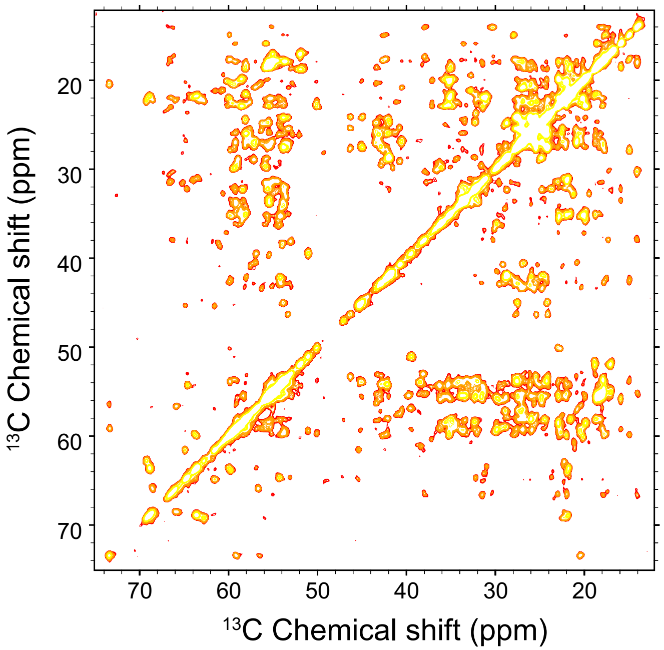
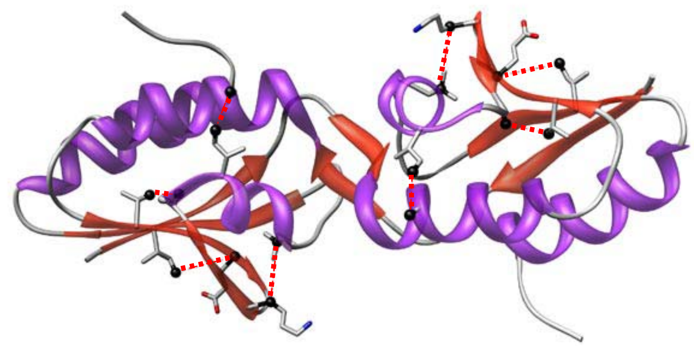
Advances in high field recoupling

Application to structure determination





Crh 2x10.4 kDa - PAR 900 MHz

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Winter school - Stowe 2008

Thanks ...

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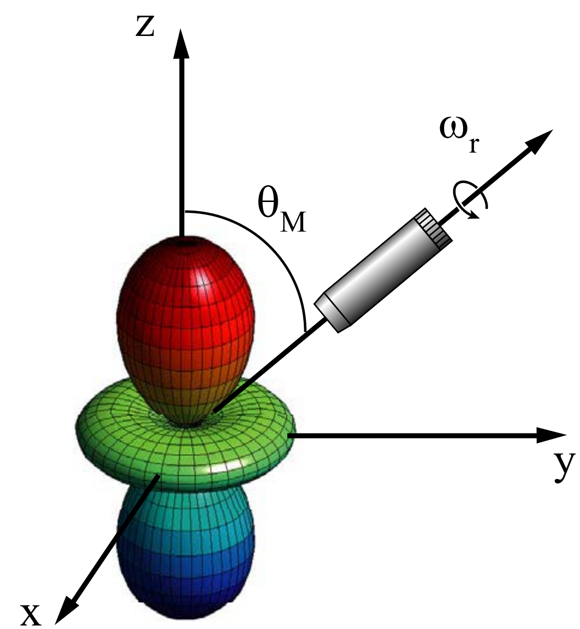
NATIONAL INSTITUTES OF HEALTH
National Institute of Biomedical Imaging and Bioengineering

1. Structure determination by MAS SSNMR

1.1 Dipolar recoupling techniques

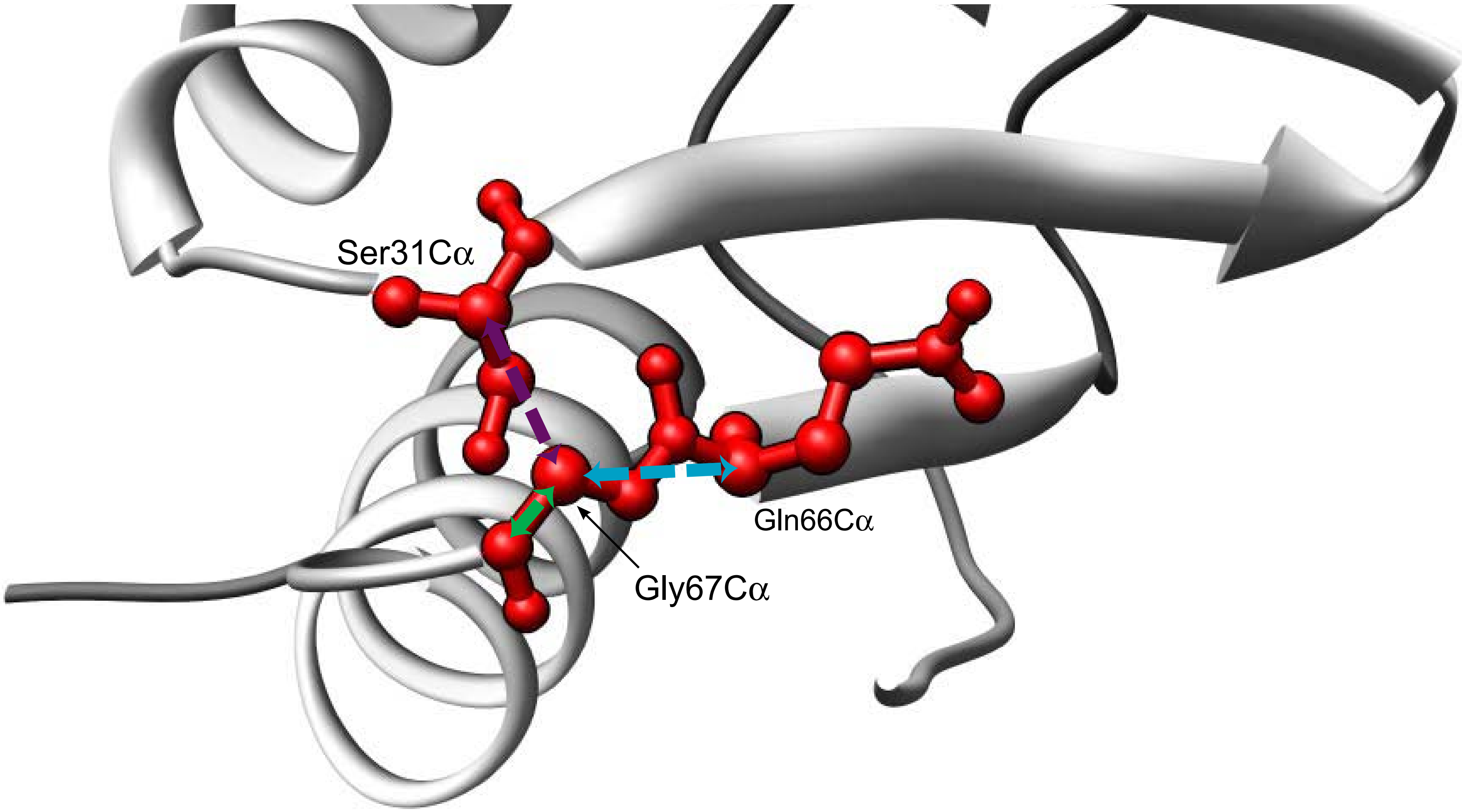
Crh dimer: 2 x 85 residues (10.4 kDa)



- Magic Angle Spinning (MAS) provides high resolution
- Recoupling sequence: reintroduction of the distance information

1. Structure determination by MAS SSNMR

1.2 Classes of distances



- Short distance contacts: one & two bond distances: e.g. Gly67Cα-Gly67C' = 1.5 Å
- Medium distance contacts: 2 Å< d <4 Å: e.g. Gly67Cα - Gln66Cα = 2.40 Å
- Long distance contacts: >4 Å: e.g. Gly67Cα- Ser31Cα = 4.03 Å

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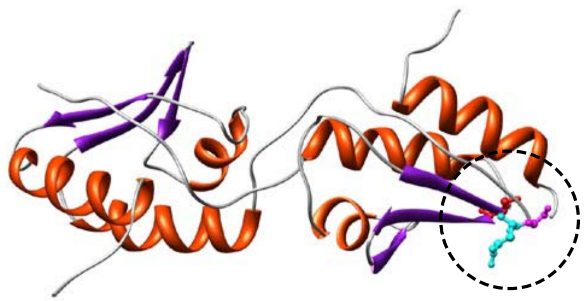
1. Structure determination by MAS SSNMR

1.3 The promises and challenges of high B_0 field



→ High field magnet → High MAS frequencies

Juy *et al.*, J Mol Biol (2003) 332.

• **Need for new methodology** a high MAS (>20 kHz) and high B_0 fields (> 600 MHz):

- able to provide efficient one-bond transfer [assignment, torsion angle]

- able to provide medium to long distance restraints [structure]

The use of AHT to design de/recoupling pulse sequence under MAS

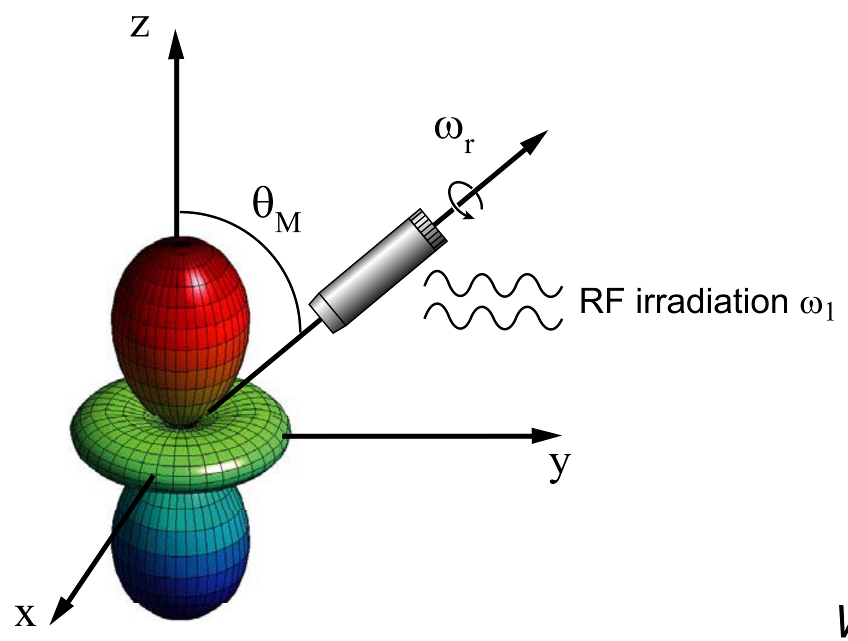
- Internal Hamiltonian: H_{int} with Magic Angle Spinning

  $\rightarrow$ Spatial part of the interations are time dependent $\left(e^{i\omega_r t}, e^{i2\omega_r t}\right)$

- Application of rf pulses $\left(H_{rf}\right)$ to decouple or recouple given interaction

- In the Rotating Frame: $H = H_{int}(t) + H_{rf}(t)$

- In the Interaction Frame defined by $H_{rf}(t)$: $H(t) \rightarrow \widetilde{H}(t)$



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The use of AHT to design de/recoupling pulse sequence under MAS

- if $\tilde{H}$ periodic, $\tilde{H}(t+T) = \tilde{H}(t)$
- with a stroboscopic detection every T period, evolution of the spin sustem can calculated using AHT*

$$\rightarrow \bar{H} = \bar{\bar{\tilde{H}}}^{(1)} + \bar{\bar{\tilde{H}}}^{(2)} + \cdots \text{ (magnus expansion}^{**}\text{)}$$

with

$$\bar{\bar{\tilde{H}}}^{(1)} = \frac{1}{T}\int_0^T \tilde{H}(t)\,dt \rightarrow \text{ first order de/recoupling}$$

$$\bar{\bar{\tilde{H}}}^{(2)} = \frac{-i}{2T}\int_0^T dt_2 \int_0^{t_2} dt_1 \left[\tilde{H}(t_1), \tilde{H}(t_2)\right] \rightarrow \text{ second order cross terms}$$

…

Examples:

1/ ^{1}H decoupling TPPM/CM : **First order decoupling** of the heteronuclear ^{1}H-^{13}C dipolar interaction
Reduced second order cross terms (notably involving CS and het. dip. interaction) (see C. Jaroniec's lecture)

2/ Dipolar recoupling sequences: **First order recoupling** of the dipolar interaction
Reduced second order cross terms
(see R. Tycko's lecture)

* U. Haeberlen and J. S. Waugh, Physical Review 175 (2), 453 (1968).
** W. Magnus, Ann. Math. 52 (1), 111 (1950). W. Magnus, Bull. Amer. Math. Soc. 55 (11), 1048 (1949).

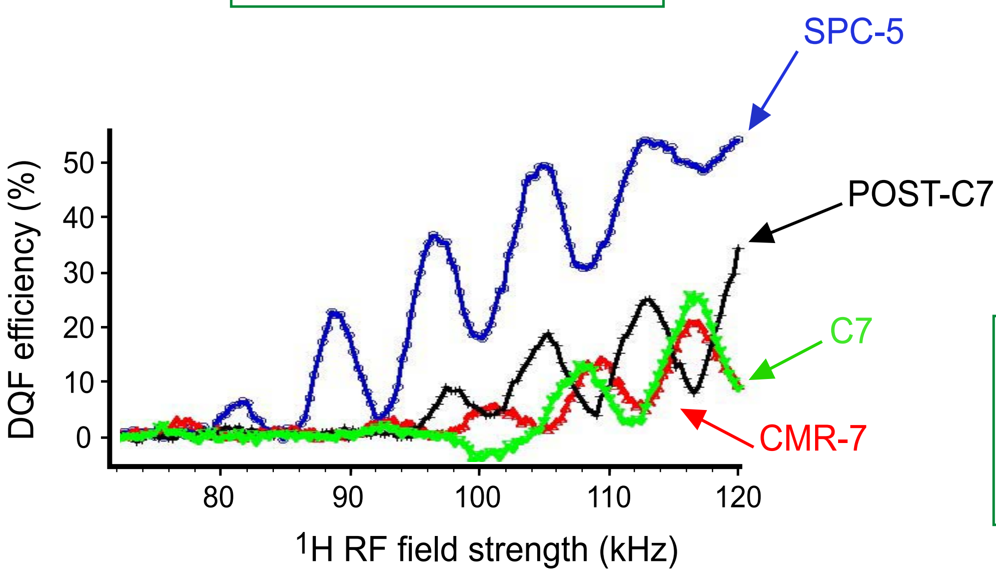
1. Structure determination by MAS SSNMR

1.4 Challenges of the fast MAS/ high B_0 field regime

1.4.1 One bond dipolar recoupling techniques

Example: DQ ^{13}C-^{13}C recoupling techniques

$\nu_{1H} > N \times \nu_r \times 3$



Adapted from Hohwy *et al.*

Experimental requirements:

ν_r=10 kHz

ν_1=50 kHz for SPC5

ν_1=70 kHz for C7, POSTC7, CMR-7

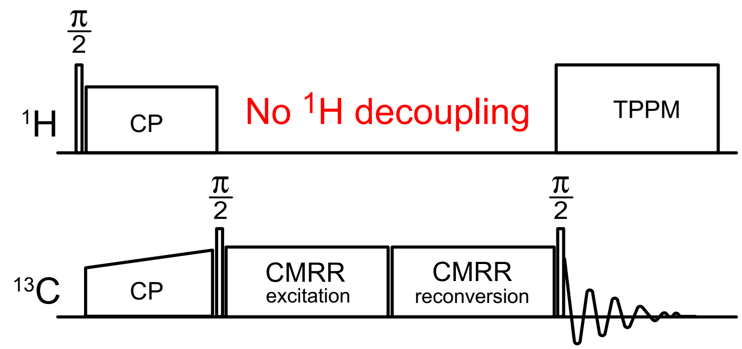
^1H decoupling field strength should be at least 3 times the ^{13}C field strength, i.e. 15-21 times the MAS frequency (i.e. 150-210 kHz)!

