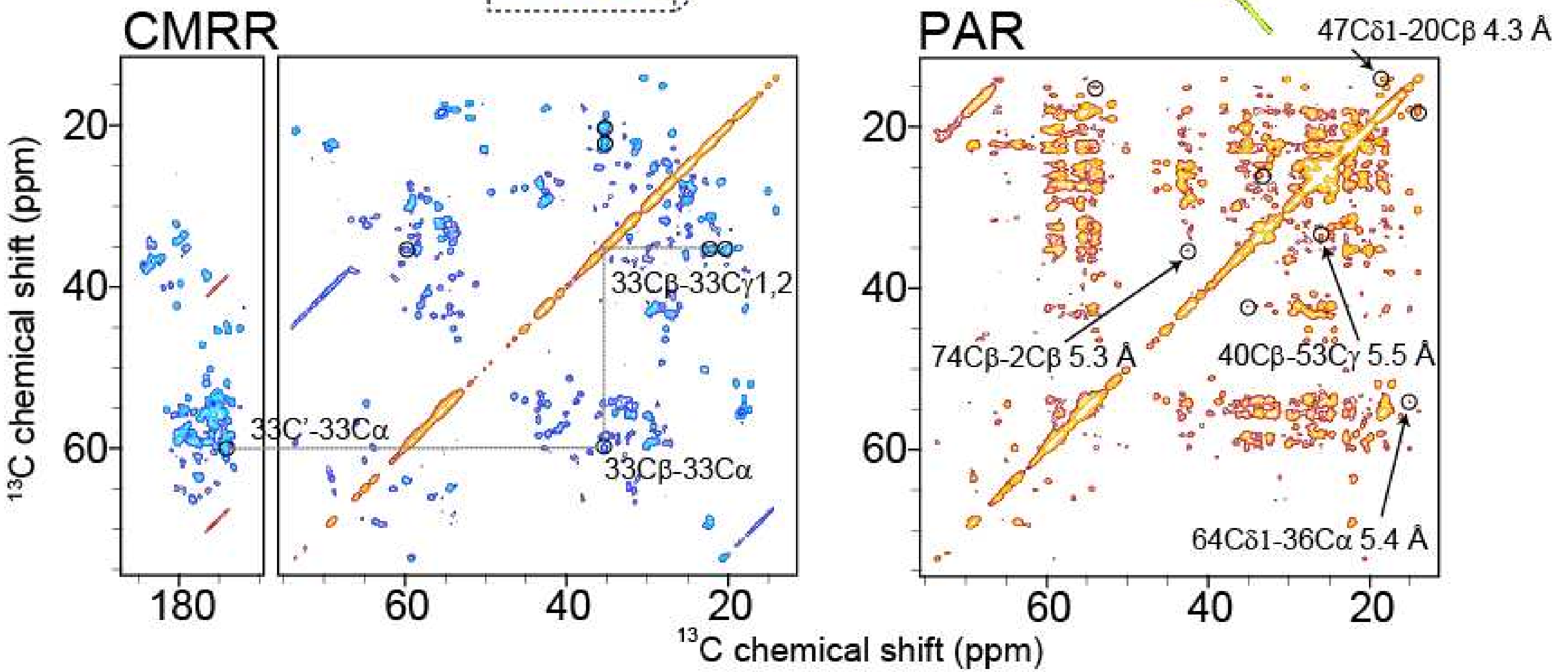
^{13}C-^{13}C correlation experiments
750/900 MHz ^{1}H Freq.
20 kHz MAS freq.



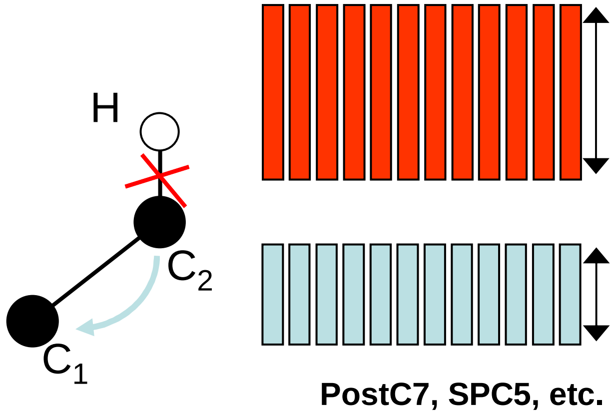


- **Who's who →** *assigning the resonances* -- **Part I - CMRR**
- **Structurally relevant restraints – Part II – TSAR mechanism**

Part I – One-bond transfer dipolar recoupling sequence

A lot of dipolar recoupling sequences have already been reported…

In practice, they necessitate concurrent ^{1}H decoupling…



$$\omega_1^H > \omega_1^C + 3\omega_R$$

$$\omega_1^C = N\omega_R$$

- @ 20 kHz $\omega_r/2\pi$, the ^{1}H decoupling field should be at least 150 kHz

→ **Beyond probe and sample limits!!**

CMRR recoupling with ^1H decoupling

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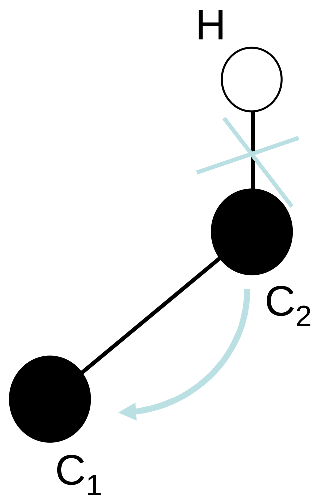
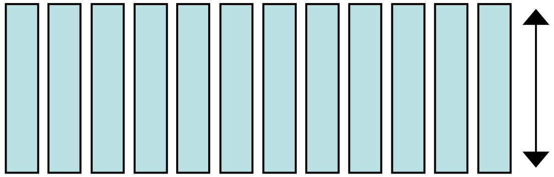
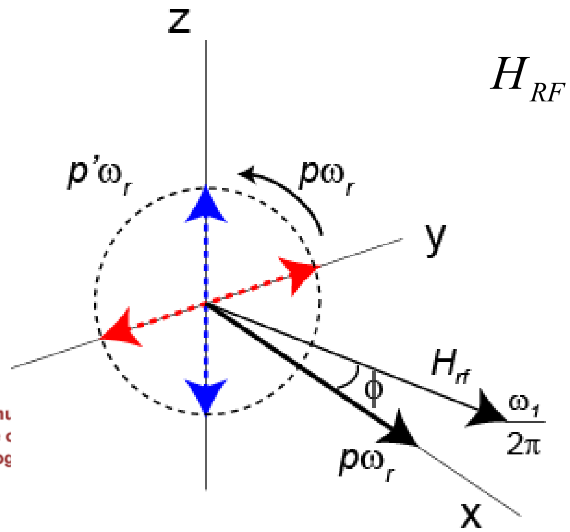
Can we design a recoupling sequence that does not require ^1H irradiation?





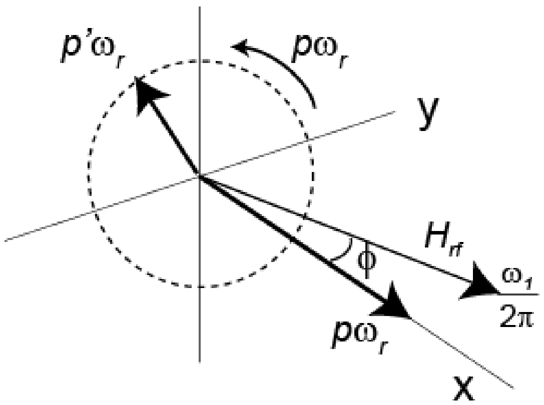
$$\omega_1^C = N\omega_R$$

Generalization of the second averaging principle



$$H_{RF} = \underline{\omega_1 \cos\phi(t)} \sum_i S_x^i + \underline{\omega_1 \sin\phi(t)} \sum_i S_y^i + \underline{\Omega(t)} \sum_i S_z^i$$

$$\begin{cases} \underline{\omega_1 \cos(\phi)} = p\omega_R \\ \underline{\omega_1 \sin(\phi)} = p'\omega_R \sin(p\omega_R t) \\ \underline{\Omega(t)} = p'\omega_R \cos(p\omega_R t) \end{cases}$$



$$\begin{cases} \phi = \arctan\left(\dfrac{p'\sin(p\omega_R t)}{p}\right) \\ \omega_1 = p\omega_R \left[1 + \left(\dfrac{p'}{p}\sin(p\omega_R t)\right)^2\right]^{1/2} \\ \Omega = p'\omega_R \cos(p\omega_R t) \end{cases}$$

$$\longrightarrow \quad U_{\mathrm{RF}} = \exp(-ip'\omega_R t C_Z)\exp(-ip\omega_R t C_X)$$

Interaction Frame

$$U_{\mathrm{RF}} = \exp\left(-ip'\omega_R t C_Z\right)\exp\left(-ip\omega_R t C_X\right)$$

Hamiltonian

$$H(t) = H_{\mathrm{RF}}(t) + \sum_{\lambda} A_{l0}^{\lambda} T_{l0}^{\lambda}$$

RF pulses → $H_{\mathrm{RF}}(t)$

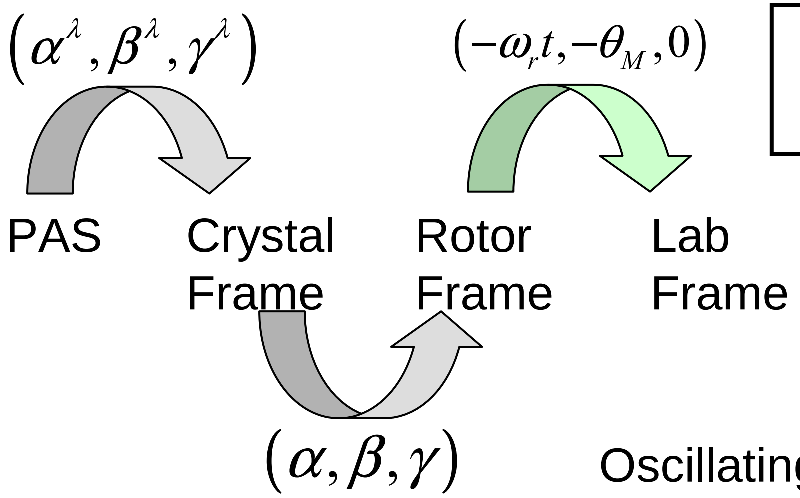
Chemical shifts, Dipolar interactions etc. → $\sum_{\lambda} A_{l0}^{\lambda} T_{l0}^{\lambda}$

Interaction Frame Hamiltonian

$$H'_{\mathrm{int}}(t) = U_{\mathrm{RF}}(t,0)^{\dagger} H_{\mathrm{int}}(t) U_{\mathrm{RF}}(t,0)$$

Homonuclear dipolar interaction:

$$H_{\mathrm{dip}} = \omega_{AB}\left[2C_{AZ}C_{BZ} - C_{AX}C_{BX} - C_{AY}C_{BY}\right] = \sqrt{6}\,\omega_{AB} T_{20}^{AB}$$



$$\left[T_{20}^{AB}\right]' = \sum_{q'q} T_{2q'}^{AB}\, e^{\left(-i(q'p'+qp)\omega_r t\right)}\, d_{0q}^{2}\left(\frac{\pi}{2}\right) d_{q'q}^{2}\left(\frac{\pi}{2}\right)$$

$$\omega_{AB} = \sum_{m=-2}^{2} \omega_{AB}^{(m)} \exp\left(-im\omega_r t\right)$$

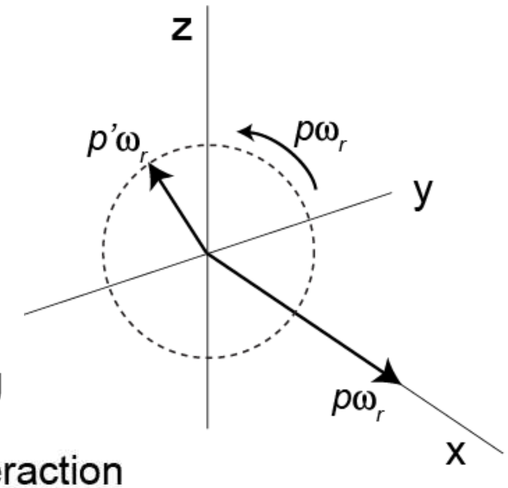
Oscillating terms at the frequencies: $(m + pq + p'q')\,\omega_R$

First order Average Hamiltonian Theory

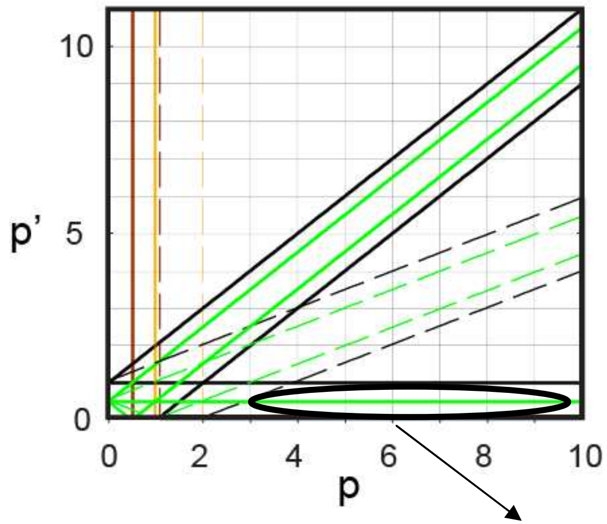
$$\overline{H'_{\text{int}}}^{(1)} = \frac{1}{T}\int_0^T dt_1 H'_{\text{int}}(t_1)$$

First order recoupling if: m + p.q + p'.q' = 0

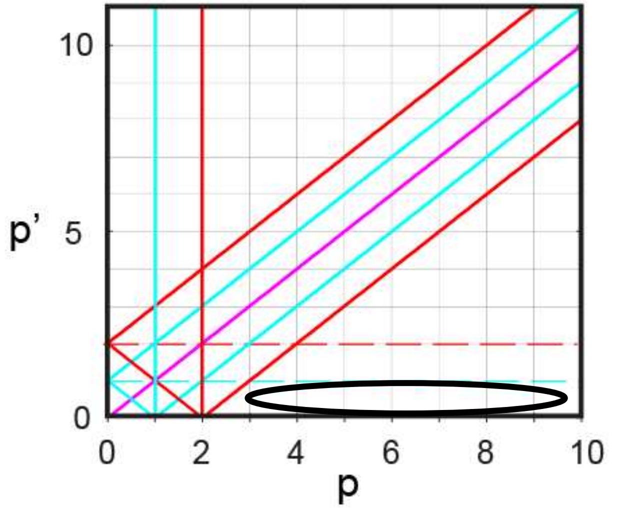
MAS — first — second averaging



Homonuclear dipolar interation



CS interaction
heteronuclear dipolar interaction



CMRR: p'=1/2 and p from 3.5 to 8

- This new scheme can be seen as a very versatile recoupling toolbox

DQ CMRR recoupling without ^{1}H decoupling

$p = 5$

$p' = 1/2$

$$\overline{H'_{\text{CMRR}}}^{(1)} = \frac{3}{8\sqrt{2}} \frac{\mu_0}{4\pi} \frac{\gamma_C^2}{r_{AB}^3} \sin(2\beta)\left[\exp(-i\gamma) T_{2-2}^{AB} + \exp(i\gamma) T_{2+2}^{AB}\right]$$

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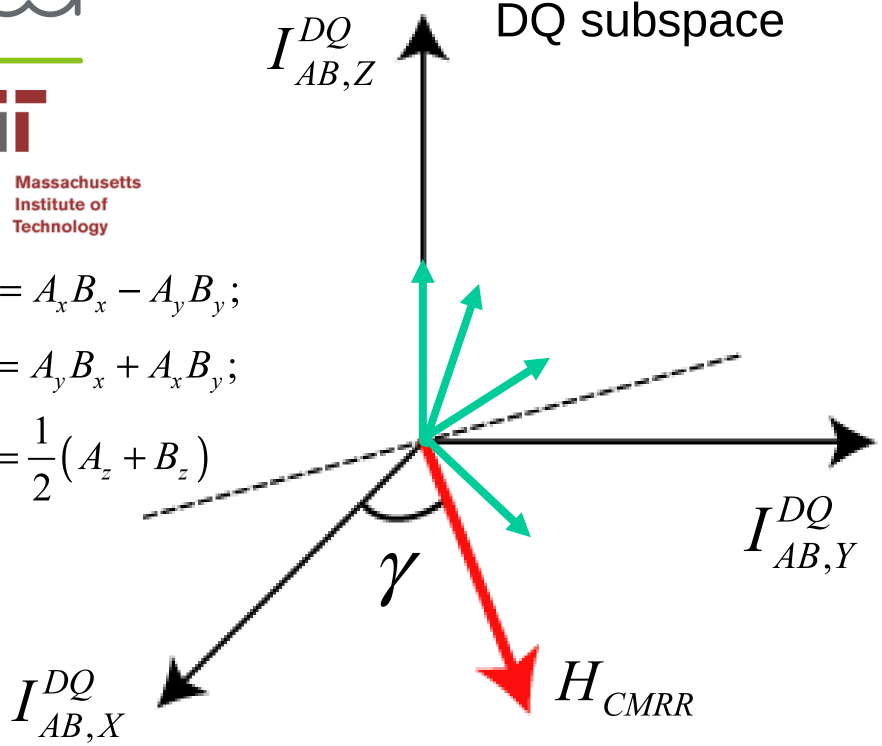
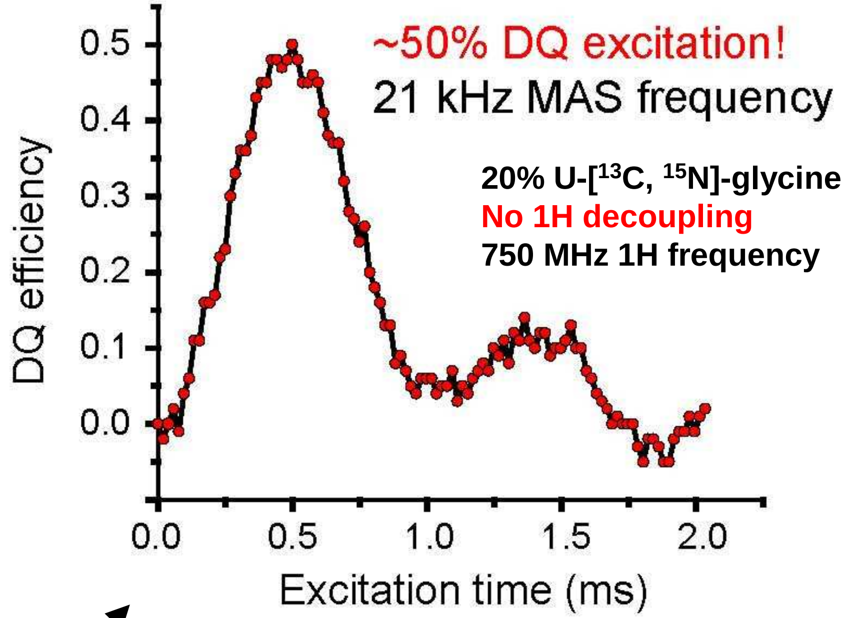
$$I_{AB,X}^{(DQ)} = A_x B_x - A_y B_y;$$

$$I_{AB,Y}^{(DQ)} = A_y B_x + A_x B_y;$$

$$I_{AB,Z}^{(DQ)} = \frac{1}{2}(A_z + B_z)$$





Powder averaging

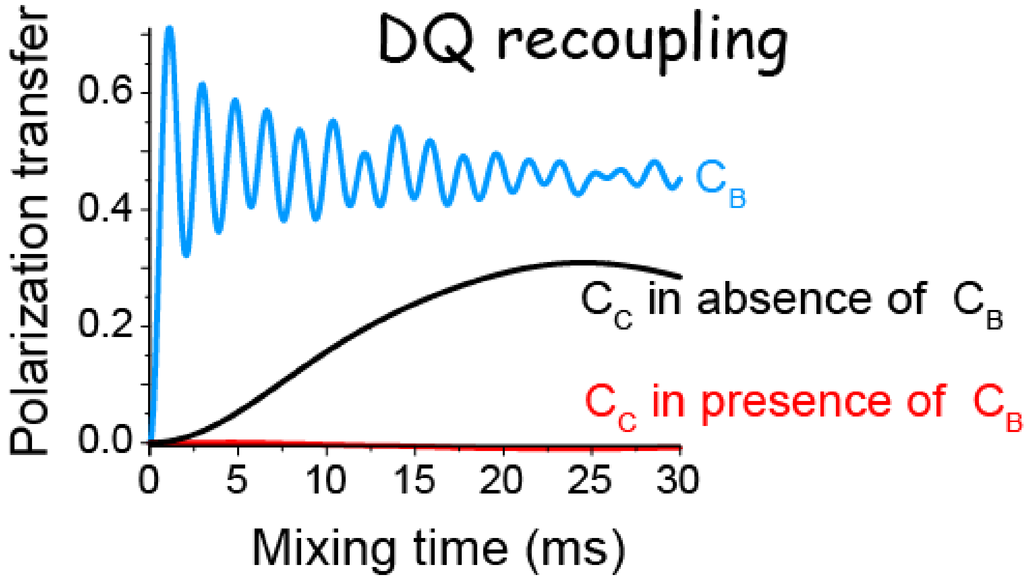
$$A_Z = \left(\frac{A_Z + B_Z}{2}\right) + \left(\frac{A_Z - B_Z}{2}\right) \longrightarrow -\left(\frac{A_Z + B_Z}{2}\right) + \left(\frac{A_Z - B_Z}{2}\right) = B_Z$$

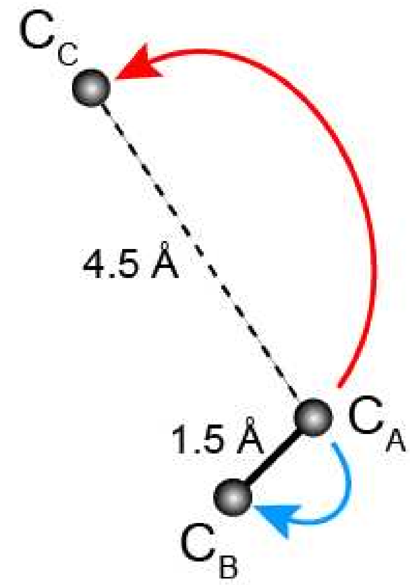
De Paëpe, Lewandowski, Griffin JCP (2008)

CMRR and dipolar truncation

$$\overline{H'_{\text{int}}}^{(1)} = \omega'_{AB}\sin(2\beta)\left[\exp(-i\gamma)T_{2-2}^{AB} + \exp(i\gamma)T_{22}^{AB}\right]$$
$$+\omega'_{AC}\sin(2\beta)\left[\exp(-i\gamma)T_{2-2}^{AC} + \exp(i\gamma)T_{22}^{AC}\right]$$
$$+\omega'_{BC}\sin(2\beta)\left[\exp(-i\gamma)T_{2-2}^{BC} + \exp(i\gamma)T_{22}^{BC}\right]$$

Non-commuting terms!