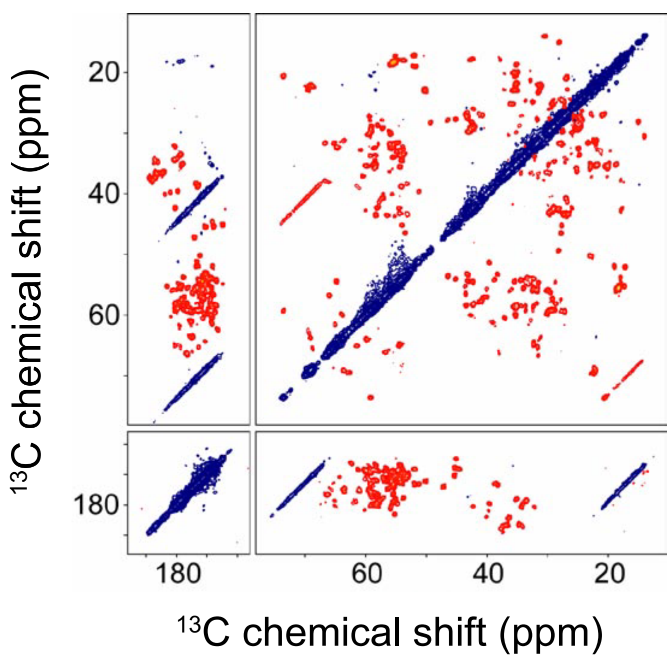
Bennett *et al.*, Ishii *et al.*, Rienstra *et al.*

- Imperfect ^1H decoupling leads to losses in ^{13}C DQ excitation efficiency
- Large RF heating for biological systems

→ New Solution: DQ-CMRR recoupling without ^1H decoupling

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





^{13}C-^{13}C recoupling without ^1H decoupling, 20 kHz MAS
CM_5RR* on [U-^{13}C, ^{15}N]-Crh - 750 MHz, 15 hours
Efficient relayed transfer mechanism

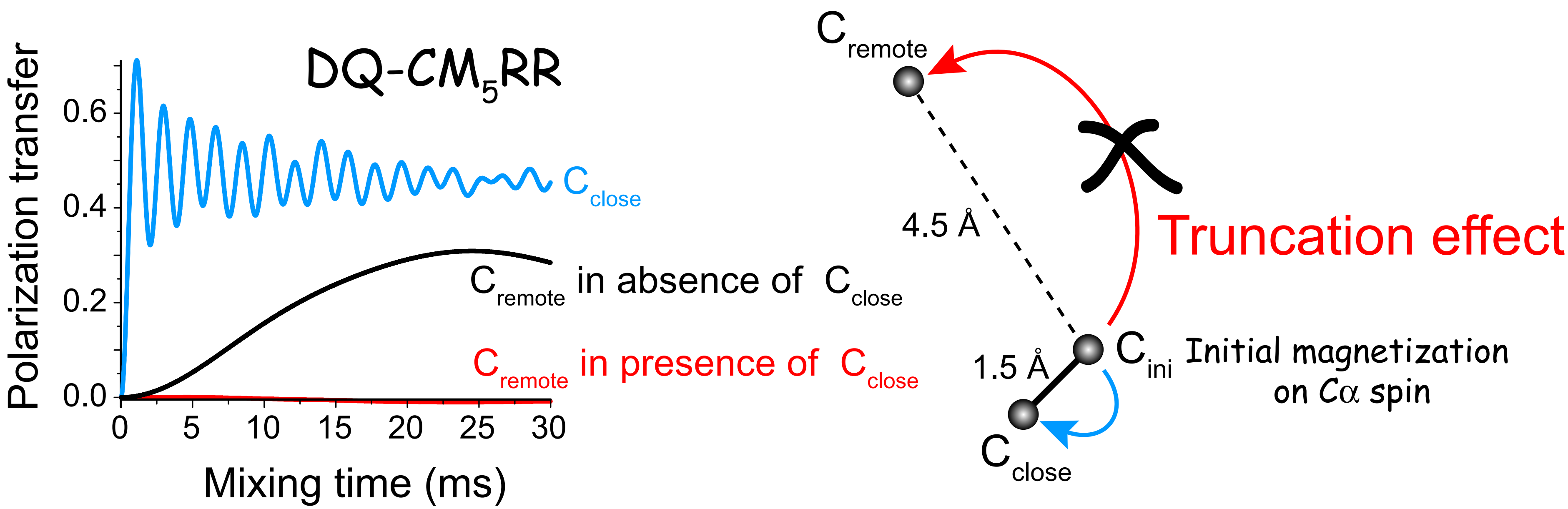
*De Paëpe *et al.*, JACS (2006)
De Paëpe *et al.*, JCP (2008)

1. Structure determination by MAS SSNMR

1.4 Challenges of the fast MAS/ high B_0 field regime

1.4.2 Dipolar truncation of the long distance transfer

Broadband recoupling



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

- CM_5RR DQ recoupling: useful for assignment
- First order ^{13}C-^{13}C recoupling: dipolar truncation phenomenon!

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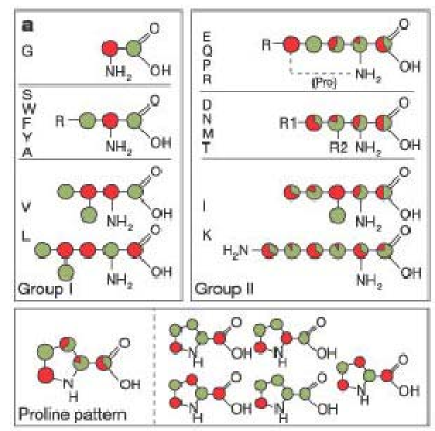
→ How to reduce dipolar truncation?- Part I

How to obtain medium to long distance contacts?

- R^2-based frequency selective techniques — Andrew *et al.*, Griffin *et al.*

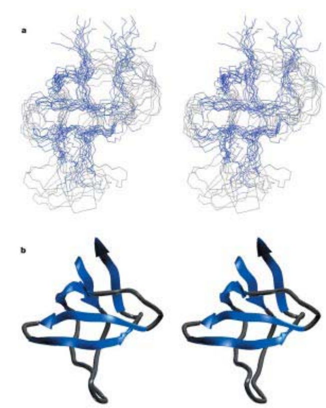
- Alternating labeling schemes:

  LeMaster *et al*., J. Biol. Chem. (1982) 257.
  LeMaster et al., JACS (1996) 118.
  Castellani *et al*., Nature (2002) 420.



- PDSD (Proton Driven Spin Diffusion) for MAS < 12 kHz

  Castellani *et al*., Nature (2002) 420.
  Castellani *et al*., Biochemistry (2003) 42.



→ Long distance transfer in: 400 ms -1 s

→ Second order recoupling: CC x CH and CC x CC cross term

Grommek *et al*., CPL (2006) 427.

→ How to reduce dipolar truncation?- Part II

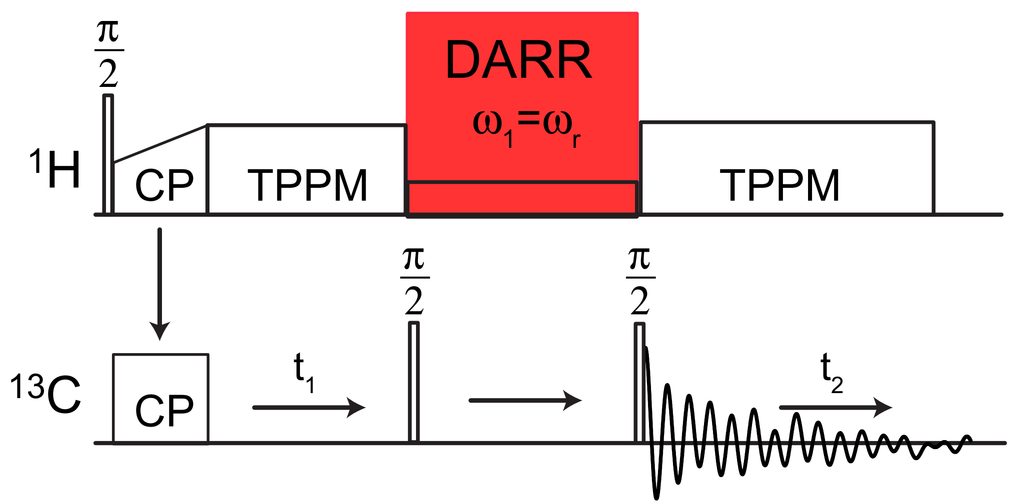
How to obtain medium to long distance contacts?

- DARR (Dipolar Assisted Rotational Resonance)

Takegoshi *et al.*, CPL (2001) 344.
Morcombe *et al.*, JACS (2004) 126.

→ Long distance transfer in: 200-400 ms (DARR at 10-20 kHz)

Zech *et al.*, JACS (2005) 1427.
Marulanda *et al.*, JPC B (2005) 109.



DARR pulse sequence

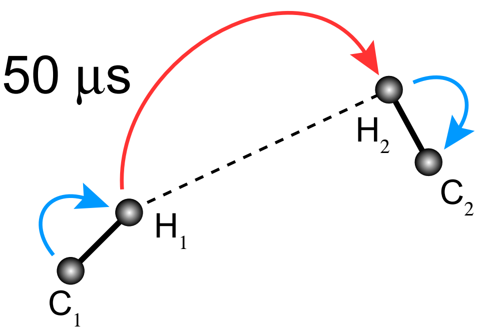
→ How to reduce dipolar truncation?- Part III

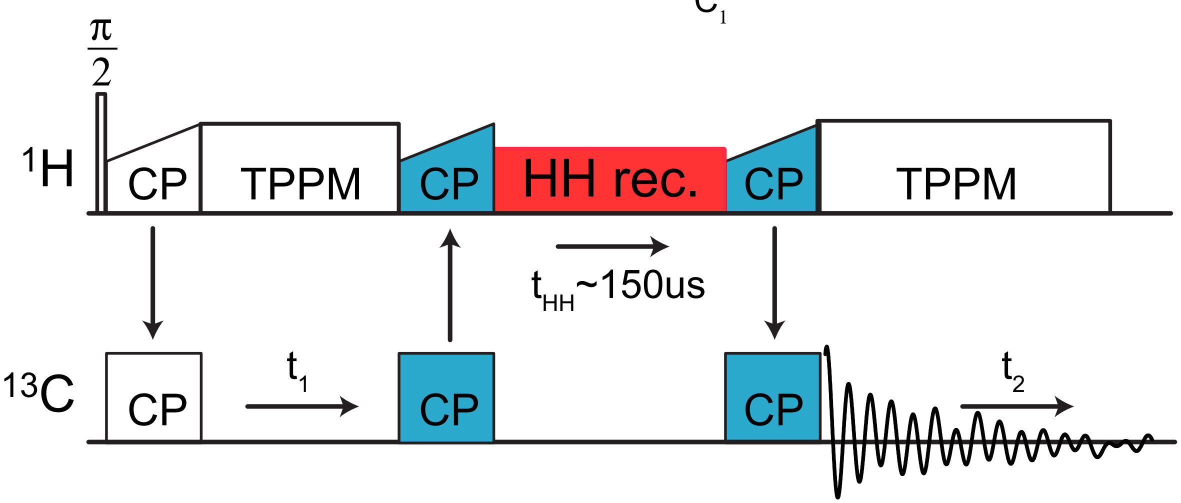
How to obtain medium to long distance contacts?

- CHHC* ^{13}C-^{13}C polarization transfer mediated by ^{1}H-^{1}H couplings

Lange *et al.*, JACS (2002) 124.
Lange *et al.* Angew Chem Int Edit (2005) 44.

→ Long distance transfer in ~150 μs

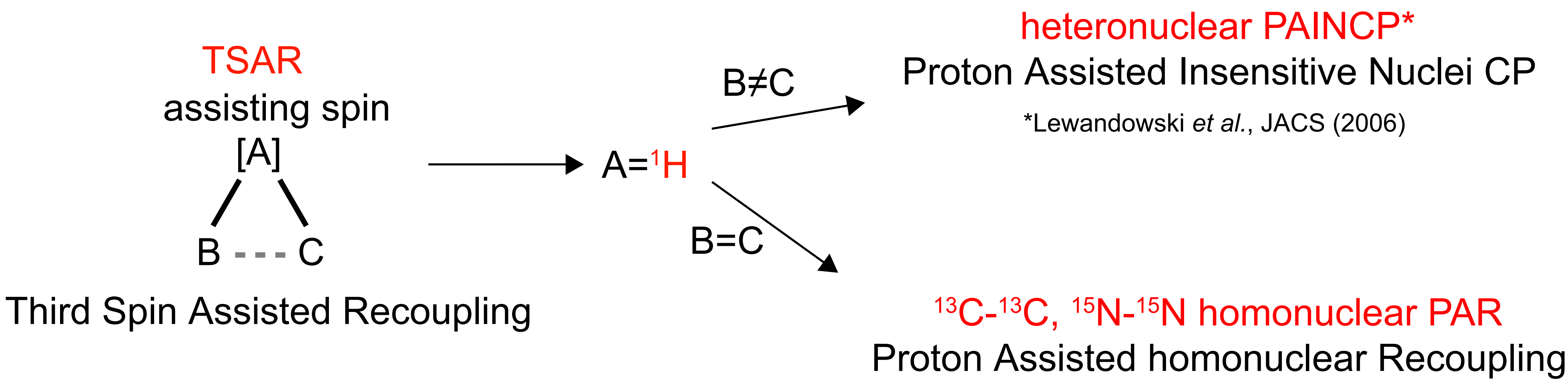




CHHC pulse sequence

→ New solution: The TSAR recoupling mechanism

- **Long distance transfer:**



- B-C recoupling assisted by surrounding protons A (BA x AC cross terms)
- Fast and efficient transfer at high MAS (>20 kHz) and high B_0 (>750 MHz)
- One-bond, sequential and long distance contacts observed in 1 to 20 ms

Recoupling without decoupling:

- DQ CMRR: the use of a ^{1}H decoupling scheme

- ZQ RFDR at high MAS frequencies

2. Homonuclear recoupling without decoupling

2.1 Idea: Using a ^{1}H-^{13}C decoupling sequence...

TPPM*/CM** - efficient ^{1}H decoupling sequence

CM r.f. phase modulation

$$\phi = a\cos(\omega_c t - \varphi_0) + \phi_0$$

$\omega_1/2\pi$ r.f. field strength
ω_C phase modulation frequency
a phase modulation amplitude

Efficient decoupling can be explained by:

- First order decoupling of the ^{1}H-^{13}C dipolar interaction
- First order decoupling of the ^{1}H CS interaction
- Reduced second order cross terms involving ^{1}H-^{13}C and ^{1}H CS interactions

- First order recoupling of the ^{1}H-^{1}H interaction**

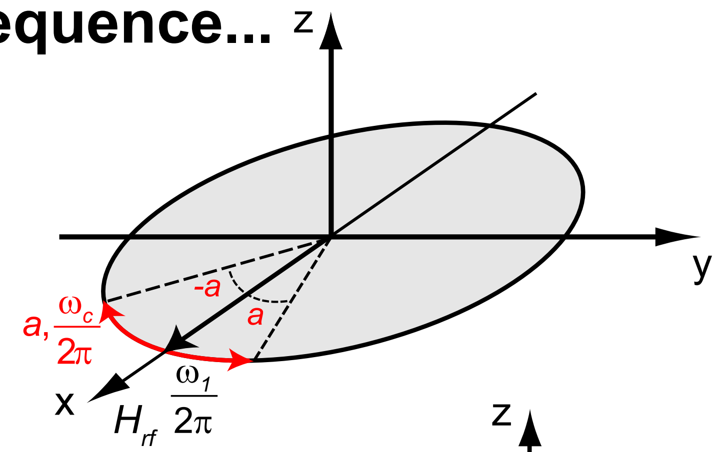
** Bennett *et al., J. Chem. Phys.* (1995).
** De Paëpe *et al., J. Chem. Phys.* (2004).

Active *self-decoupling* mechanism!

- ideal candidate for ^{13}C-^{13}C recoupling without decoupling!