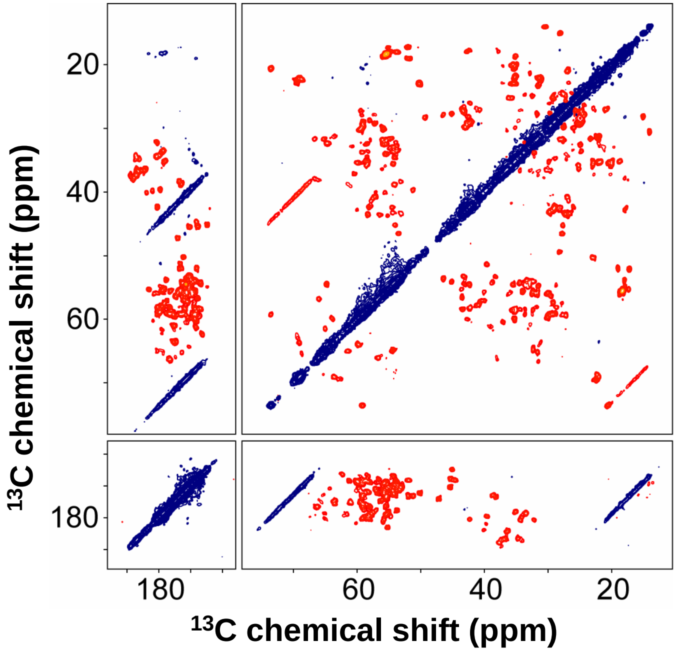


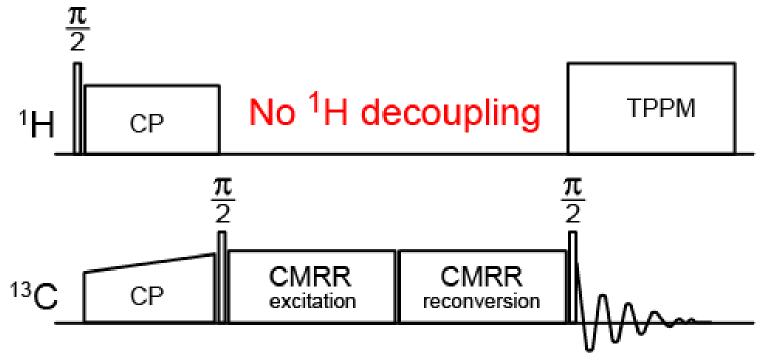
SPINEVOLUTION, Veshtort et al., JMR (2006) 178.

- Spin dynamics dominated by one-bond couplings
- very useful for assignment!

DQ-CMRR recoupling without ^{1}H decoupling

Red cross peaks = one-bond transfer only!





^{13}C-^{13}C recoupling without ^{1}H decoupling
20 kHz MAS CM5RR* on [U-^{13}C, ^{15}N]-Crh
750 MHz, 15 hours

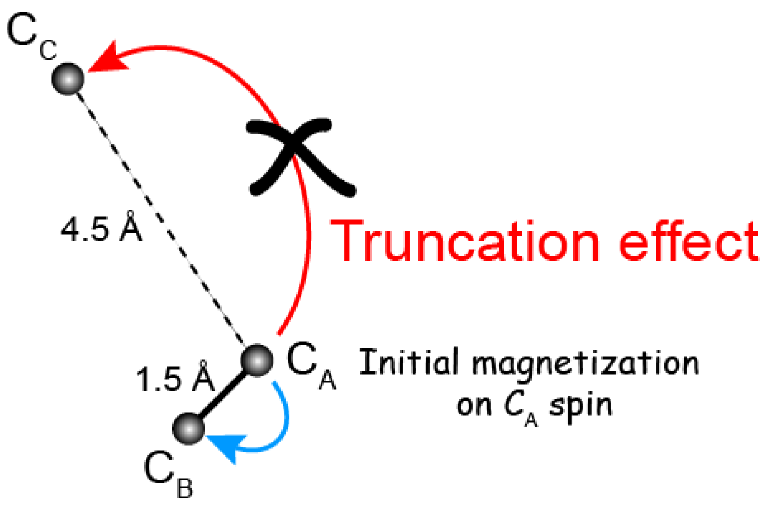
***De Paëpe, Bayro, Lewandowski, Griffin, JACS (2006)**
De Paëpe, Lewandowski, Griffin, JCP 128 (2008)

Properties/advantages:

- Single channel irradiation
- Attenuation of r.f. sample heating
- Efficient at high B_0, high $\omega_r/2\pi$

Challenges to obtain long distance transfer

- DQ/ZQ broadband ^{13}C-^{13}C dipolar recoupling yields truncation



→ useless for long distance transfer!!

Part II - TSAR mechanism: use of assisting spins!

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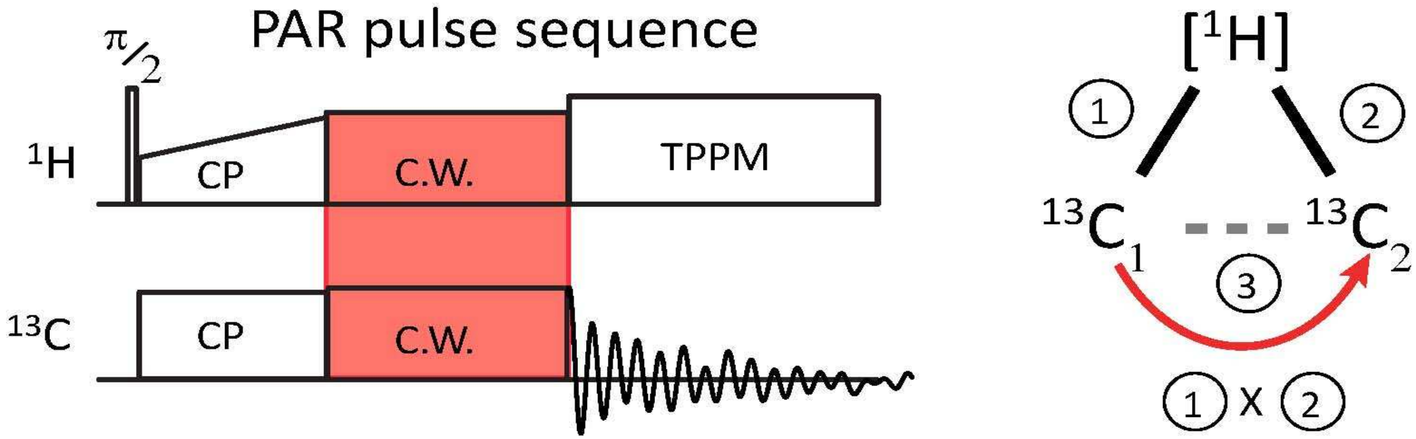

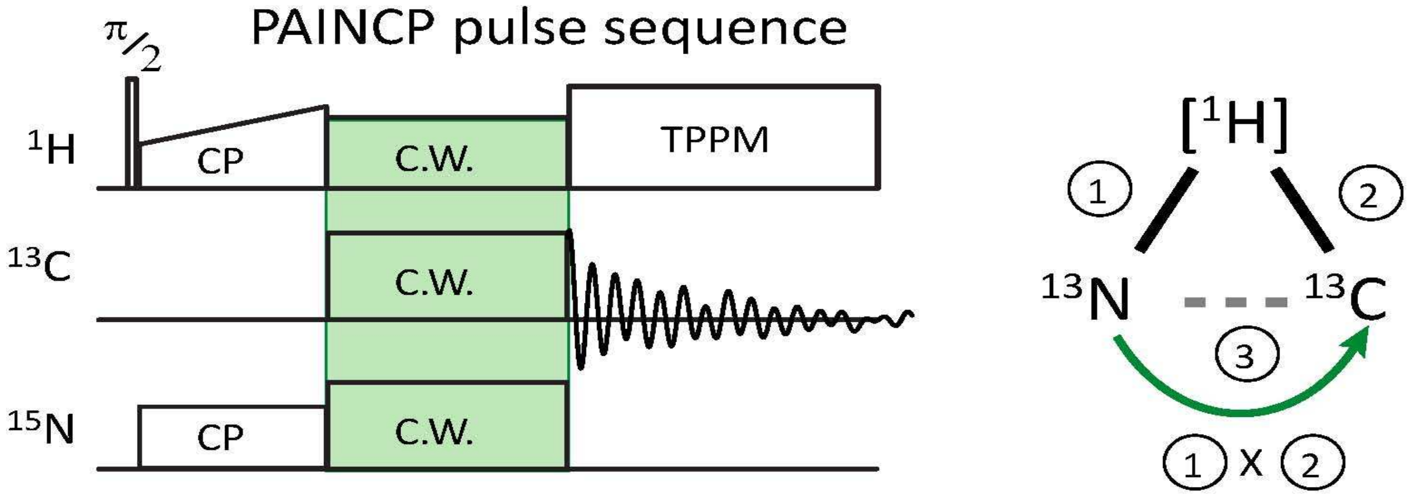

Third Spin Assisted Recoupling - Principles

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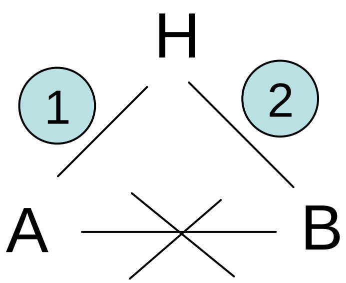
Assisting Spin: e.g. 1H's



A,B ^{13}C spins

$$H_{\mathrm{RF}} = \omega_{1C} A_X + \omega_{1C} B_X + \omega_{1H} H_X$$
$$= \omega_R \left(p_C A_X + p_C B_X + p_H H_X \right)$$

$$U_{\mathrm{RF}} = \exp\left\{ -i \left(\underline{p_C \omega_R t} A_X + \underline{p_C \omega_R t} B_X + \underline{p_H \omega_R t} H_X \right) \right\}$$

$$\omega_{ij} = \sum_{m=-2}^{2} \omega_{ij}^{(m)} \exp\left(-\underline{i m \omega_r t} \right)$$

$$H_{\mathrm{int}} = \omega_{AH} 2 A_Z H_Z \quad (1)$$
$$+ \omega_{HB} 2 H_Z B_Z \quad (2)$$

$$\xrightarrow{\exp\left(-i \omega_r X t \right)} \begin{cases} X_1 = m_1 \pm p_C \pm p_H & \rightarrow (1) \\ X_2 = m_2 \pm p_H \pm p_C & \rightarrow (2) \end{cases}$$

Average Hamiltonian Theory – Magnus Expansion

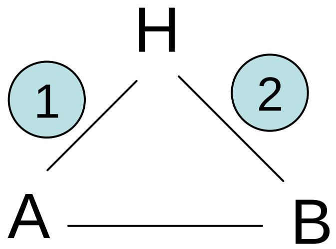
$$\overline{H'} = \overline{H'_{\mathrm{int}}}^{(1)} + \overline{H'_{\mathrm{int}}}^{(2)} + \overline{H'_{\mathrm{int}}}^{(3)} + \ldots$$

$$\overline{H'_{\mathrm{int}}}^{(1)} = \frac{1}{T} \int_0^T dt_1 H'(t_1)$$ zero if X ≠ 0

Let's calculate the second order!

De Paëpe *et al.*, J. Chem. Phys. (2008).

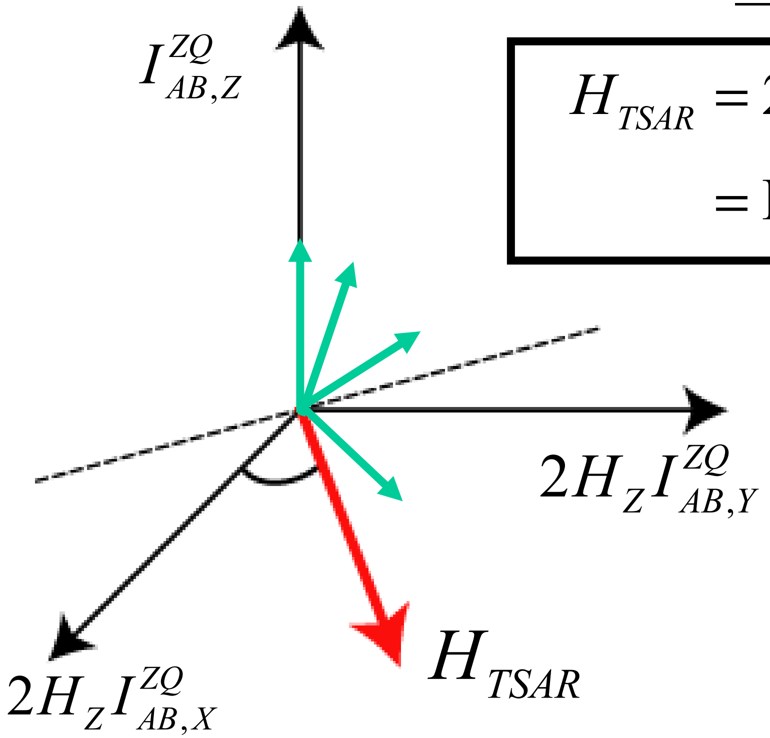
Second order Average Hamiltonian Theory - TSAR term



$$\overline{H'_{\text{int}}}^{(2)} = \frac{1}{2iT}\int_0^T dt_1 \int_0^{t_1} dt_2 \left[H(t_1), H(t_2)\right]$$

Second order TSAR Hamiltonian (1) X (2)

$$\begin{aligned} H_{TSAR} &= 2\omega_{TSAR} A^+ B^- H_Z + 2\omega^*_{TSAR} A^- B^+ H_Z \\ &= \mathrm{Re}\left(\omega_{TSAR}\right) 2 I^{(ZQ)}_{AB,X} H_Z + \mathrm{Im}\left(\omega_{TSAR}\right) 2 I^{(ZQ)}_{AB,Y} H_Z \end{aligned}$$



$$\omega_{TSAR} = \frac{1}{\omega_r}\left[\underbrace{\mathrm{Re}\left(\omega^1_{AH}\ \omega^{-1}_{HB}\right)\lambda(1, p_C, p_H) + i\,\mathrm{Im}\left(\omega^1_{AH}\ \omega^{-1}_{HB}\right)\sigma(1, p_C, p_H)}_{\omega^1{}_{TSAR}} + \underbrace{\mathrm{Re}\left(\omega^2_{AH}\ \omega^{-2}_{HB}\right)\lambda(2, p_C, p_H) + i\,\mathrm{Im}\left(\omega^2_{AH}\ \omega^{-2}_{HB}\right)\sigma(2, p_C, p_H)}_{\omega^2{}_{TSAR}} \right]$$

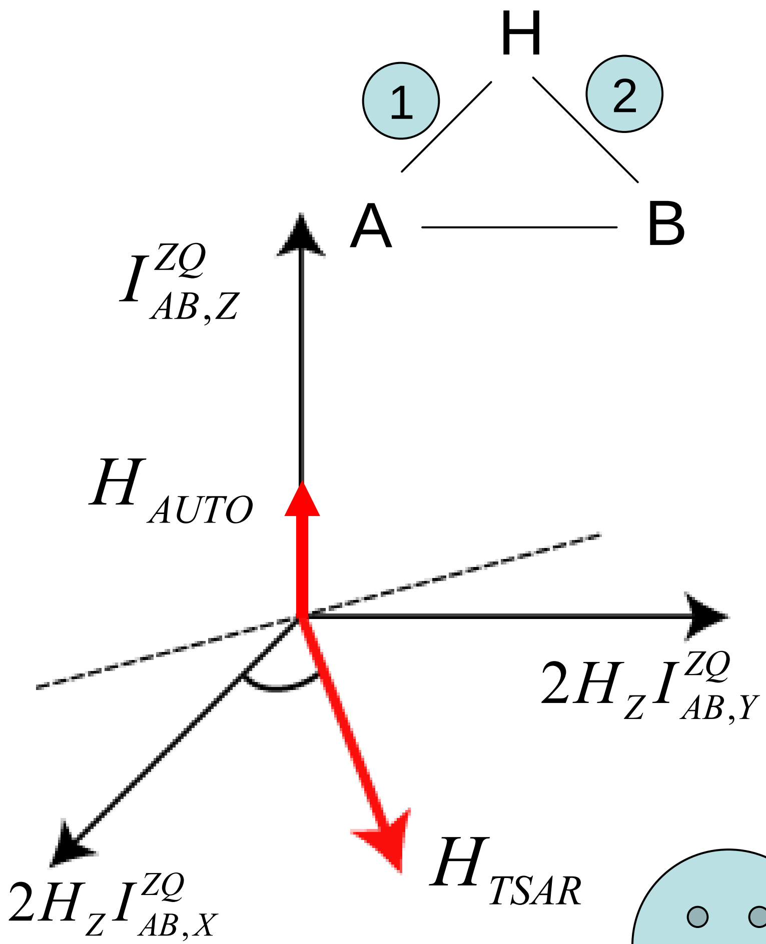
$$\lambda(m, p_C, p_H) = \left(\frac{-(p_C + p_H)}{m^2 - (p_C + p_H)^2} + \frac{-(p_H - p_C)}{m^2 - (p_H - p_C)^2} \right)$$

$$\sigma(m, p_C, p_H) = \left(\frac{m}{m^2 - (p_H + p_C)^2} - \frac{m}{m^2 - (p_H - p_C)^2} \right)$$

•TSAR subspace:

→coupled basis between a fictitious ZQ spin and an assisting proton spin.

Second order Average Hamiltonian Theory - TSAR term



$$\overline{H'_{\text{int}}}^{(2)} = \frac{1}{2iT}\int_0^T dt_1 \int_0^{t_1} dt_2 \left[H(t_1), H(t_2)\right]$$

Second order TSAR Hamiltonian

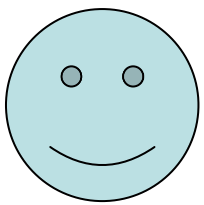
1 X 1

2 X 2

$$H_{AUTO} = \omega_{AUTO} I^{(ZQ)}_{AB,Z}$$

$$\omega_{AUTO} = \frac{1}{\omega_r}\left[\begin{array}{l}\left(\omega^{1}_{C_1H}\ \omega^{-1}_{HC_1} - \omega^{1}_{C_2H}\ \omega^{-1}_{HC_2}\right)\chi(1, p_C, p_H) \\ +\left(\omega^{2}_{C_1H}\ \omega^{-2}_{HC_1} - \omega^{2}_{C_2H}\ \omega^{-2}_{HC_2}\right)\chi(2, p_C, p_H)\end{array}\right]$$

$$\chi(m, p_C, p_H) = -\frac{1}{2}\left(\frac{(p_H + p_C)}{m^2 - (p_H + p_C)^2} - \frac{(p_H - p_C)}{m^2 - (p_H - p_C)^2}\right)$$

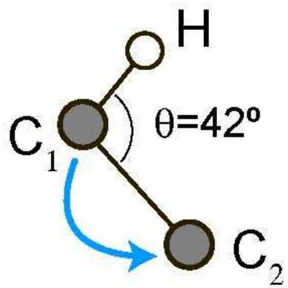


$\chi(m, p_C, p_H) = 0$ if $p_H = \sqrt{p_C^2 - m^2}$

- **Auto-cross terms leads to longitudinal off-resonance contributions!**
- **But these off-resonance contributions can be minimized!**