2. Homonuclear recoupling without decoupling

2.1 Idea: Using a ^{1}H-^{13}C decoupling sequence...



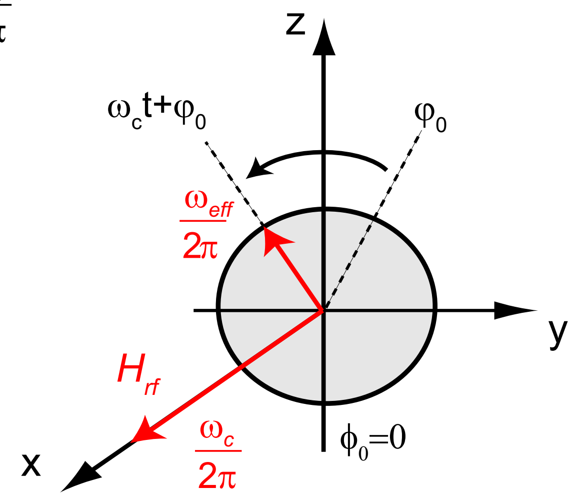
$$H_{rf} = \omega_1 \cos(\phi(t)) \sum_i S_x^i + \omega_1 \sin(\phi(t)) \sum_i S_y^i$$

with $\phi(t) = a \sin(\omega_c t)$

If $a \ll 1: H_{rf} \approx \omega_1 (1 - a^2/4) \sum_i S_x^i + \omega_1 a \sin(\omega_c t) \sum_i S_y^i$

If $a \ll 1: H_{rf} \approx \omega_1 (1 - a^2/4) \sum_i S_x^i + \omega_1 a \left(\frac{e^{i\omega_c t} - e^{-i\omega_c t}}{2i} \right) \sum_i S_y^i$

If $\omega_1 (1 - a^2/4) = \omega_c : H_{rf} \approx \omega_c \sum_i S_x^i + \frac{\omega_1 a}{2i} e^{i\omega_c t} \sum_i S_y^i - \underbrace{\frac{\omega_1 a}{2i} e^{-i\omega_c t} \sum_i S_y^i}_{\text{non resonant term}}$

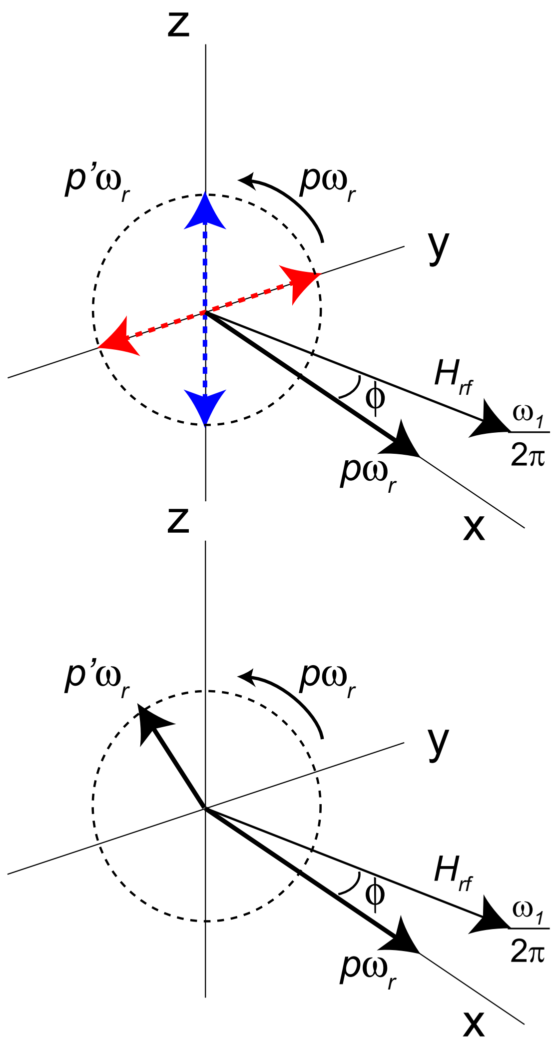


$$\omega_c = \omega_1 (1 - a^2/4)$$
$$\omega_{eff} = a\omega_1/2$$

- This type of rf scheme induces a two steps averaging process:
  - CW averaging at $\omega_c = p\ \omega_r$
  - Resonant audio field averaging at $\omega_{eff} = p'\ \omega_r$

2. Homonuclear recoupling without decoupling

2.2 Generalization of the second averaging principle



$$H_{rf} = \omega_1 \cos\left(\phi\left(t\right)\right)\sum_i S_x^i + \omega_1 \sin\left(\phi\left(t\right)\right)\sum_i S_y^i + \Omega\cos\left(\omega_c t\right)\sum_i S_z^i$$

$$\begin{cases} \omega_1 \cos\left(\phi\right) = p\omega_r \\ \omega_1 \sin\left(\phi\right) = p'\omega_r \sin\left(p\omega_r t\right) \\ \Omega = p'\omega_r \end{cases} \Longrightarrow \begin{cases} \phi = \arctan\left(\dfrac{p'\sin\left(p\omega_r t\right)}{p}\right) \\ \omega_1^{(0)} = p\omega_r\left[1+\left(\dfrac{p'}{p}\sin\left(p\omega_r t\right)\right)^2\right]^{1/2} \\ \Omega = p'\omega_r \end{cases}$$

$$H_{rf} = \quad p\omega_r \sum_i S_x^i + p'\omega_r \sin\left(p\omega_r t\right)\sum_i S_y^i + p'\omega_r \cos\left(p\omega_r t\right)\sum_i S_z^i$$

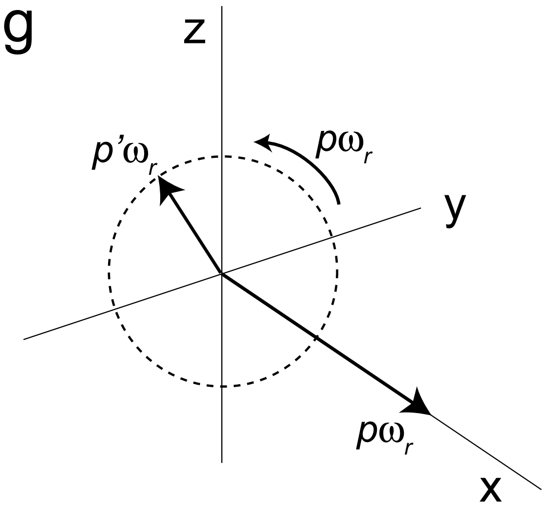
- This new scheme generalizes the concept of second averaging!
- The irradiation scheme is completely defined by p, p', and ω_r

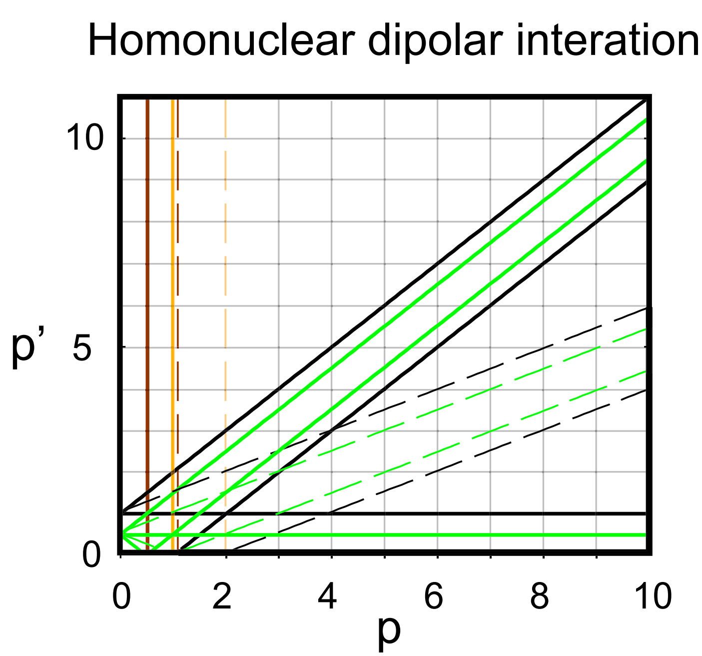
2. Homonuclear recoupling without decoupling

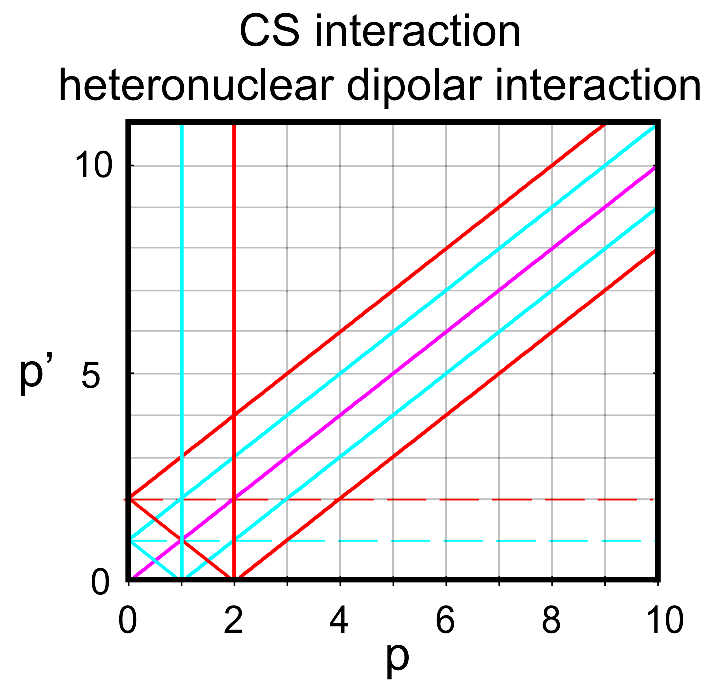
2.3 First order recoupling

$$m + p.q + p'.q' = 0$$

MAS → m; first averaging → $p.q$; second averaging → $p'.q'$



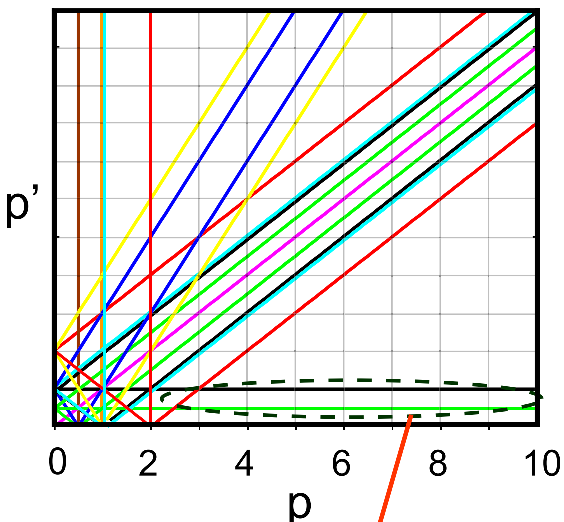


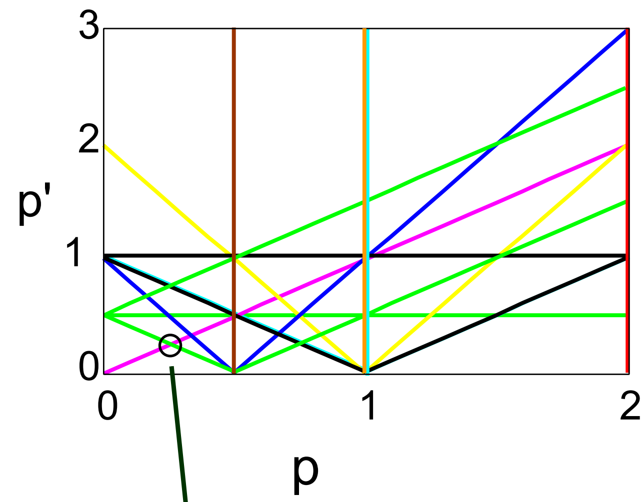


- This new scheme can be seen as a very verstatile recoupling toolbox

2. Homonuclear recoupling without decoupling

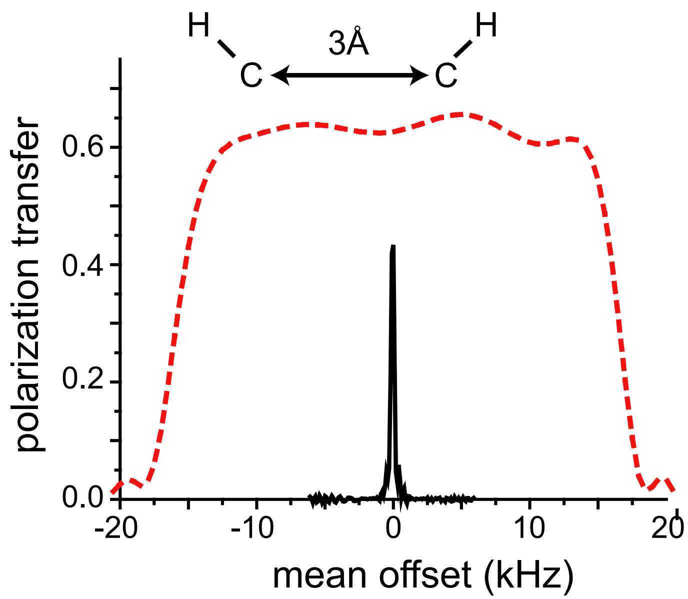
2.4 Broadband CMRR and narrowband COMICS





CM$_5$RR
p'=0.5

- 3.5 < p < 10
- Broadband
- no ^{1}H decoupling required!

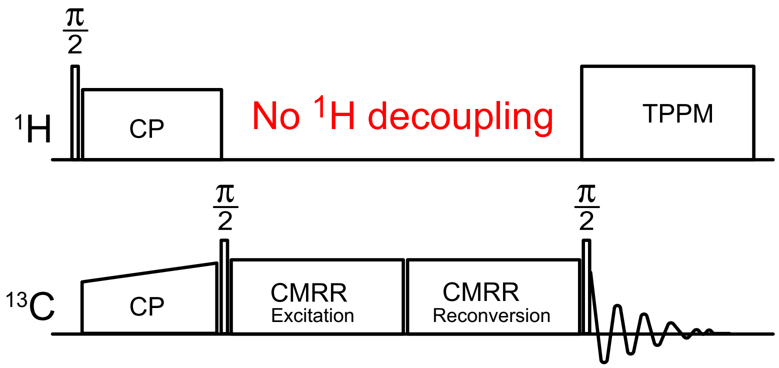


COMICS
p=0.25
p'=0.25

- Narrowband, frequency selective
- ^{1}H decoupling required

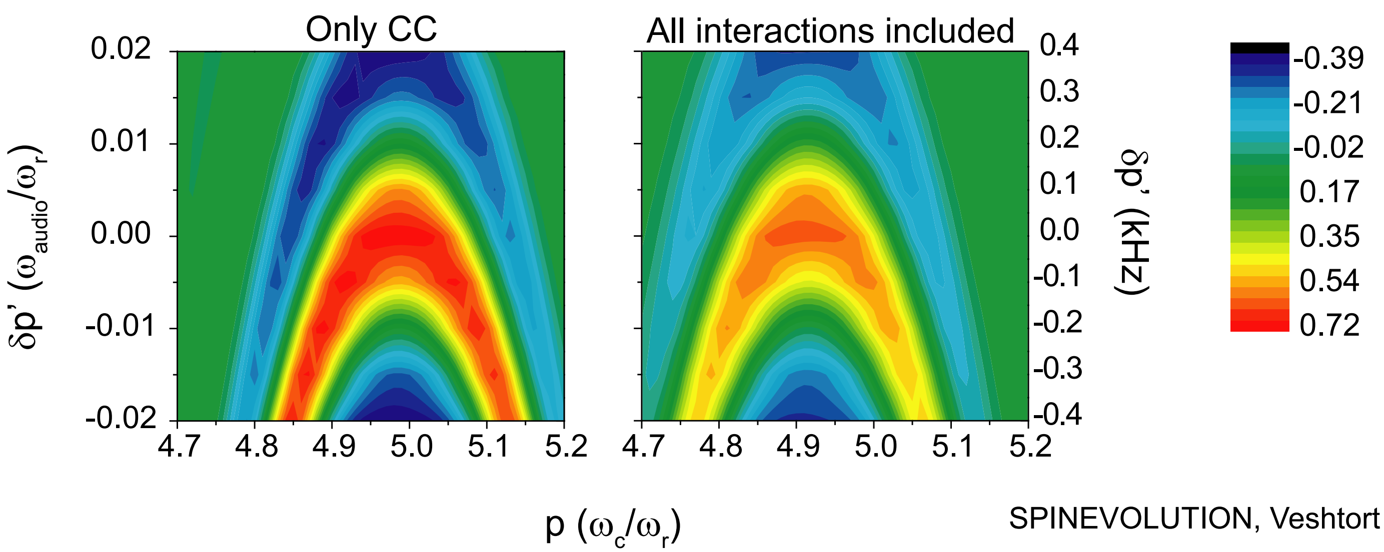
2. Homonuclear recoupling without decoupling

2.5 optimization maps - CMpRR



Spin System

NH_3^+-$^{13}CH_2$-$^{13}CO_2^-$



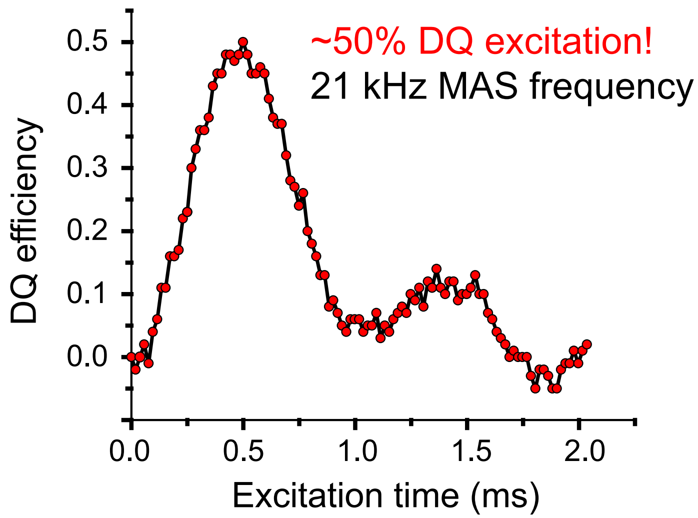
SPINEVOLUTION, Veshtort et al., JMR (2006) 178.

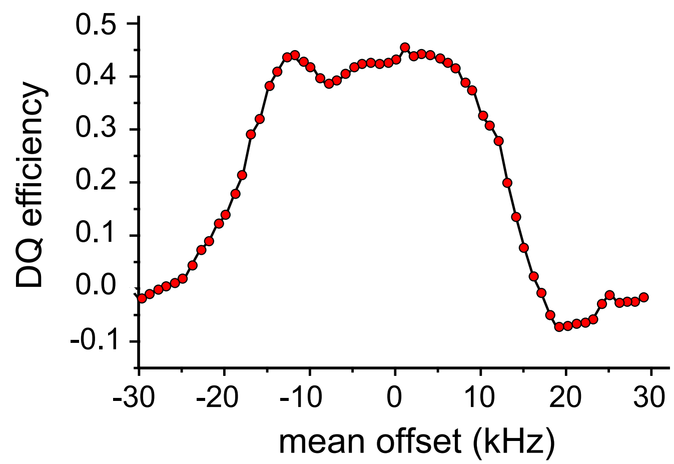
- Fine reoptimization can compensate second and third order effects!

2. Homonuclear recoupling without decoupling

2.6 Scaling factor and bandwidth

20% U-^{13}C,^{15}N-glycine
21 kHz MAS, CM_5RR
No ^{1}H decoupling
750 MHz



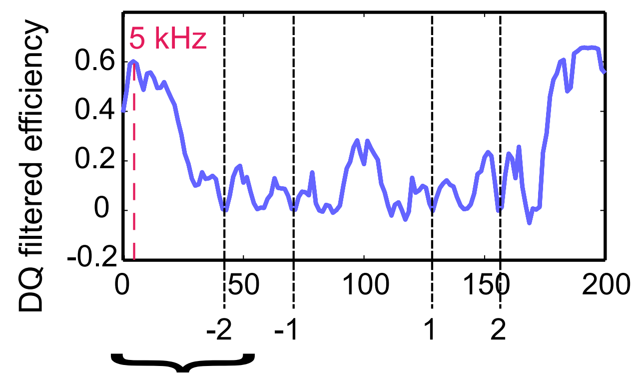


- no ^{1}H decoupling applied - excellent scaling factor - $5 < p < 10$ -
- Broadband DQ recoupling sequence even at 750 - 900 MHz!

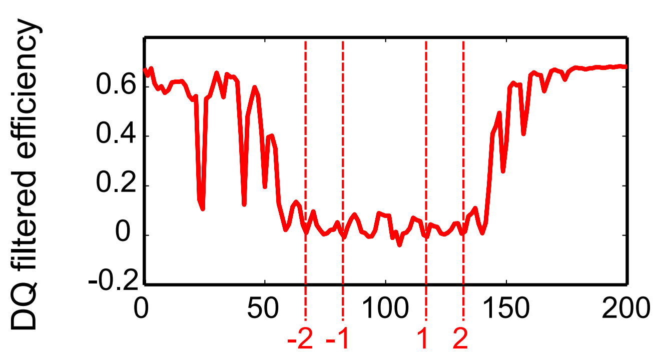
2. Homonuclear recoupling without decoupling

2.7 ^{1}H dependency at constant ω_c

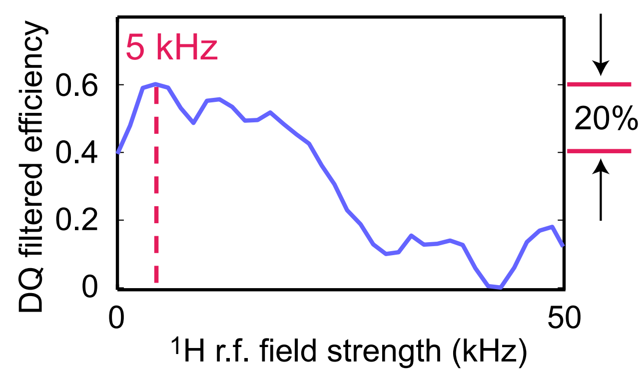
• ν_c = 100 kHz, p = 3.5, ν_r = 28.5 kHz



• ν_c = 100 kHz, p = 6, ν_r = 16.7 kHz



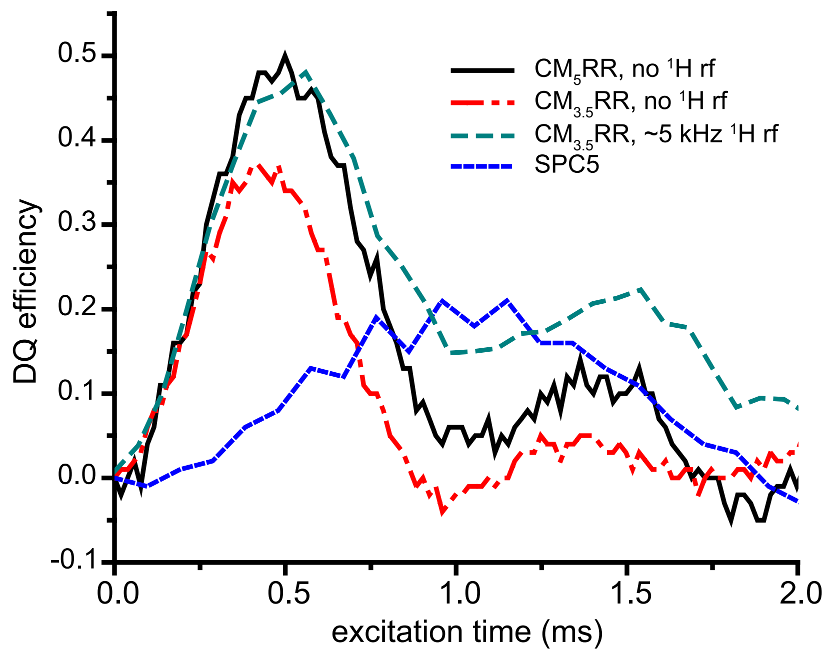
~5 kHz of ^{1}H CW enhances the DQ efficiency by ~20%!!



• Low ^{1}H power irradiation (~3kHz) extends the range of applicability of CMpRR to smaller p indices (3.5 to 5) and larger MAS spinning frequencies ($\omega_r = \omega_c/p$)

2. Homonuclear recoupling without decoupling

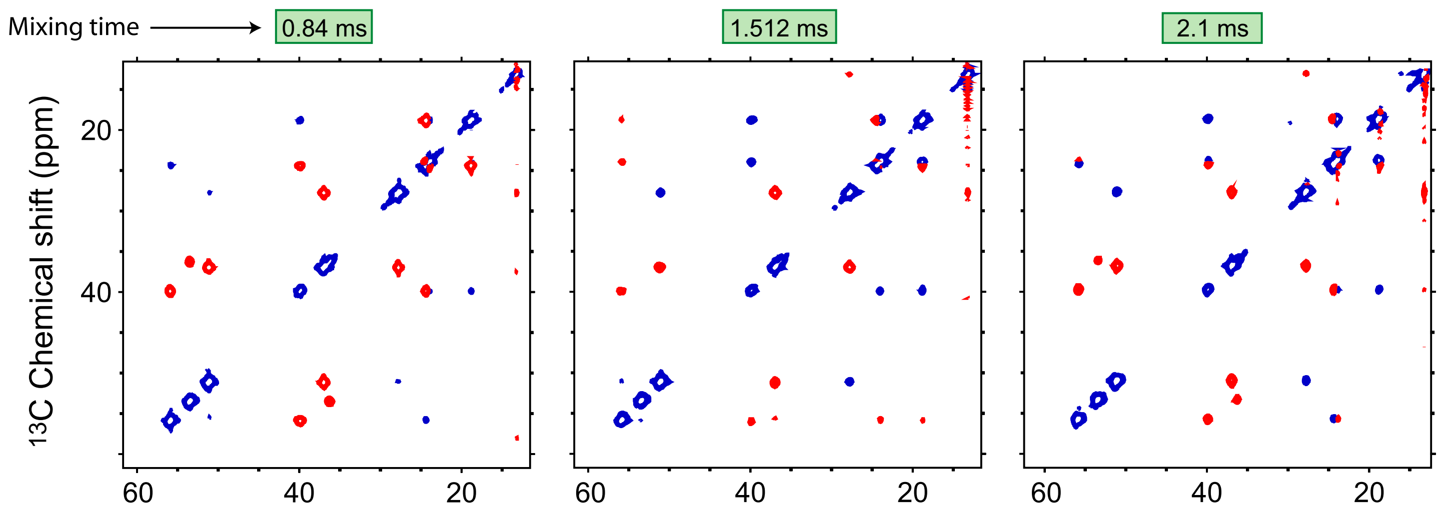
2.8 Experimental results

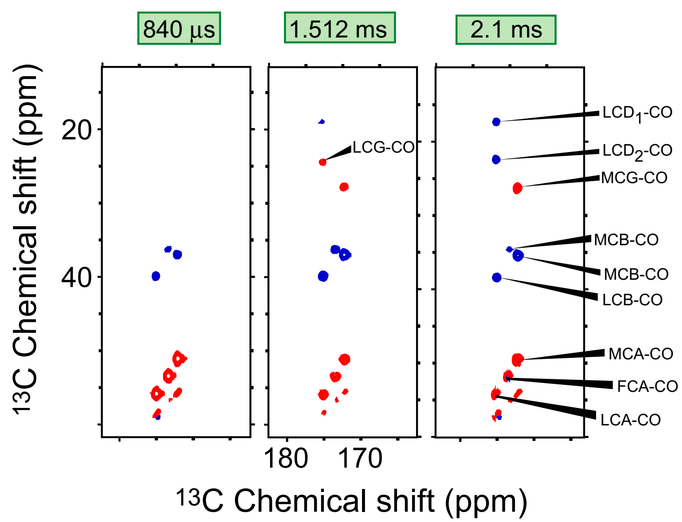


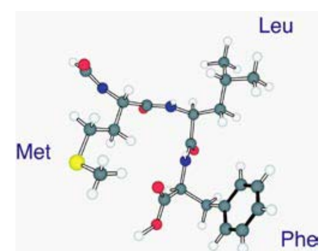
- Very efficient DQ recoupling up to 30 kHz MAS!

3. Biomolecular ^{13}C-^{13}C 2D spectra without ^{1}H decoupling

3.1 ^{13}C N-f-MLF-OH $CM_{3.5}RR$ spectra at 900 MHz







MAS 23.8 kHz
N-*f*-MLF-OH 10%

- Broadband DQ ^{13}C-^{13}C recoupling at 900 MHz with 3-5 kHz ^{1}H irradiation
- Relayed transfer mechanism

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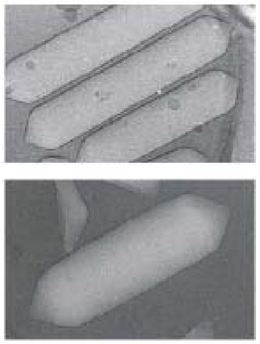
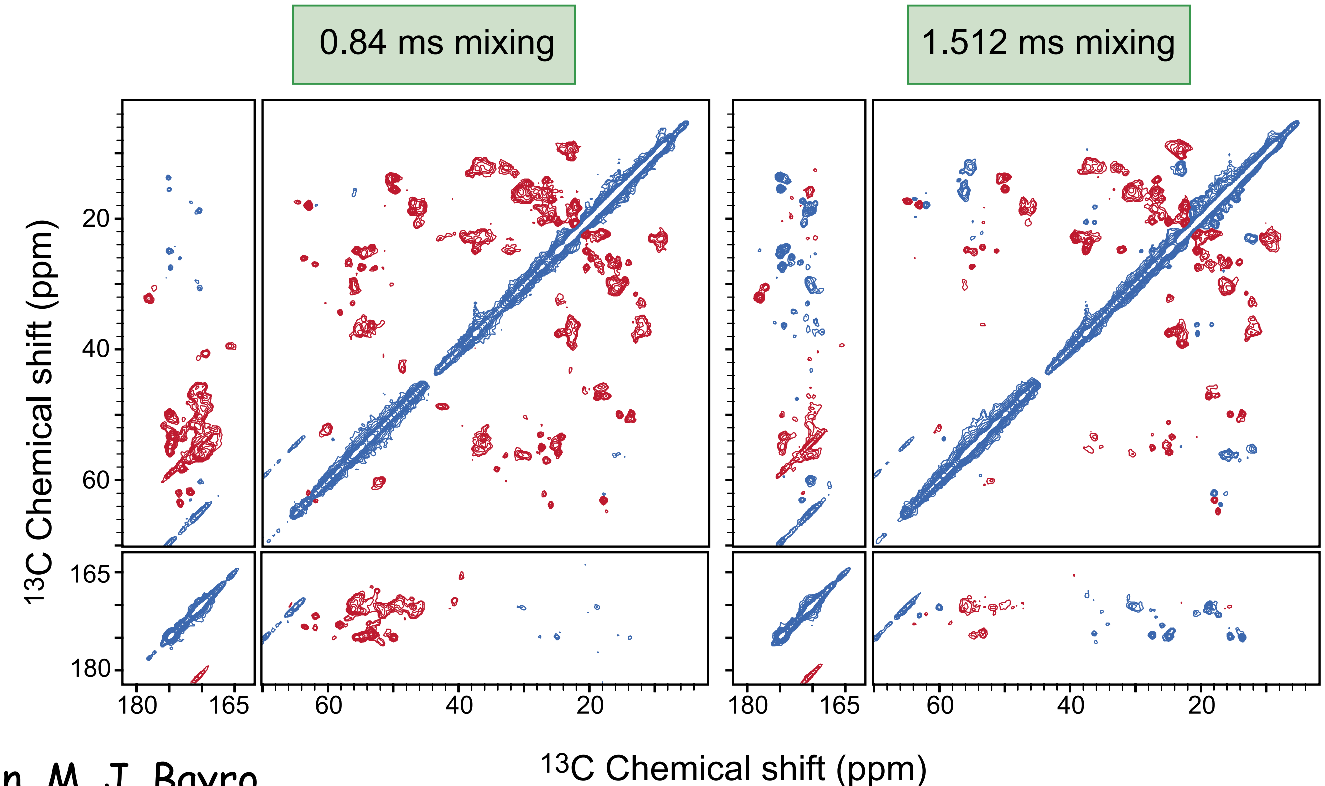
3. Biomolecular ^{13}C-^{13}C 2D spectra without ^{1}H decoupling

3.2 ^{13}C GVpa $CM_{3.5}RR$ spectra at 900 MHz



GVpa

$CM_{3.5}RR$
MAS 23.1 kHz
83 kHz ^{13}C pulses
~3-5 kHz ^{1}H CW

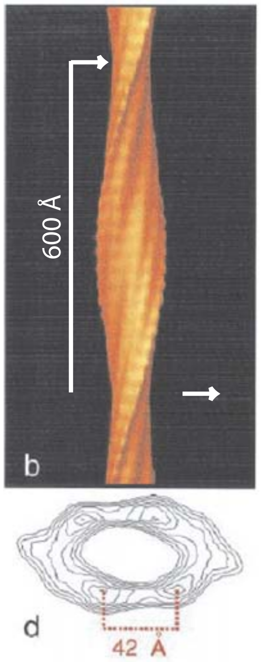


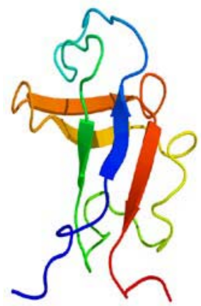
• GVpa project:
M. Belenky, A. Sivertsen, M. J. Bayro,
J. Herzfeld, R. G. Griffin

• Broadband ^{13}C-^{13}C recoupling at 900 MHz with 3-5 kHz ^{1}H irradiation
• Relayed transfer mechanism

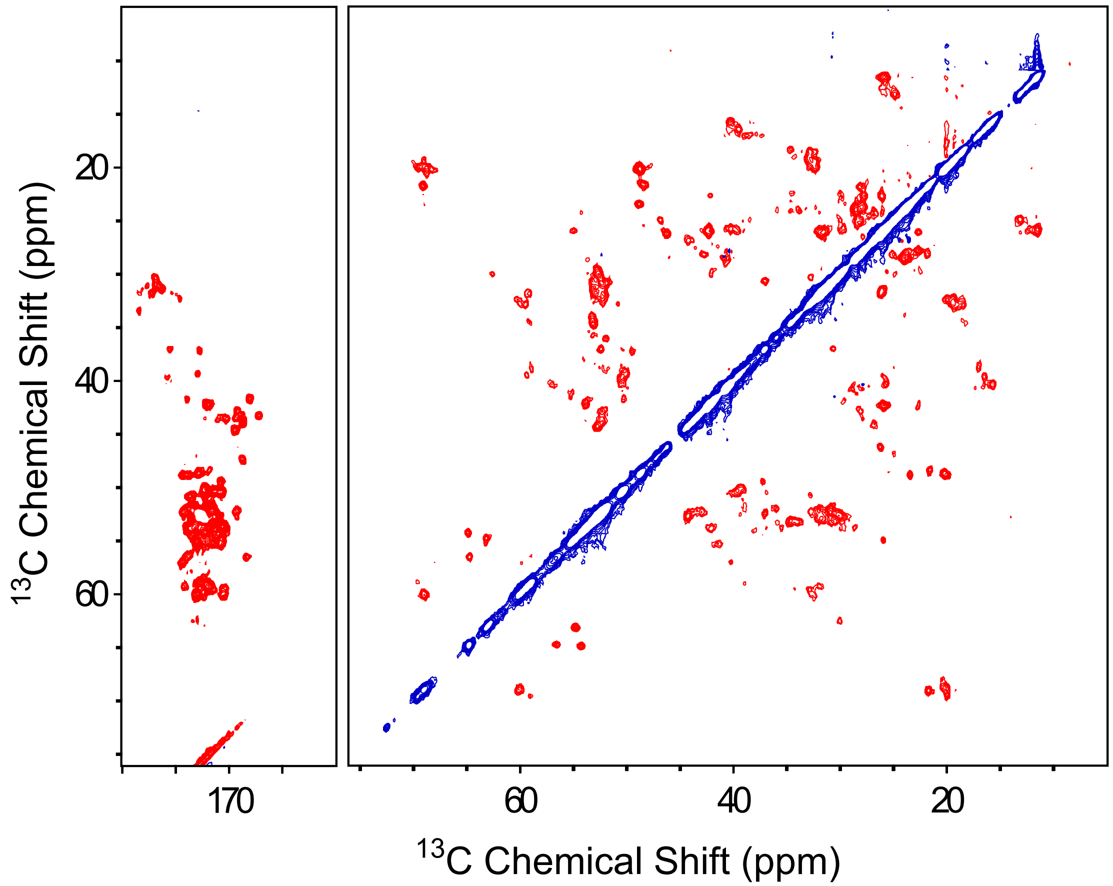
3. Biomolecular ^{13}C-^{13}C 2D spectra without ^{1}H decoupling