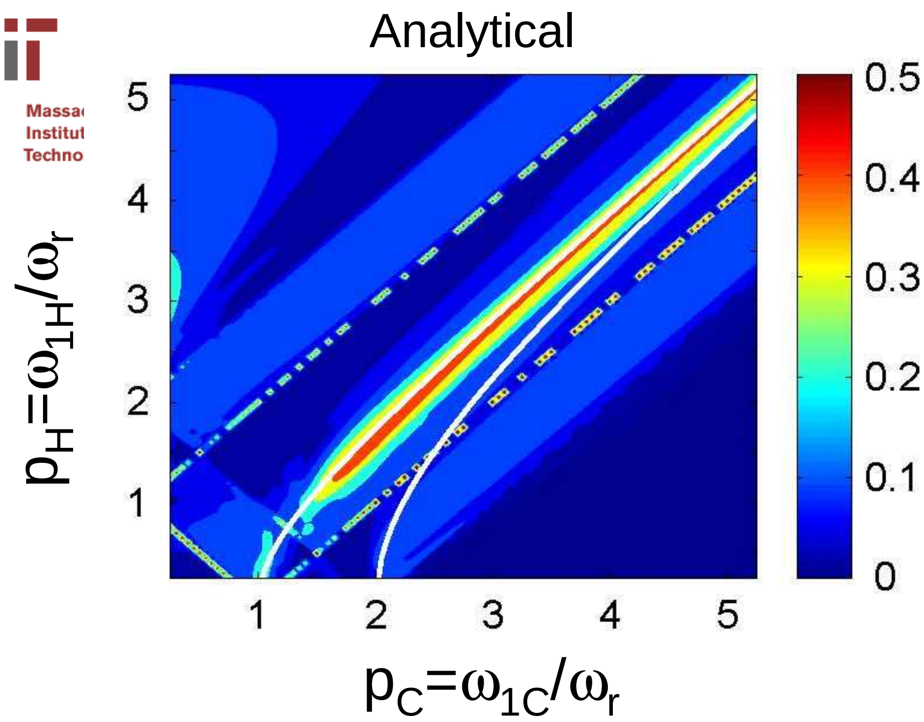
Analytical versus numerical simulations

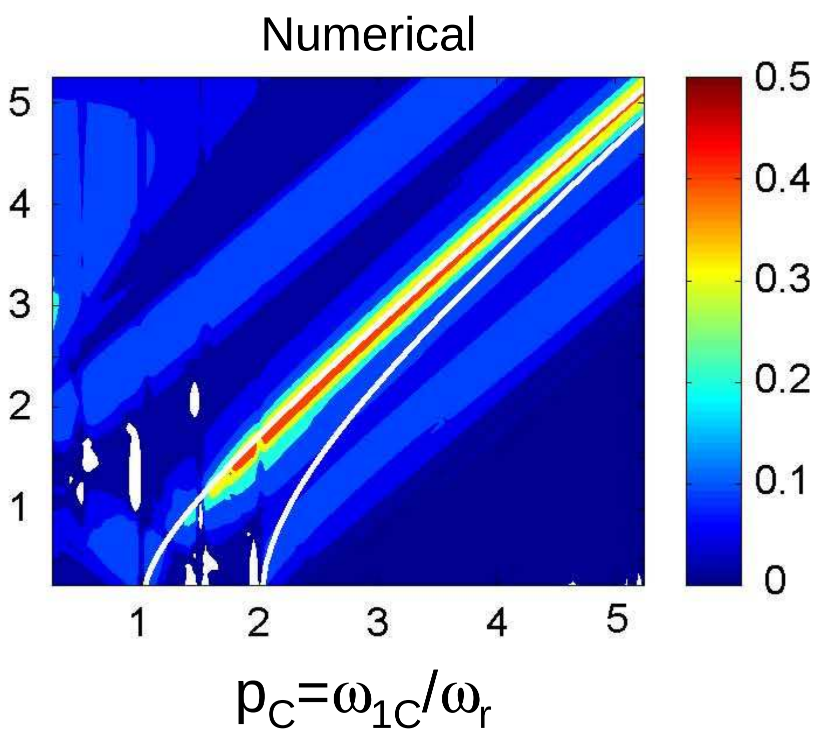
Homonuclear case



$$p_H = \sqrt{p_C^2 - 1}$$

$$p_H = \sqrt{p_C^2 - 4}$$



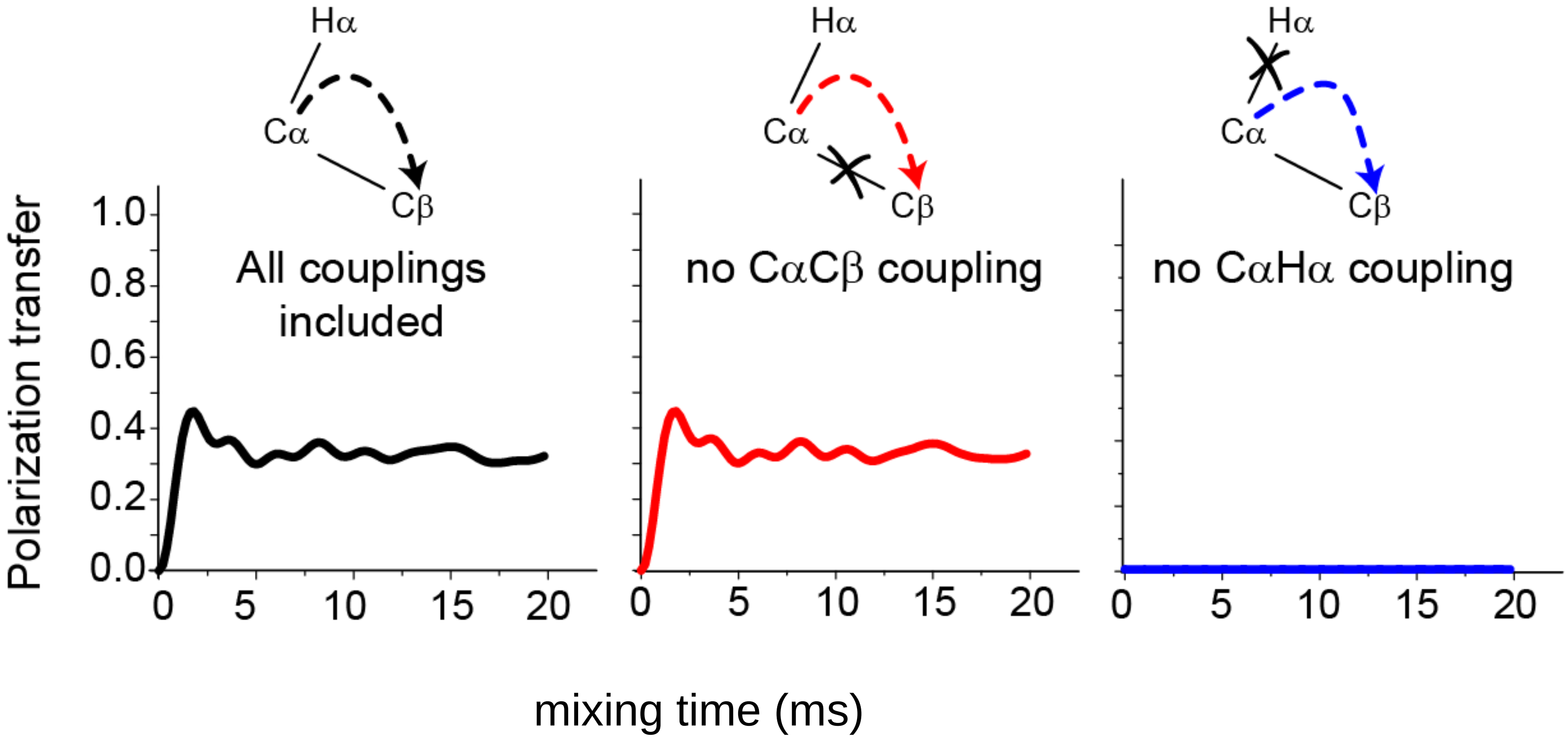


- Second order AHT explains the numerical maps!