3.2 ^{13}C PI3-SH3 fibrils CM_4RR at 900 MHz





PI3-SH3



CM_4RR at 900 MHz ^{1}H freq.
ν_r = 20.1 kHz
ν_1 = 81.5 kHz
no ^{1}H decoupling
793 μs excitation time
64 scans

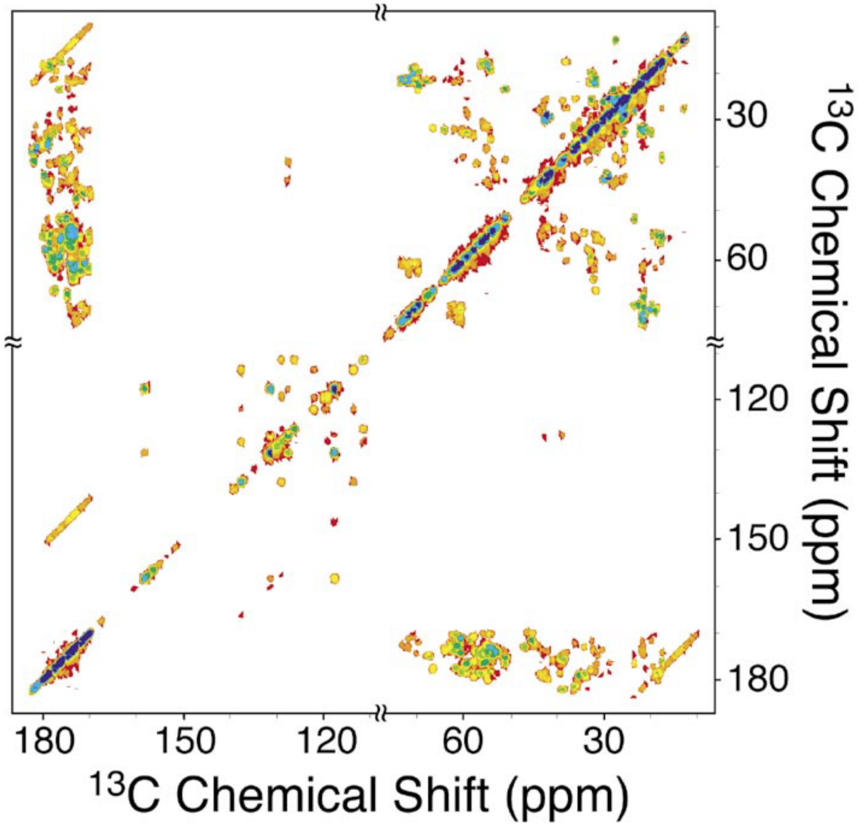
- U-^{13}C,^{15}N-PI3-SH3 fibril (82 residues)

- PI3-SH3 project:

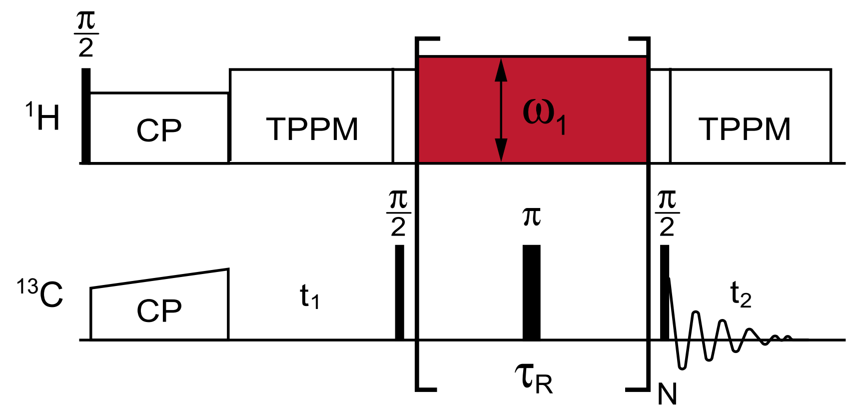
T. Maly, M. J. Bayro, M. Caporini, R. G. Griffin
N. Birkett, C. Dobson, C. MacPhee, M. Vendrosolo

3. Biomolecular ^{13}C-^{13}C 2D spectra without ^{1}H decoupling

3.2 ^{13}C GB$_1$ RFDR in the high MAS/ high field regime



Bayro *et al.*, JCP (2008)



Bennett *et al.*, JCP, **96**, 8624 (1992)

Bennett *et al.*, JCP, **108**, 9463 (1998)

Ishii *et al.*, JCP, **114**, 8473 (2001)

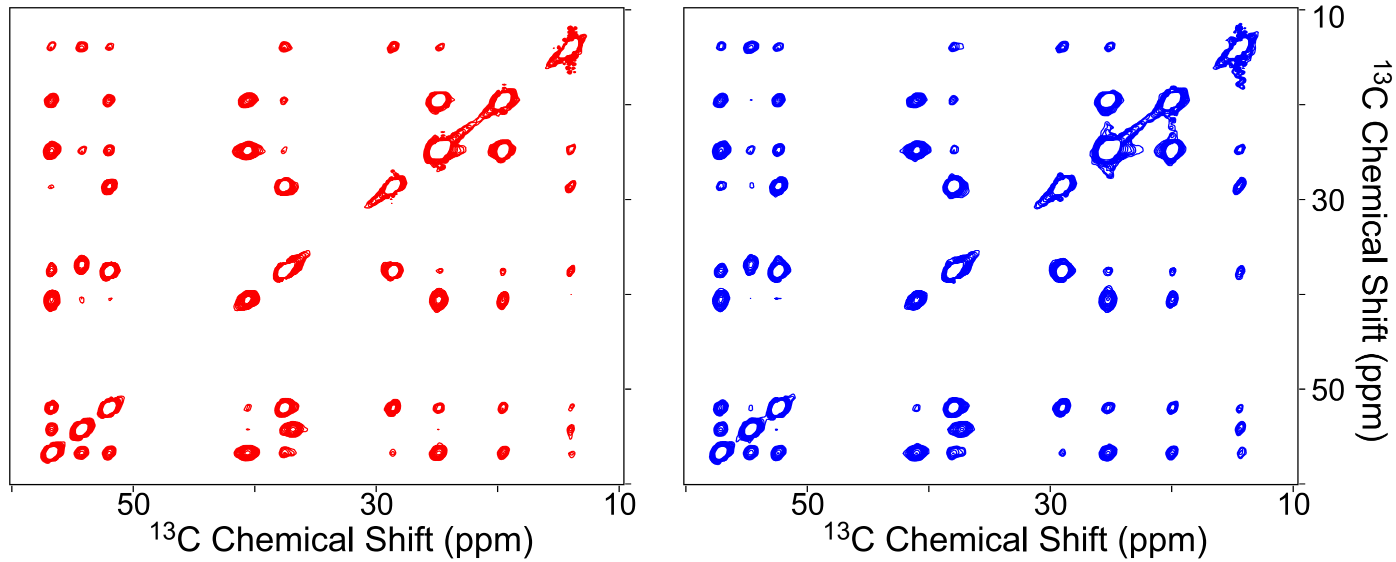
U-^{13}C, ^{15}N GB1
2 ms RFDR
30 kHz MAS
100 kHz ^{13}C π pulses

• Broadband ZQ ^{13}C-^{13}C RFDR recoupling at 750 MHz without ^{1}H irradiation

3. Biomolecular ^{13}C-^{13}C 2D spectra without ^{1}H decoupling

3.2 ^{13}C MLF-OH RFDR in the high MAS/ high field regime

8ms RFDR spectra of U-^{13}C, ^{15}N MLF-OH at 30 KHz MAS



RFDR with and without Heteronuclear Decoupling

- Left: Optimized ^{1}H decoupling (102/83 kHz) during mixing
- Right: No ^{1}H decoupling irradiation during mixing
- At high MAS frequencies, RFDR can be applied for long mixing times

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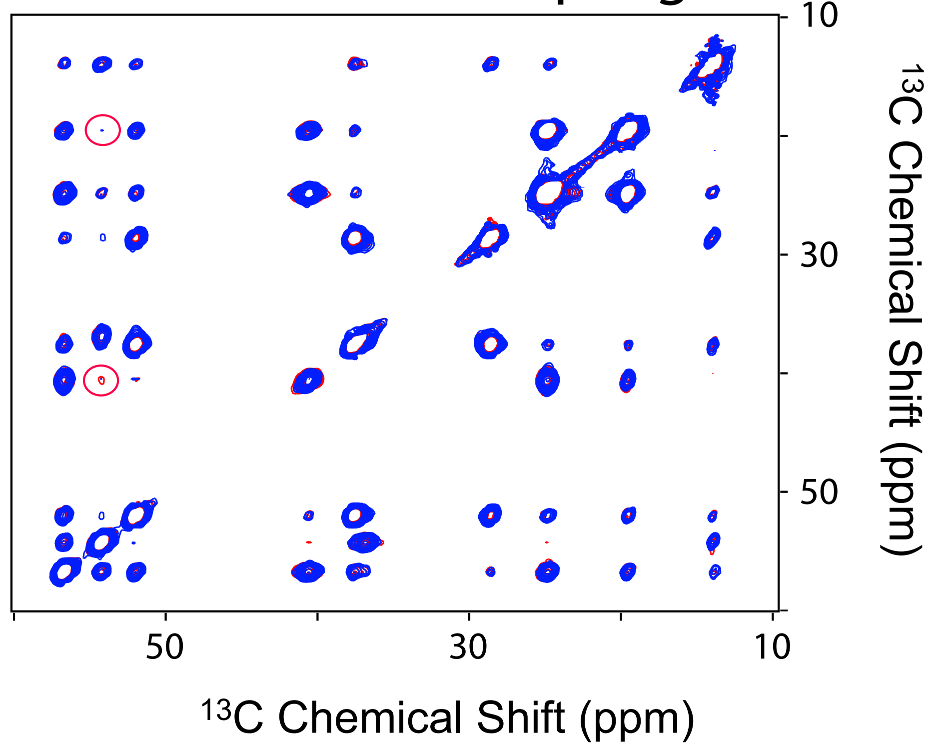
3. Biomolecular ^{13}C-^{13}C 2D spectra without ^{1}H decoupling

3.2 ^{13}C MLF-OH RFDR in the high MAS/ high field regime

superposition
sans/avec decoupling



U-^{13}C, ^{15}N MLF-OH
Overlaid spectra
8 ms mixing
30 kHz MAS

At high MAS rates, RFDR can be applied for long mixing times without decoupling

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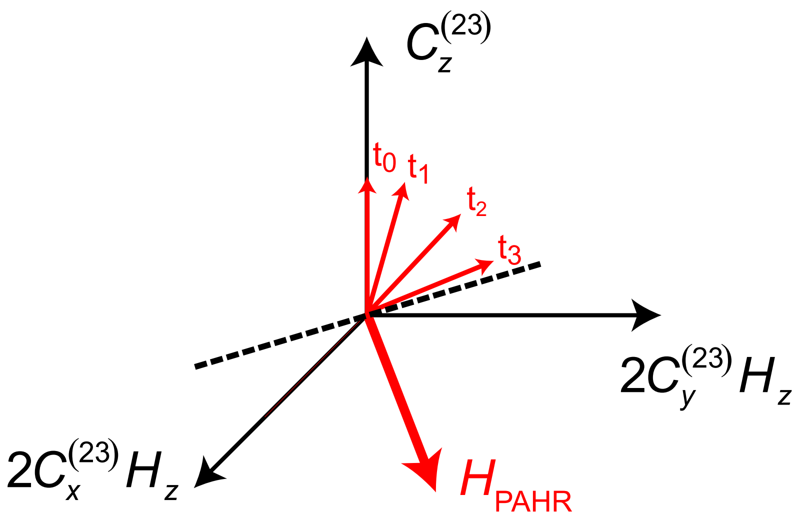
4. Long distance constraints

4.1 Third Spin Assisted Recoupling mechanism (TSAR)

4.1.1 Principle and TSAR subspace

- ^{13}C-^{13}C, ^{15}N-^{15}N or ^{15}N-^{13}C recoupling assisted by surrounding protons
- Second order recoupling: CH x CH, NH x HN or NH x HC cross terms
- Applicable at high B_0 and high MAS spinning frequencies
- Very efficient for short, medium and long distance transfer

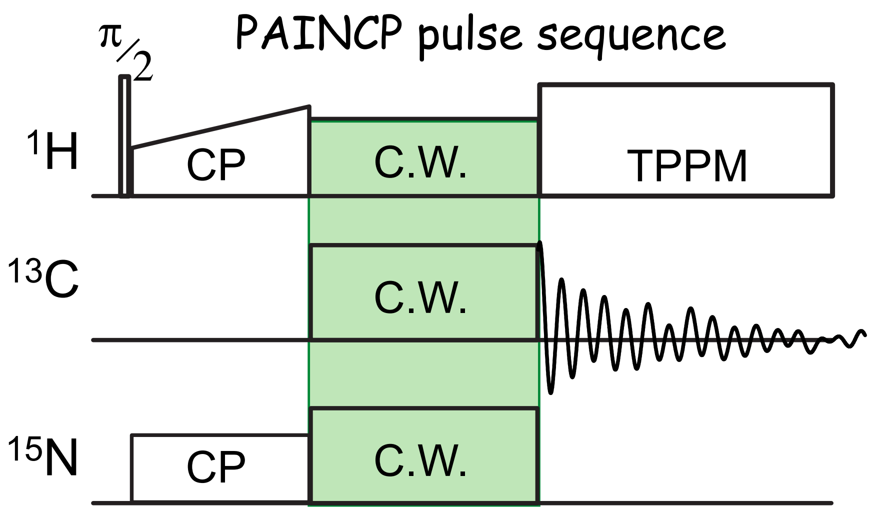


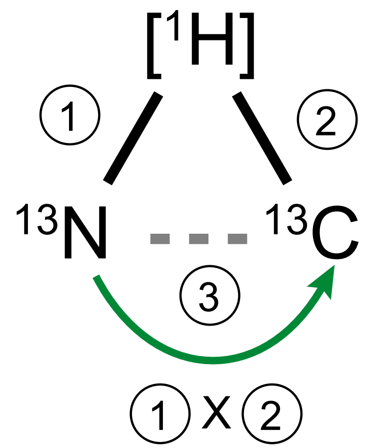
- TSAR subspace: coupled basis between a fictituous ZQ spin (C_1-C_2, N_1-N_2, N_1-C_2) and the assisting proton spin.