Insight in the PAR experiment

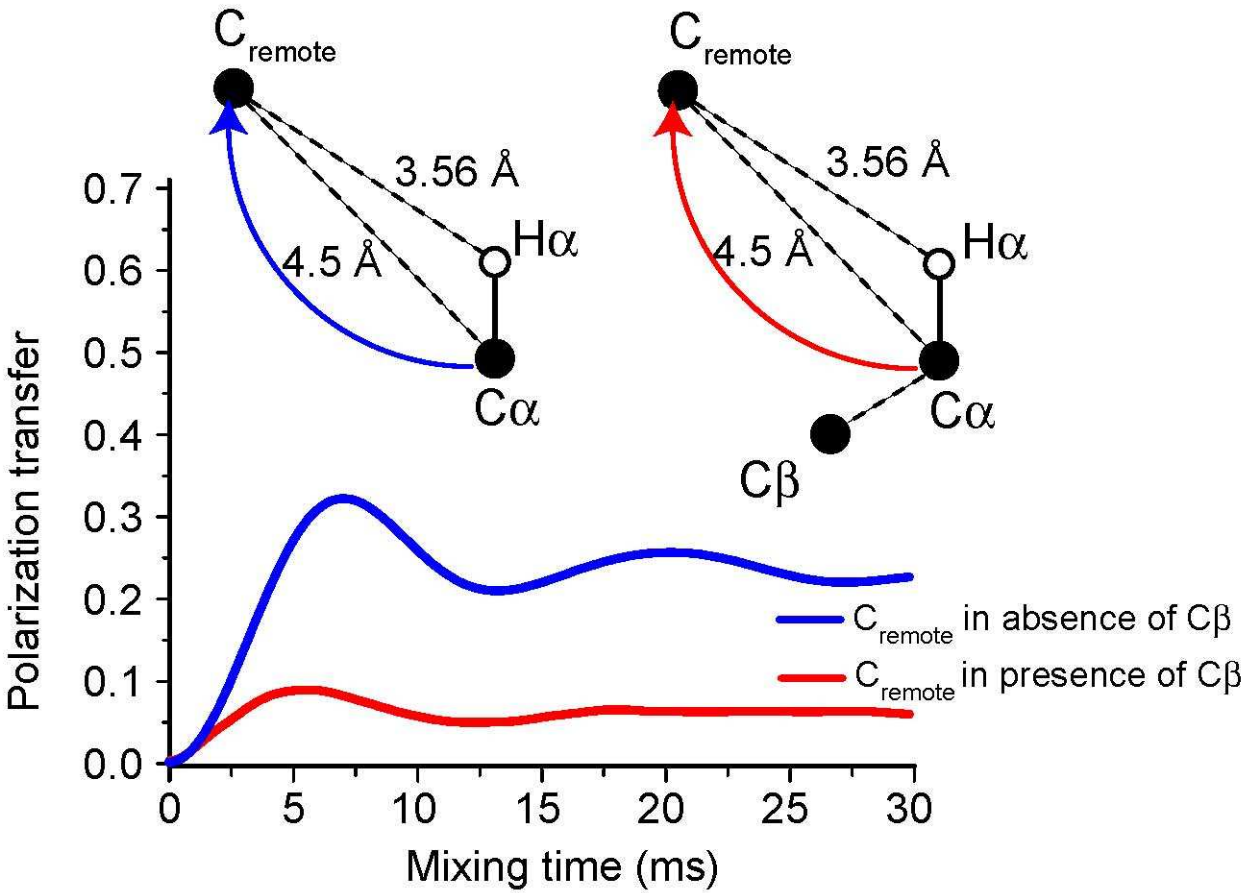
Homonuclear case

$\omega_r/2\pi$=20 kHz $\omega_0/2\pi$=750 MHz



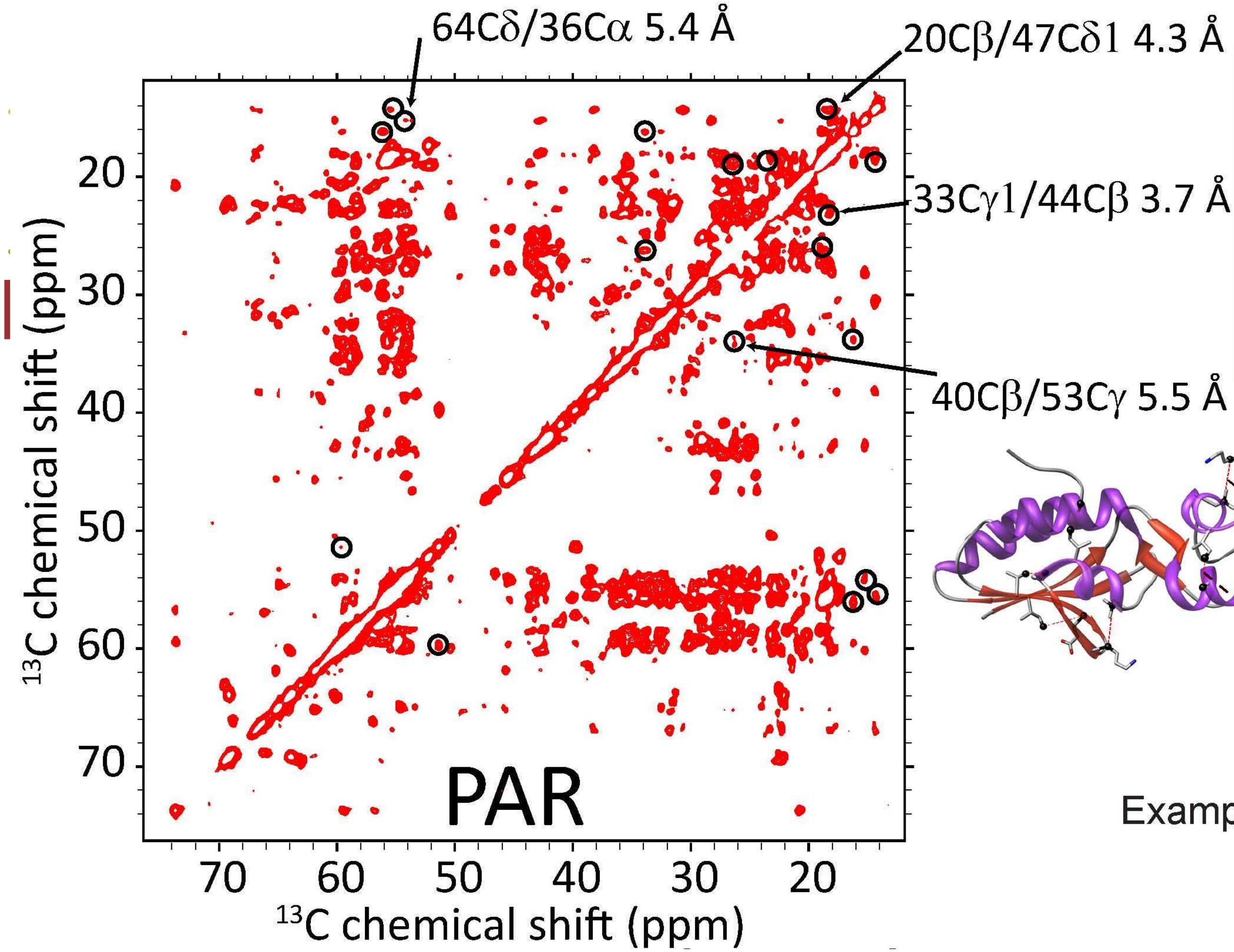
- PAR recoupling does not rely on CC coupling!

PAR versus dipolar truncation



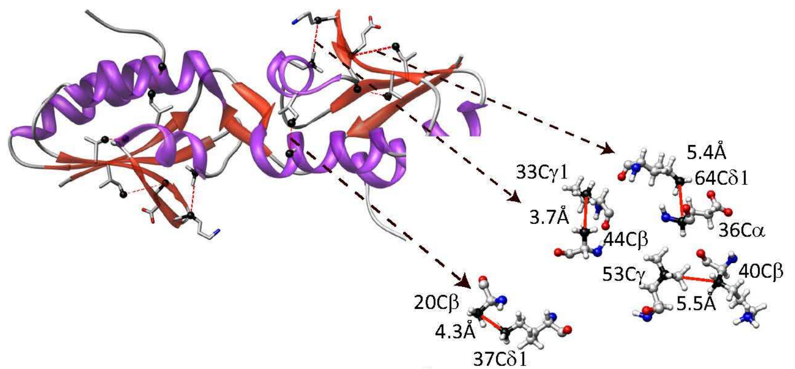
- Long distance transfer (~4.5 Å) in presence of directly bonded carbon: **reduction of the dipolar truncation!**

PAR on [U-^{13}C,^{15}N]-Crh at 750 MHz, 20kHz MAS



Crh, 2 x 85 residues

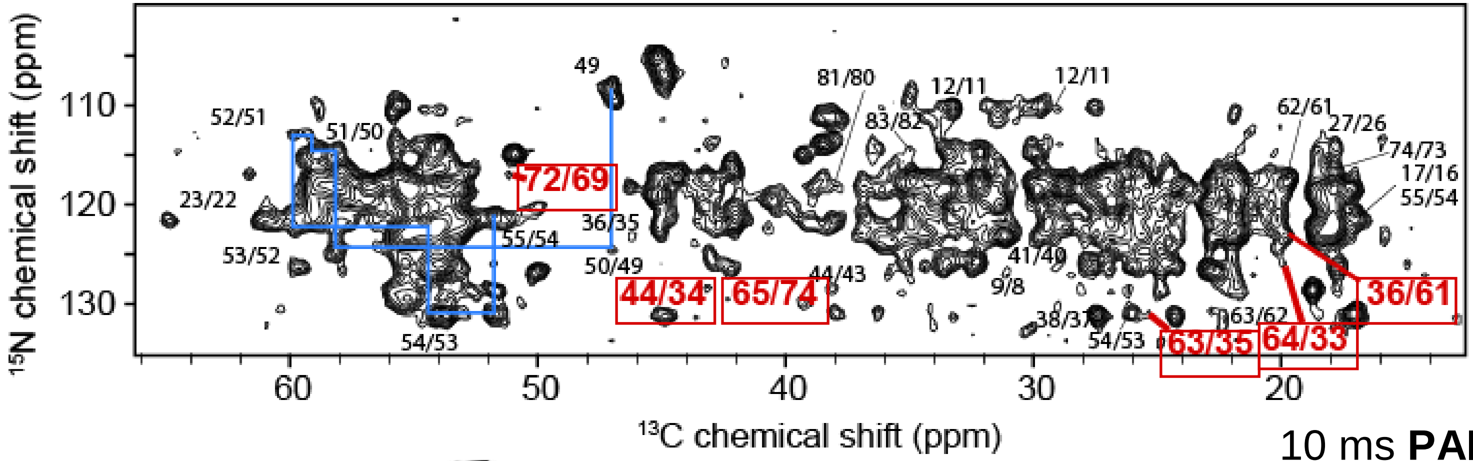
$\omega_r/2\pi$ = 20 kHz
ω_{1c} ~ 50 kHz
ω_{1h} ~ 50 kHz