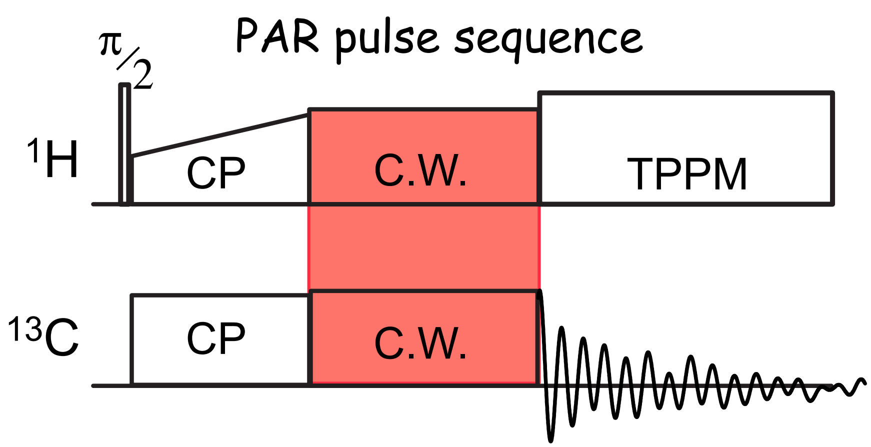
4. Long distance constraints

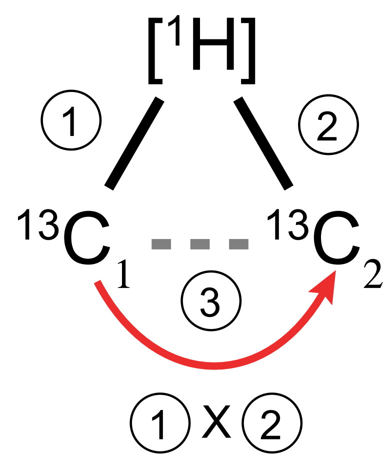
4.1 Third Spin Assisted Recoupling mechanism (TSAR)

4.1.2 Pulse sequences





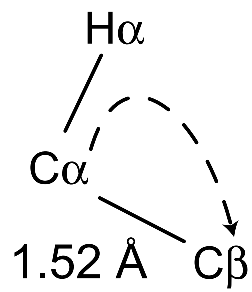




4. Long distance constraints

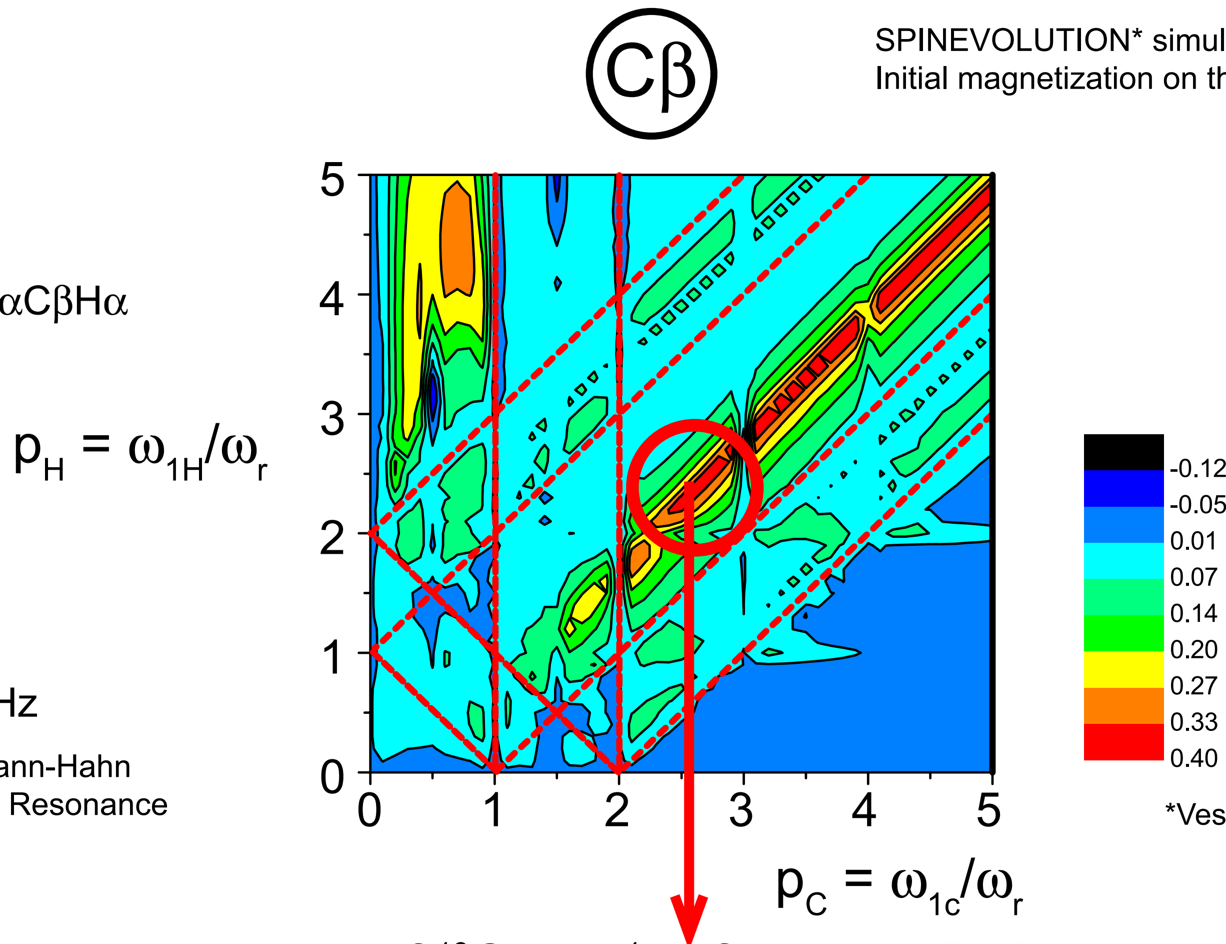
4.2 Homonuclear case: insight in the PAR experiment

4.2.1 Optimization maps



PAR: 3 spin case - CαCβHα

SPINEVOLUTION* simulations
Initial magnetization on the Cα spin



$\omega_r/2\pi$ = 20 kHz

$\omega_0/2\pi$ = 750 MHz

H.H. - Hartmann-Hahn
R.R. - Rotary Resonance

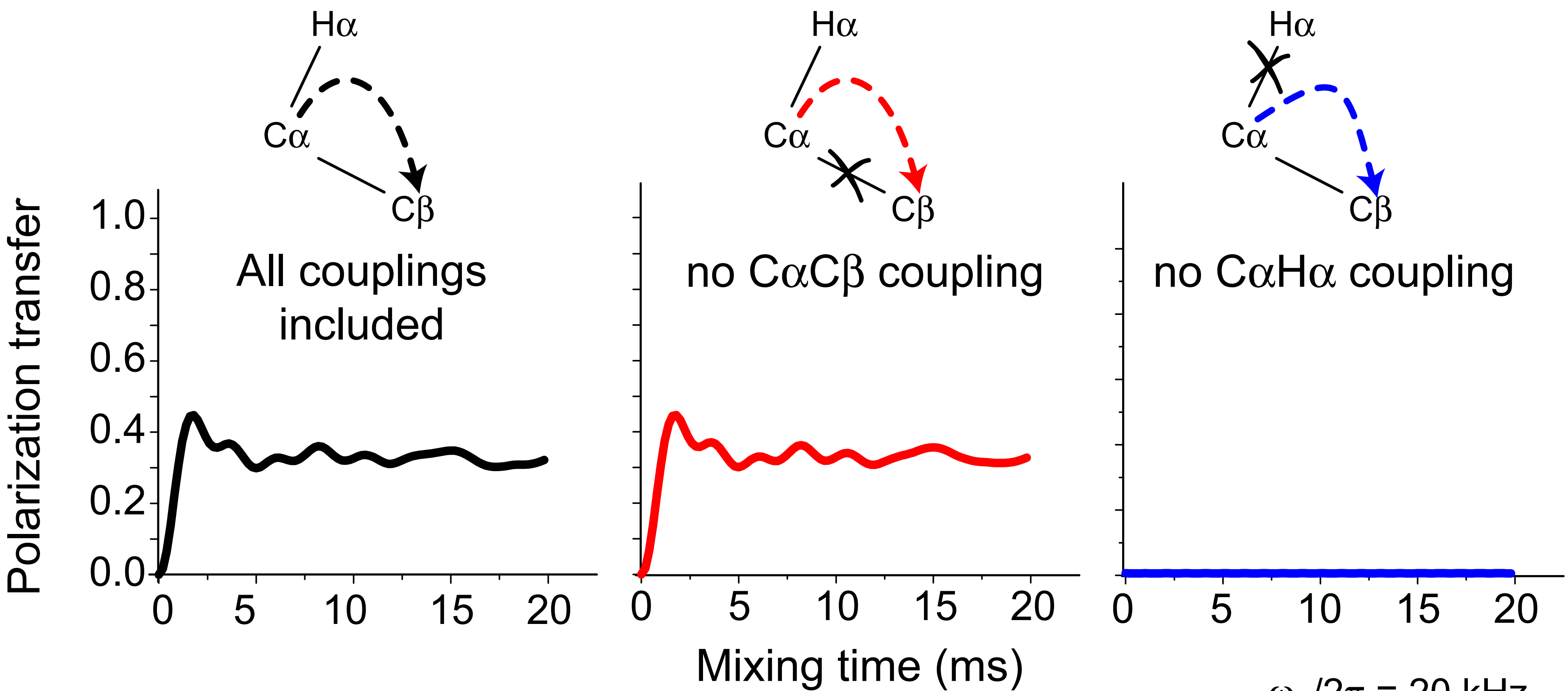
*Veshtort et al., JMR (2006) 178.

- Appropriate choice of ^{13}C and ^{1}H rf leads to PAR recoupling!

4. Long distance constraints

4.2 Homonuclear case: insight in the PAR experiment

4.2.2 Buildups



$\omega_r /2\pi$ = 20 kHz

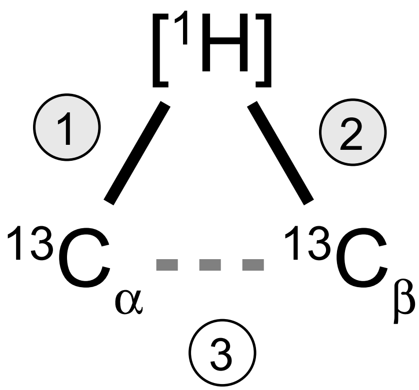
$\omega_0 /2\pi$ = 750 MHz

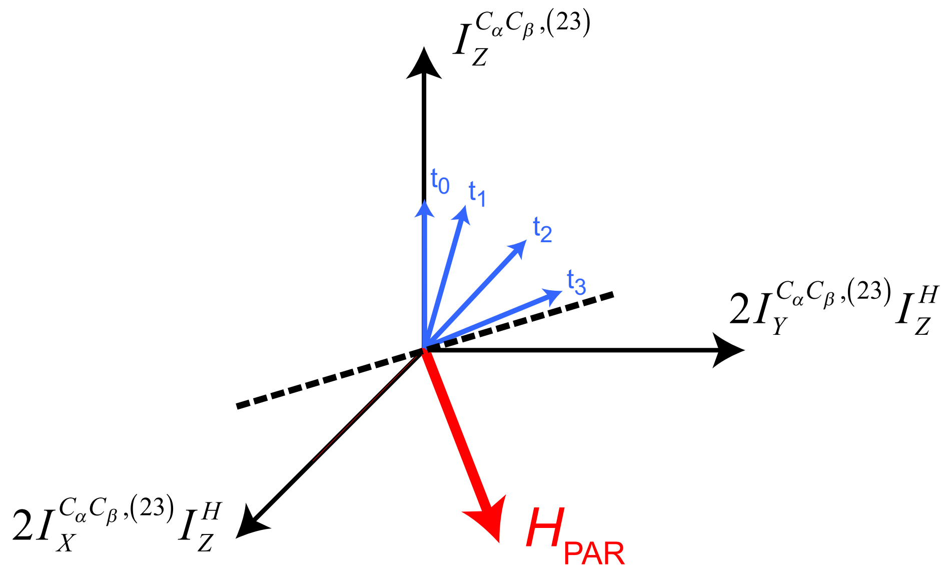
• PAR recoupling does not rely on CC coupling!

4. Long distance constraints

4.3 TSAR mechanism: the PAR case

4.3.1 Second order AHT TSAR term





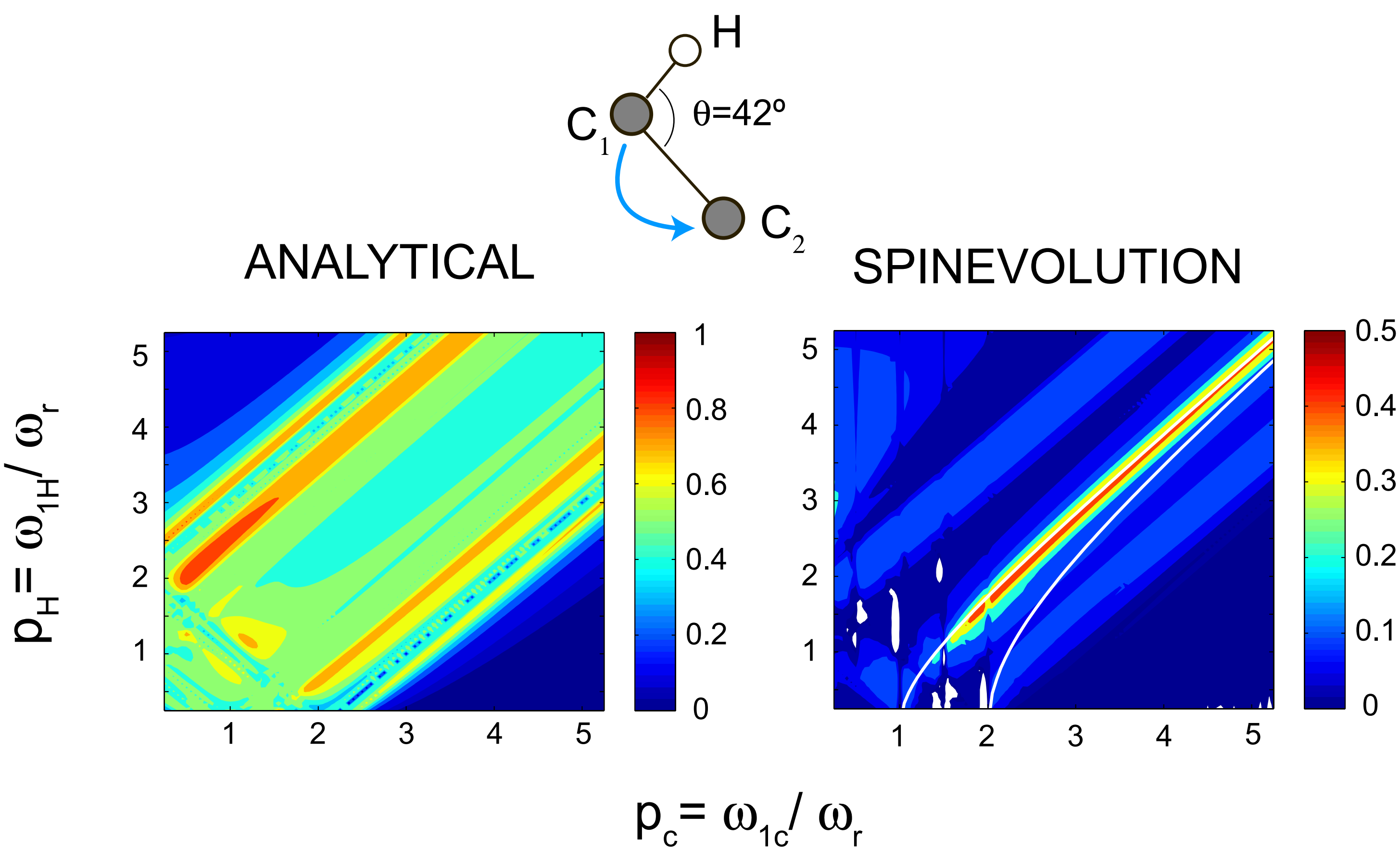
3-spin ZQ-PAR recoupling subspace

$$\bar{\tilde{\mathrm{H}}}_{\mathrm{PAR}}^{(2)} = \mathrm{Re}(\lambda)\, 2I_X^{C_\alpha C_\beta,(23)} I_Z^H + \mathrm{Im}(\lambda)\, 2I_Y^{C_\alpha C_\beta,(23)} I_Z^H$$

4. Long distance constraints

4.3 TSAR mechanism: the PAR case

4.3.2 Analytical versus numerical simulations - TSAR term

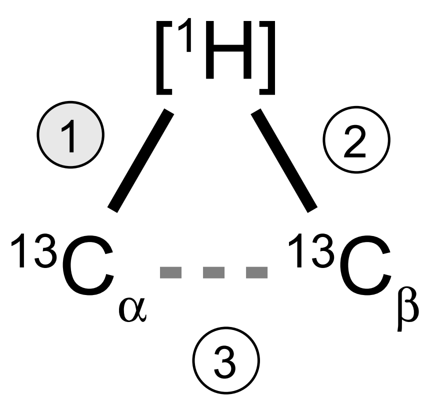


• Second TSAR term alone is not able to explain numerical simulations!

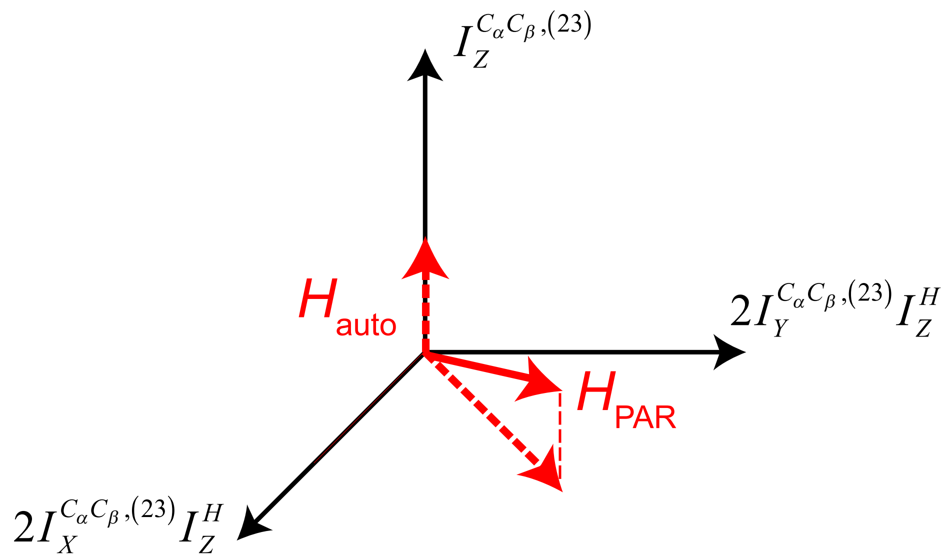
4. Long distance constraints

4.3 TSAR mechanism: the PAR case

4.3.3 Second order AHT including auto cross-term



3-spin ZQ-PAHR recoupling subspace

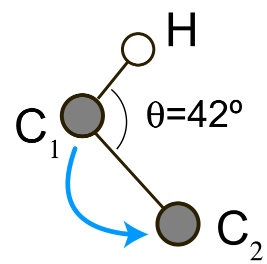


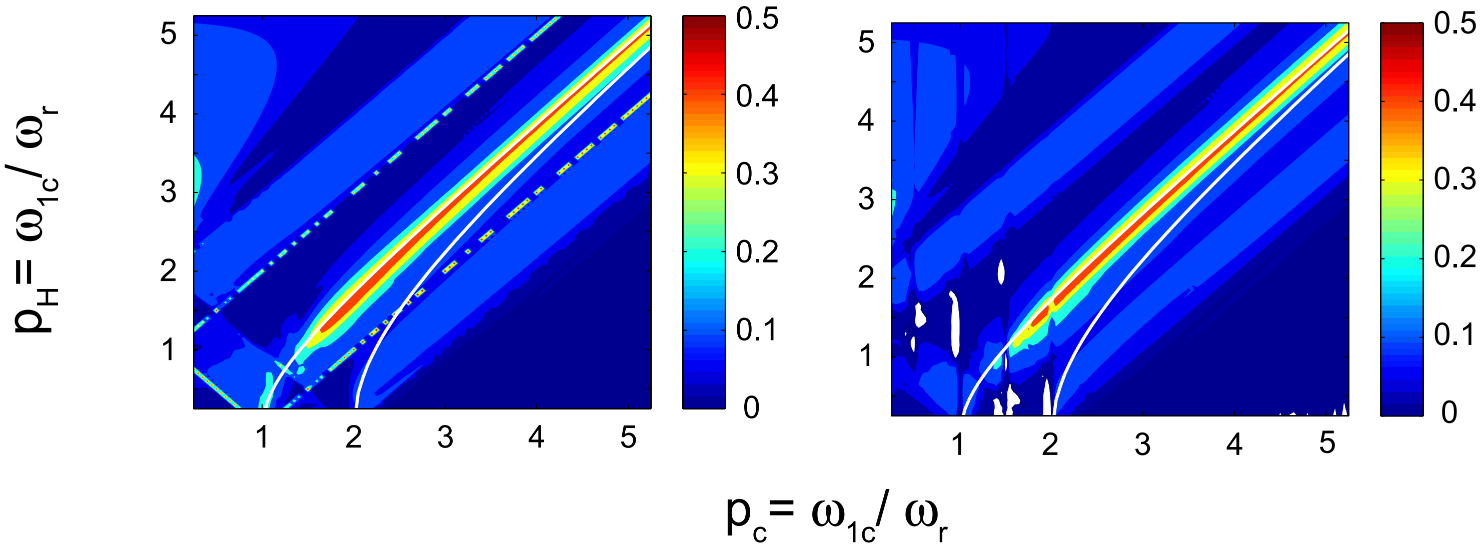
$$\bar{\tilde{\mathrm{H}}}^{(2)}_{\text{auto}} = \frac{1}{2\omega_r}\left[\omega^{1}_{C_\alpha H}\;\omega^{-1}_{HC_\alpha}\,\chi\left(1,p_C,p_H\right)+\omega^{2}_{C_\alpha H}\;\omega^{-2}_{HC_\alpha}\,\chi\left(2,p_C,p_H\right)\right]\left(\frac{T_{10}^{C_\alpha}-T_{10}^{C_\beta}}{2}\right)$$

4. Long distance constraints

4.3 TSAR mechanism: the PAR case

4.3.4 Analytical versus numerical simulations



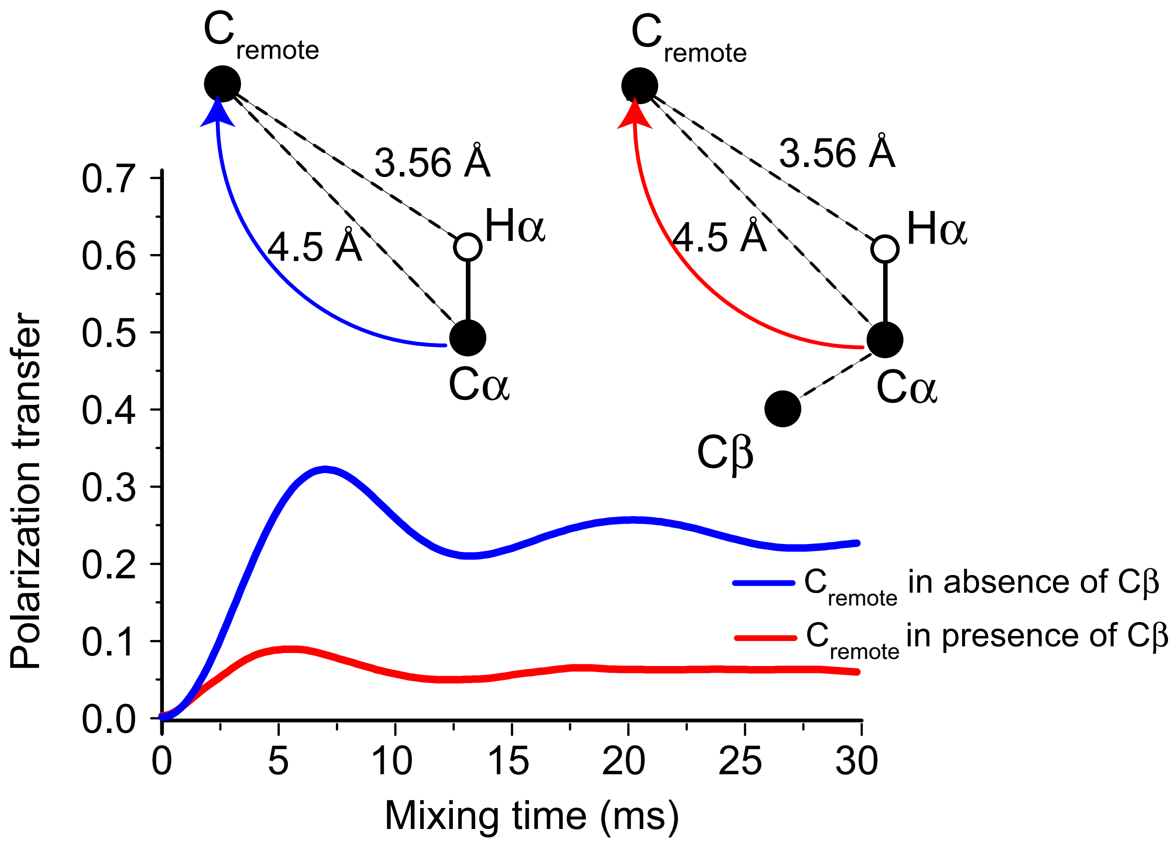


• Second order AHT explains the numerical maps!

4. Long distance constraints

4.4 Long distance transfer

4.4.2 PAR versus dipolar truncation



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

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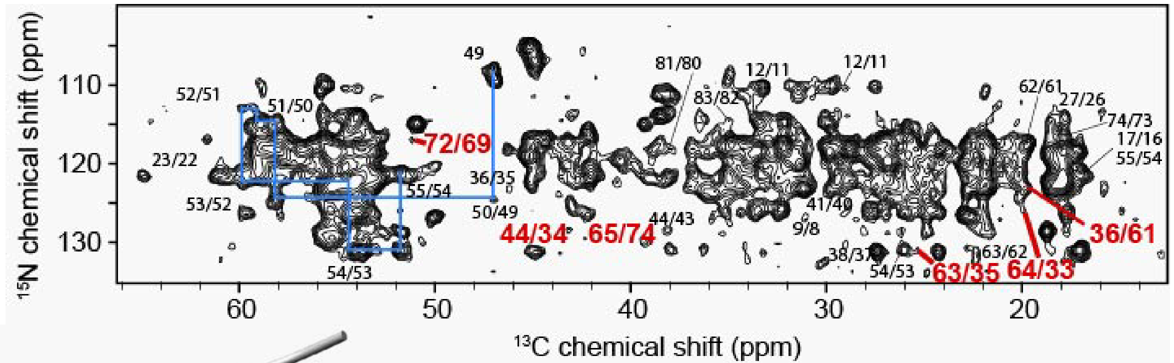
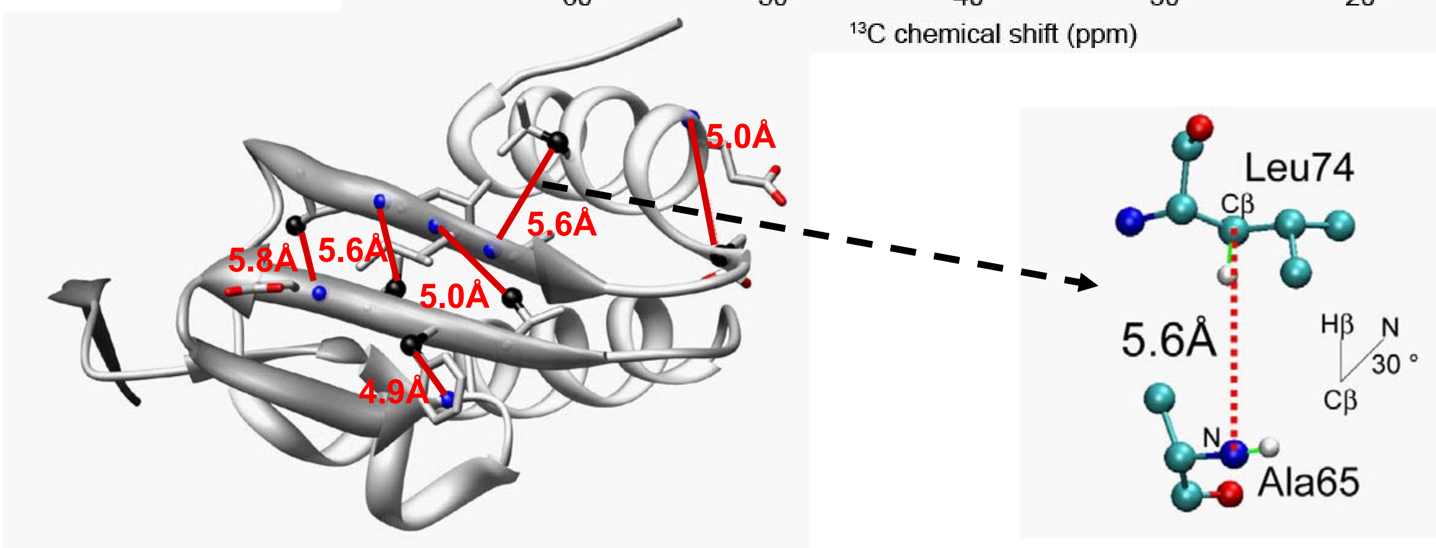
5. New methods for protein structure determination: Crh 2x10.4 kDa

5.1 High field (750/900 MHz) Crh spectra

5.5.1 PAIN-CP

PAIN-CP:
10 ms
13C rf - 15 kHz
15N rf - 15 kHz
1kHz ramp
1H rf – 53 kHz



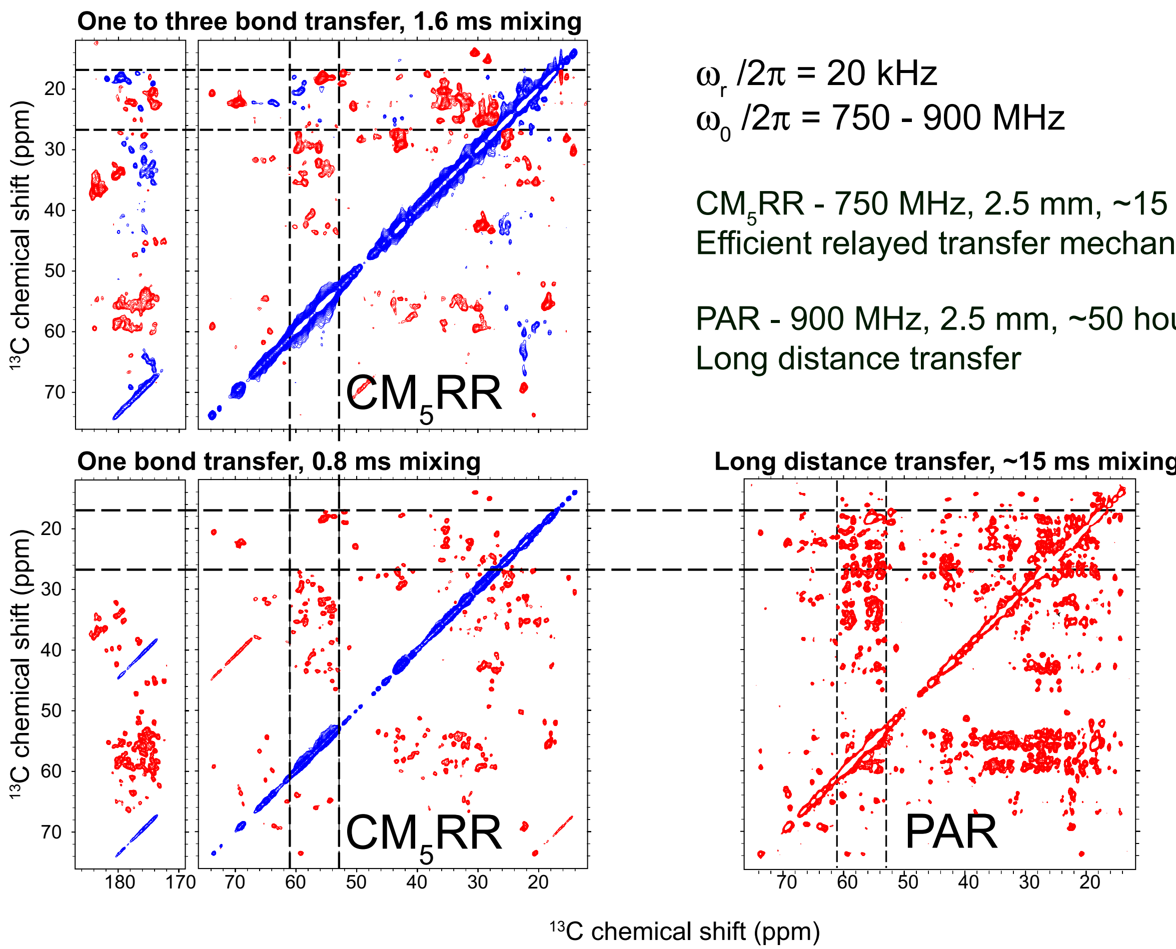


• >5Å ^{15}N-^{13}C contacts between secondary structure elements

5. New methods for protein structure determination: Crh 2x10.4 kDa

5.1 High field (750/900 MHz) Crh spectra

5.1.2 CMRR and PAR



$\omega_r/2\pi$ = 20 kHz
$\omega_0/2\pi$ = 750 - 900 MHz

CM$_5$RR - 750 MHz, 2.5 mm, ~15 hours
Efficient relayed transfer mechanism

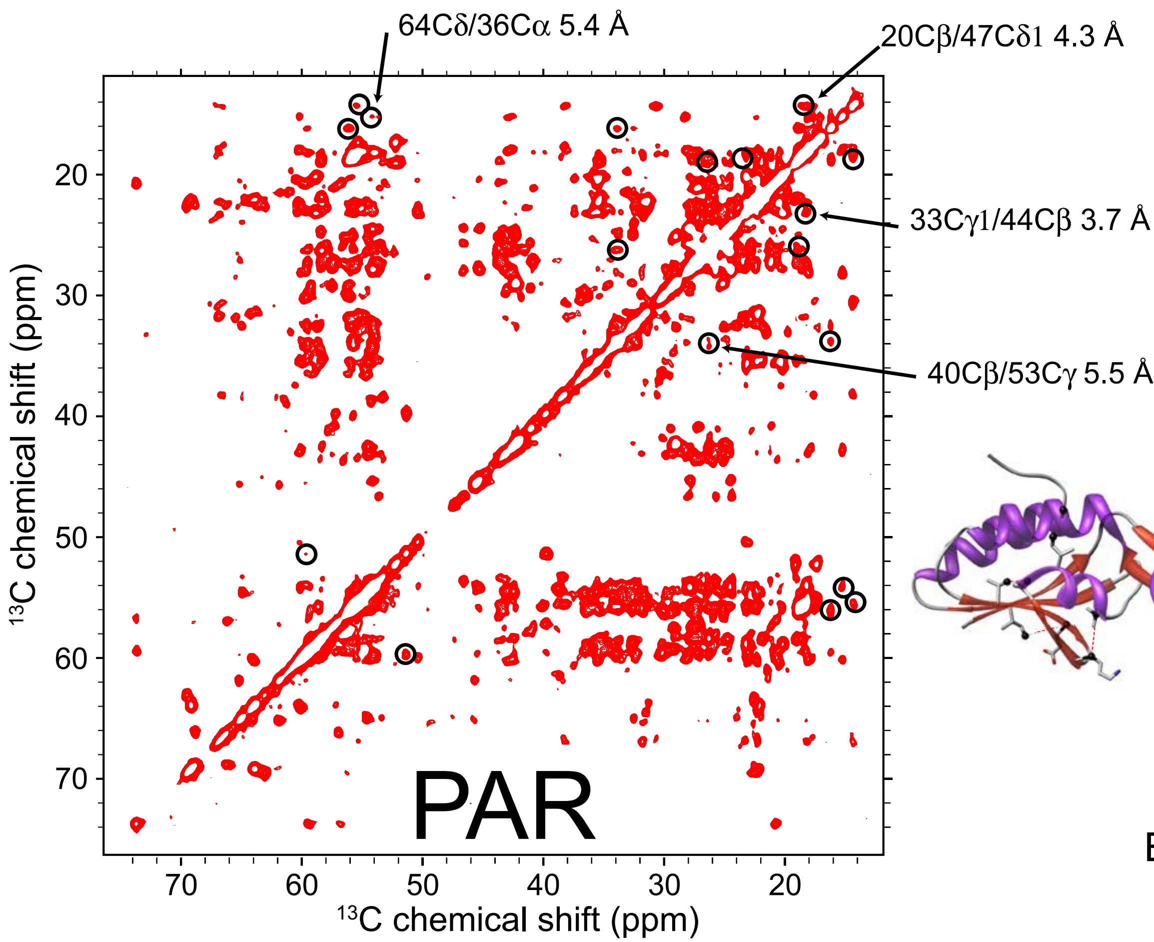
PAR - 900 MHz, 2.5 mm, ~50 hours
Long distance transfer

- one to three bond ^{13}C-^{13}C relayed transfer in the CMRR spectrum
- >5Å ^{13}C-^{13}C contacts in the PAR spectrum!