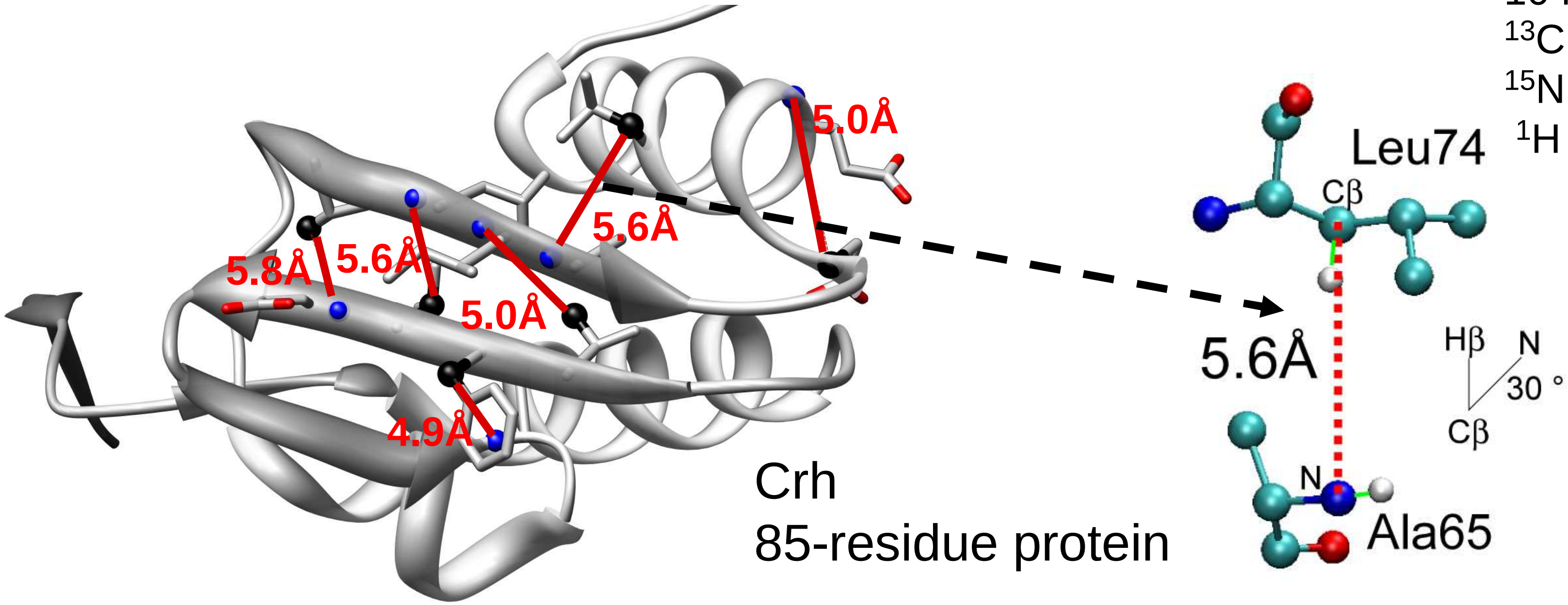
Examples of interresidue contacts

- Long distance transfer in **uniformly labeled protein!**

PAINCP on [U-^{13}C,^{15}N]-Crh at 750 MHz, 20kHz MAS

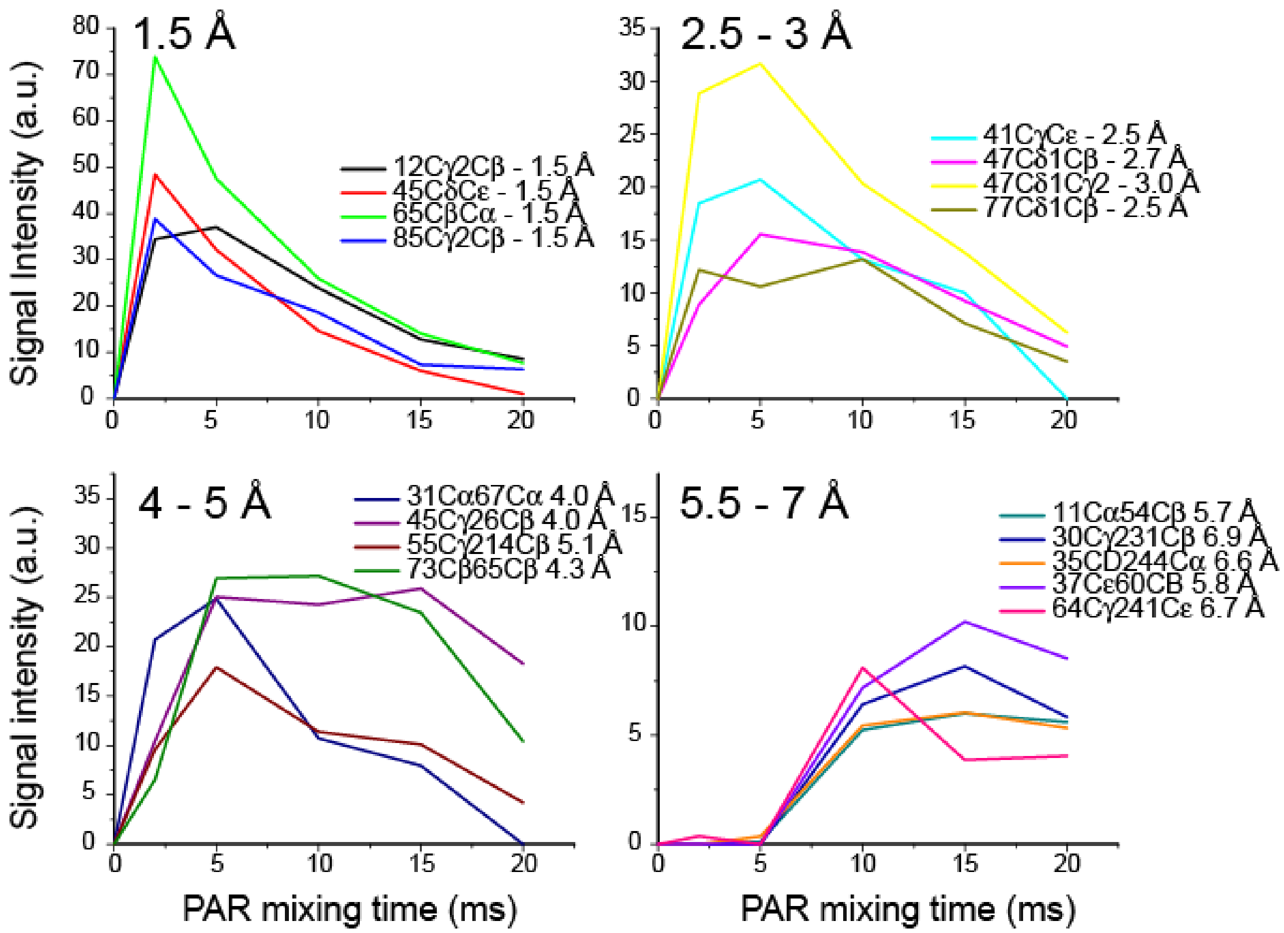


10 ms **PAINCP**
^{13}C rf - 15 kHz
^{15}N rf - 15 kHz
^{1}H rf – 53 kHz



- >5Å ^{15}N-^{13}C contacts between secondary structure elements

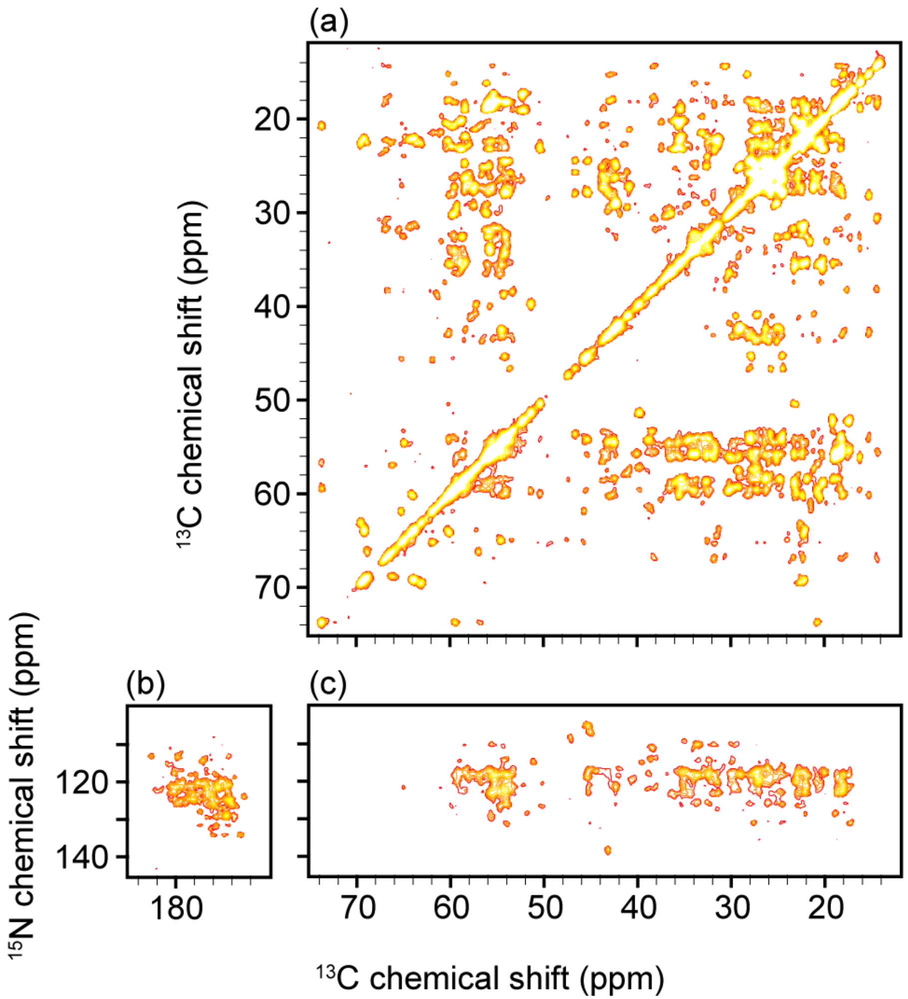
Structure determination by SSNMR



PAR 900 MHz
2, 5, 10, 15 & 20 ms
PAR irradiation
$\omega_r/2\pi = 20$ kHz
$\omega_{1C}/2\pi \sim 50$ kHz
$\omega_{1H}/2\pi \sim 50$ kHz

- **Observed buildups can be categorized into different distance classes**
- **Upper bond distance can be estimated**

De novo structure determination – Crh dimer (2 x 10.4 kDa)



PAR & PAINCP @ 900 MHz

Collaboration with A. Böckmann et al. at IBCP, France

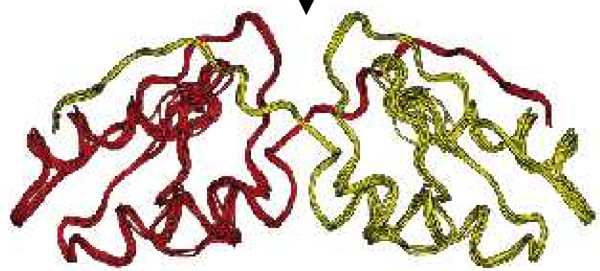
Crh assignment (e.g. CMRR)
+ ^{13}C-^{13}C PAR (~15 ms)
+ ^{15}N-^{13}C PAINCP (~15 ms)

↓ ARIA

795 unambiguous CC constraints
(269 long range)

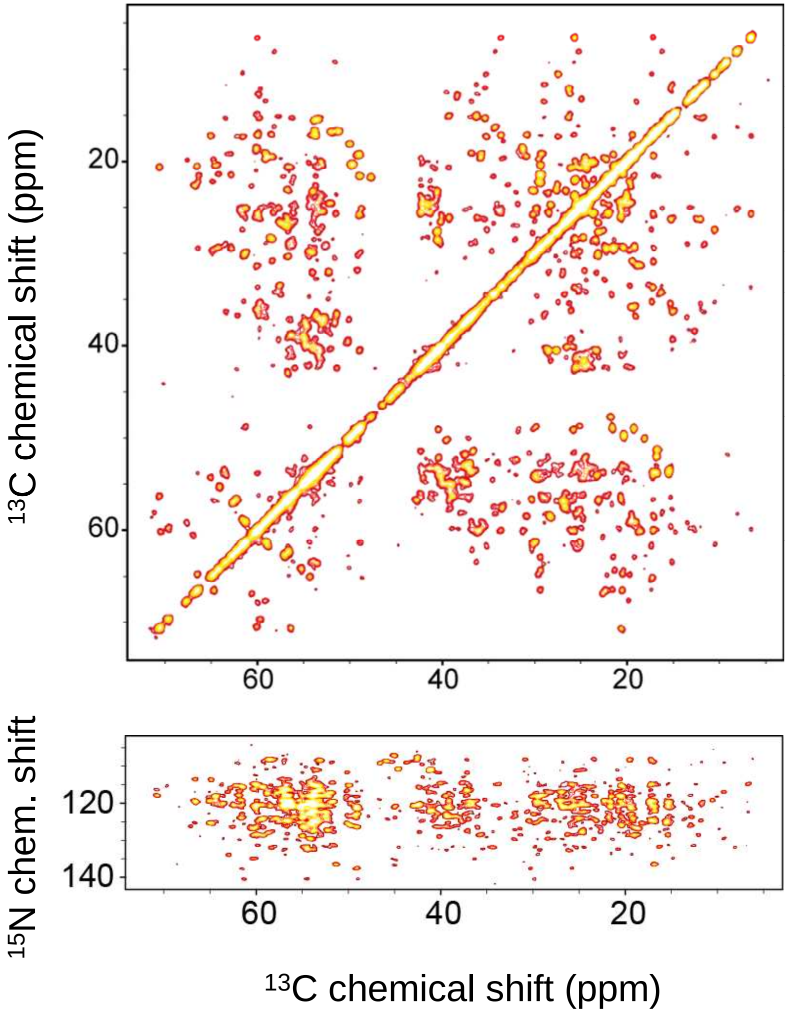
400 unambiguous NC constraints
(130 long range)

↓ TALOS
+ XPLOR-NIH



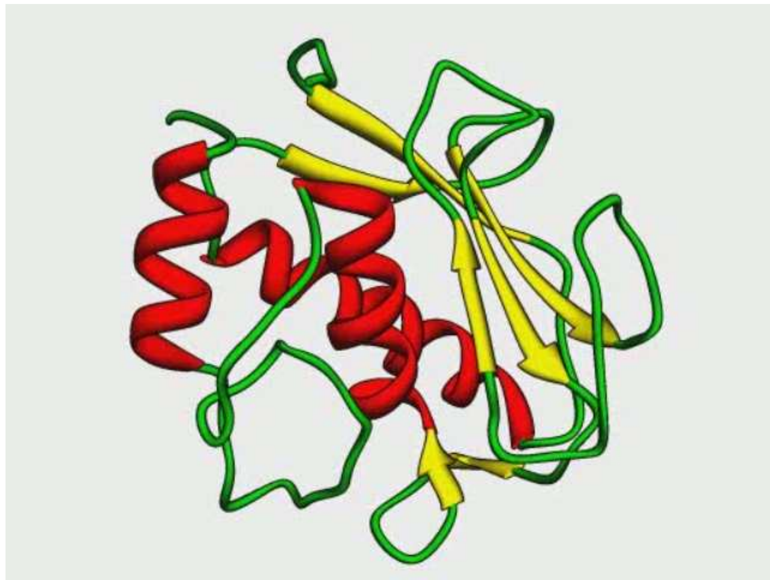
RMSD 0.58 Å

De novo structure determination – MMP-12 (17.6 kDa)



$\omega_r / 2\pi$ = 20 kHz $\omega_{1H} / 2\pi$ = 900 MHz

PAR + PAINCP



MMP-12 protein (17.6 kDa)

Collaboration with I. Bertini et al. at CERM, Italy

- Application to larger and larger systems…

Conclusions

- **DQ-CMRR efficient one-bond relayed recoupling sequence for assignment (without 1H decoupling)**

- **TSAR recoupling:**

  - ^{13}C-^{13}C, ^{15}N-^{15}N or ^{15}N-^{13}C recoupling assisted by surrounding protons

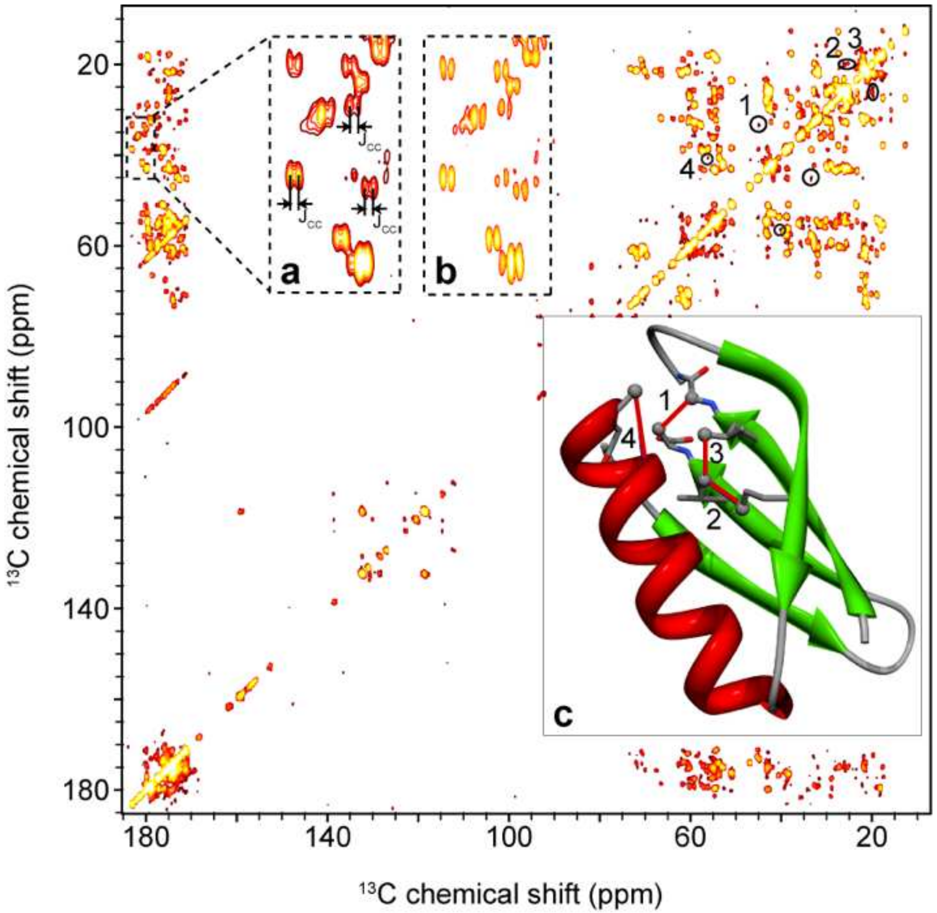
  - Very efficient for short, medium and long distance transfer

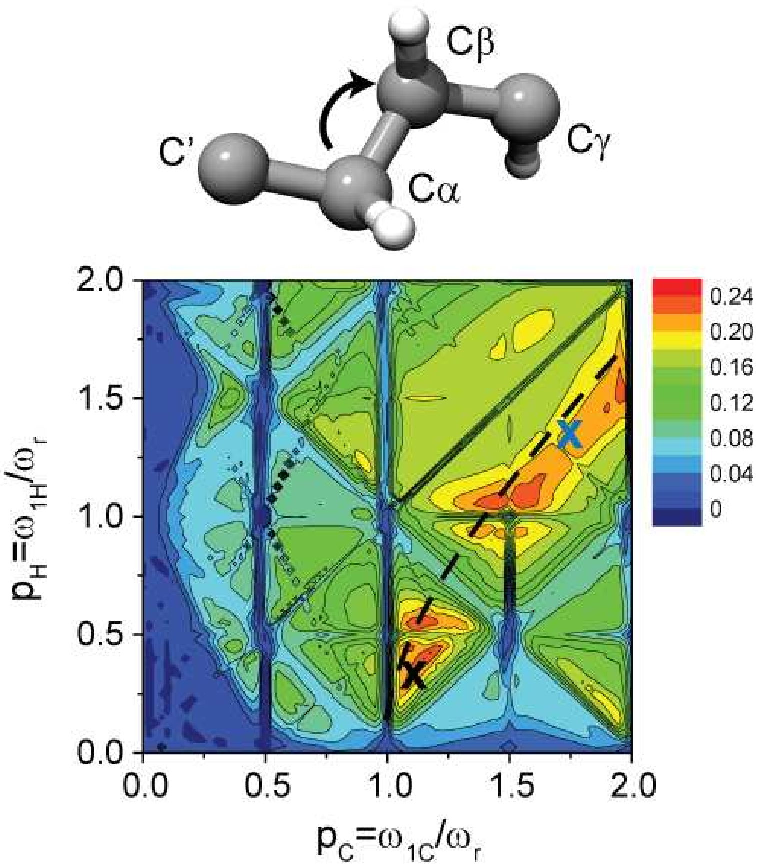
  - Applicable to *de novo* structure determination

- **Promising techniques for *de novo* atomic structure determination of challenging systems: membrane proteins, fibrils, etc… that are not accessible by other techniques.**

Massachusetts Institute of Technology

^{13}C-^{13}C PAR at 65 kHz MAS Frequency on [U-^{13}C,U-^{15}N]GB1

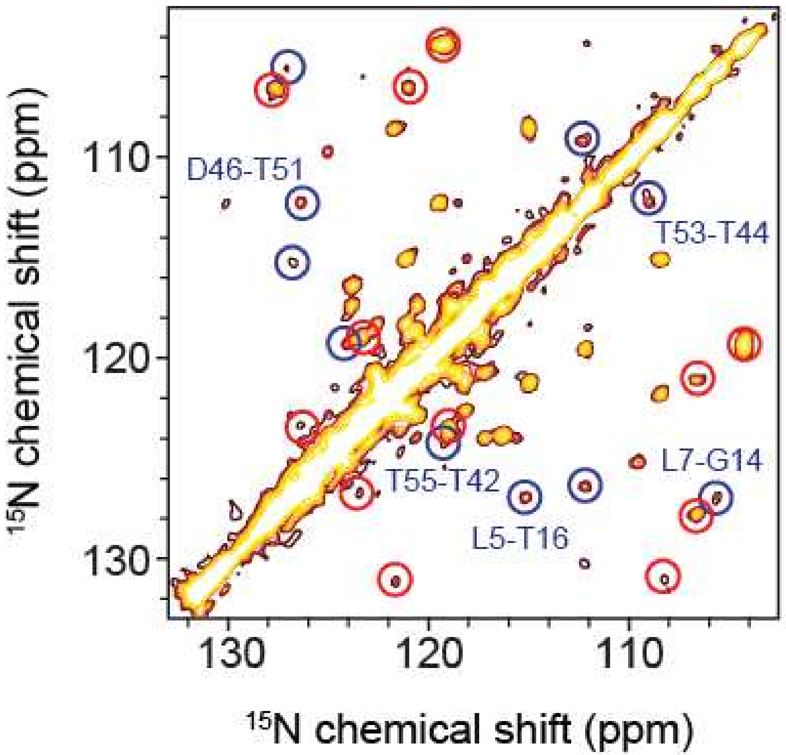




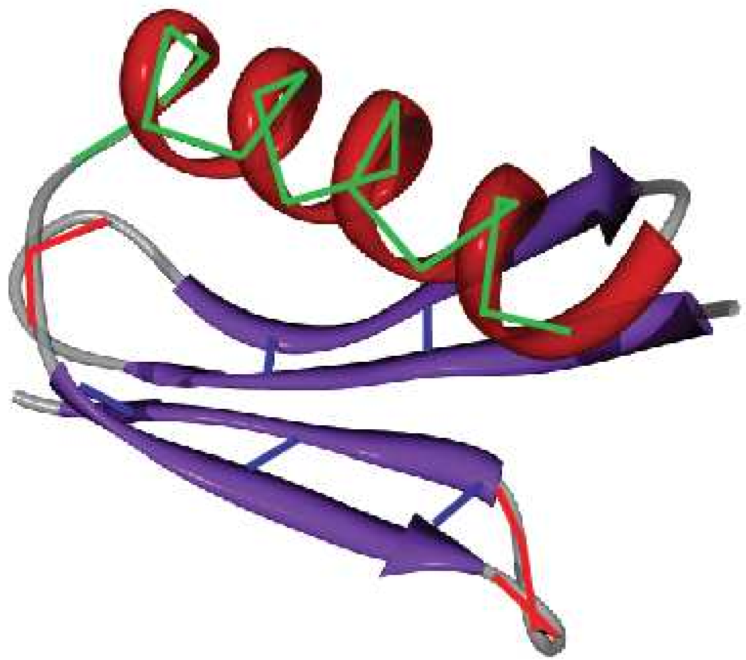
Collaboration with W. Maas et al. at Bruker, MA

- **Despite being a second order recoupling sequence, PAR is still applicable at 65 kHz MAS !**

^{15}N-^{15}N PAR on [1,3-^{13}C,U-^{15}N]GB1



Lewandowski *et al.* (2008) JACS



18 ms mixing time

$\omega_r/2\pi$ = 20kHz, $\omega_{0H}/2\pi$ = 900MHz

- direct information about secondary and tertiary structure
- identification of α-helix and connectivity of strands in β-sheets

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