5. New methods for protein structure determination: Crh 2x10.4 kDa

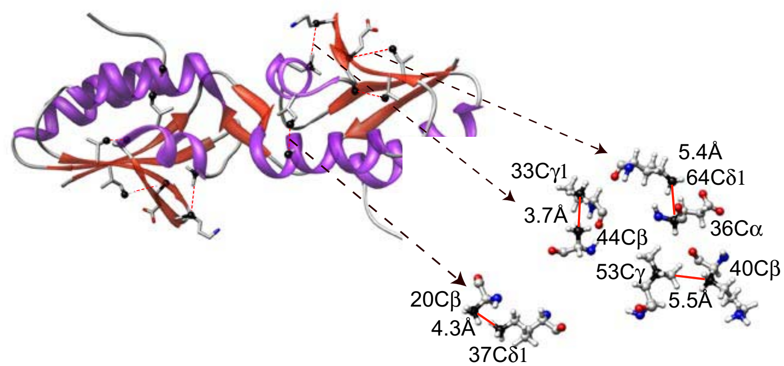
5.1 High field (750/900 MHz) Crh spectra

5.5.2 PAR



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**

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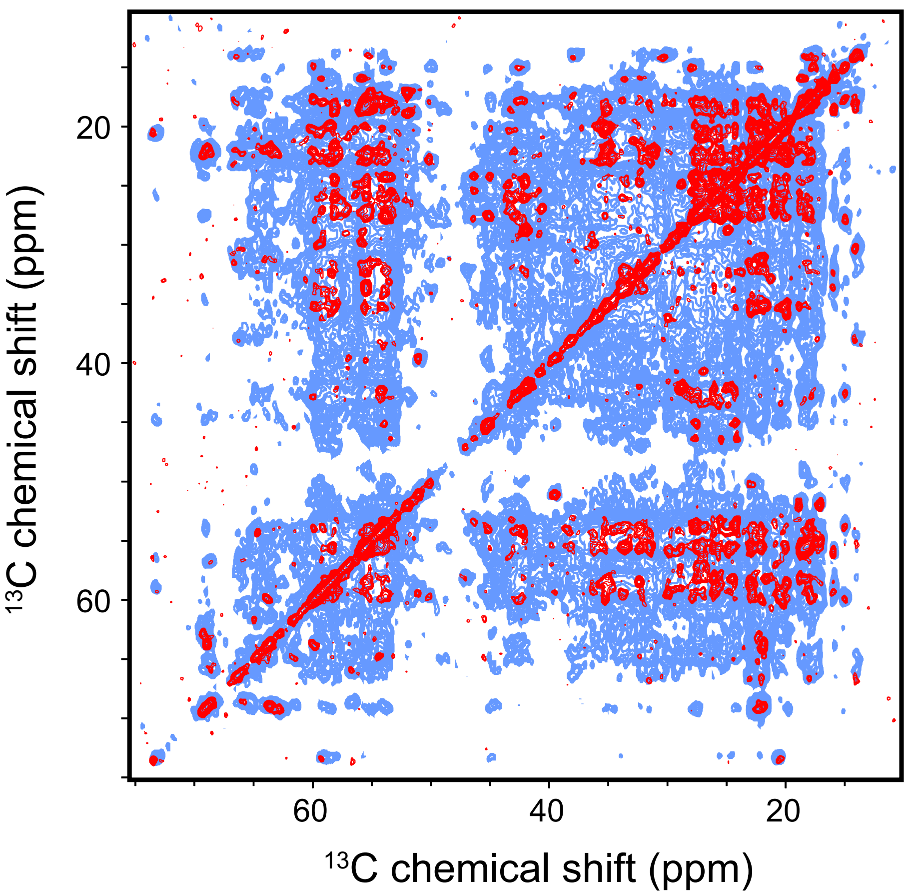
5. New methods for protein structure determination: Crh 2x10.4 kDa

5.2 Alternative to available techniques

5.2.1 DARR versus PAR



DARR 500 MHz
200 ms irradiation
$\omega_r/2\pi$ = 10 kHz

PAR 750 MHz
9 ms irradiation
$\omega_r/2\pi$ = 20 kHz
ω_{1c} ~ 50 kHz
ω_{1h} ~ 50 kHz

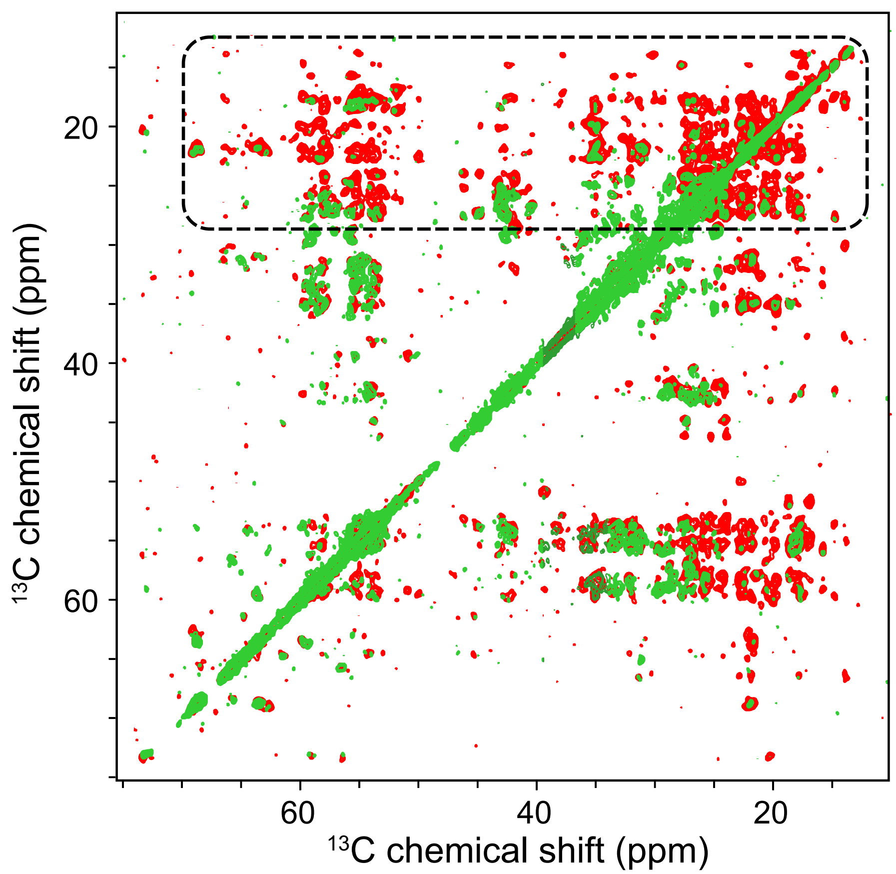
• DARR spectra: crowded at long mixing times:
difficult to extract sufficient number of unambiguous restraints in uniformly labeled systems!

Marulanda *et al.*, JPC B (2005) 109.

5. New methods for protein structure determination: Crh 2x10.4 kDa

5.2 Alternative to available techniques

5.2.2 CHHC versus PAR



CHHC 500 MHz
4 mm (20 mg), ~46 h
150 µs irradiation
$\omega_r/2\pi$ = 10 kHz

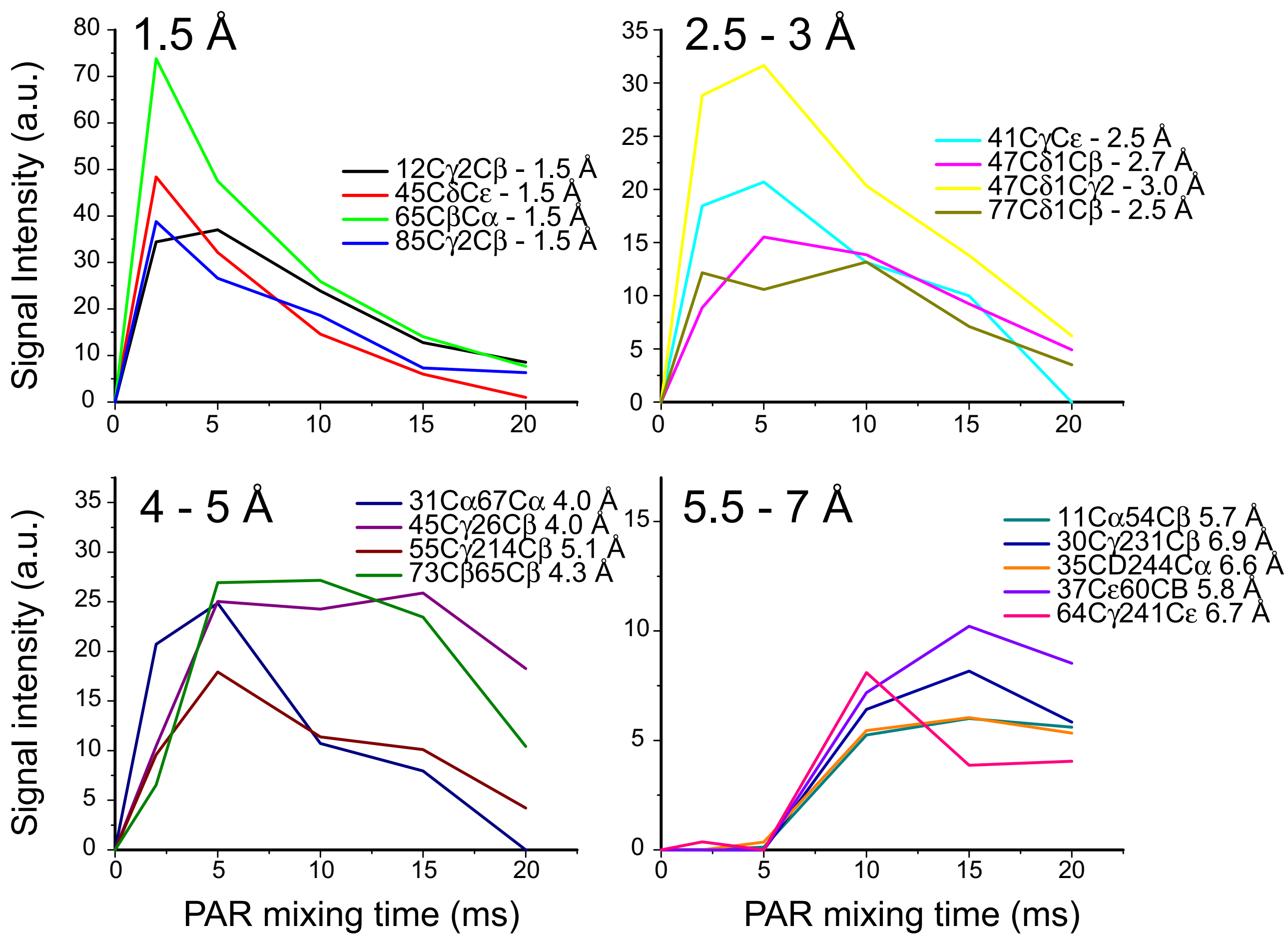
PAR 750 MHz
2.5 mm (6 mg), ~18 h
9 ms irradiation
$\omega_r/2\pi$ = 20 kHz
ω_{1c} ~ 50 kHz
ω_{1h} ~ 50 kHz

- CHHC and PAR yield similar information except for CH3 groups!
- PAR has better sensitivity than CHHC

5. New methods for protein structure determination: Crh 2x10.4 kDa

5.3 Structure detremination by SSNMR

5.3.2 *de novo* 3D structure determination of the Crh dimer



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

- Observed buildups can be categorized in different distance classes
- Analogy with NOE in solution NMR

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5. New methods for protein structure determination: Crh 2x10.4 kDa

5.3 Structure detremination by SSNMR

5.3.2 *de novo* 3D structure determination of the Crh dimer

Crh assignment

+

TALOS

+

4 Chemical shifts lists

↑

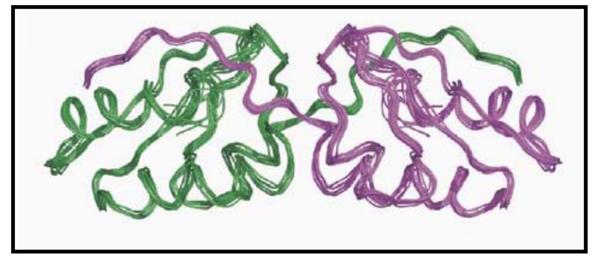
A: CP in PAR 2ms only
B: CP in PAR 20 ms only
C: CP in PAR 2 & 20 ms
D: CP in PAINCP 15 ms

ARIA →

795 unambiguous C-C constraints (269 long-range)

400 unambiguous N-C constraints (130 long-range)

CNS →



RMSD 0.63 Å

Less than 6 days of spectrometer time!

Conclusions ...

New methodologies for high B_0 and high MAS structural studies:

• First order ^{13}C-^{13}C recoupling without decoupling

- DQ CMRR: efficient relayed transfer mechanism (10-30 kHz MAS)
- ZQ RFDR: efficient mechanism at high MAS (> 30 kHz MAS)

• New recoupling mechanism: TSAR (Third Spin Assisted Recoupling)

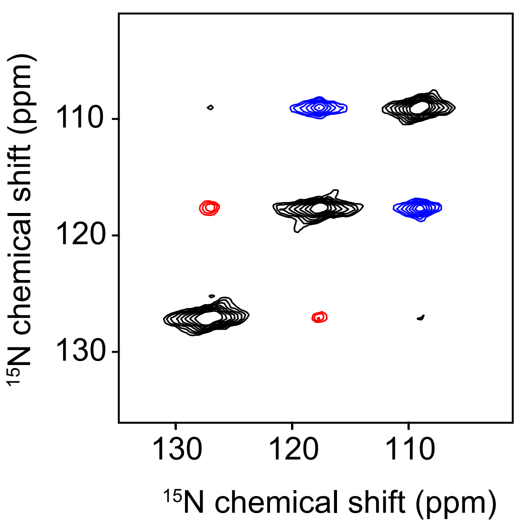
  • New pulse sequences: PAR (homonuclear) and PAINCP (heteronuclear)
  • Reduced dipolar truncation
  • Efficient for short, medium, and long range transfer in uniformly labeled systems

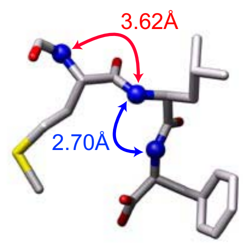
→ *de novo* structure determination of uniformly labeled protein (Crh, 2 x 85 res.)

... and perspectives

- Application of the TSAR mechanism for ^{15}N-[^{1}H]-^{15}N correlation experiments
- Exploit the TSAR mechanism at higher MAS frequencies (> 30 kHz)

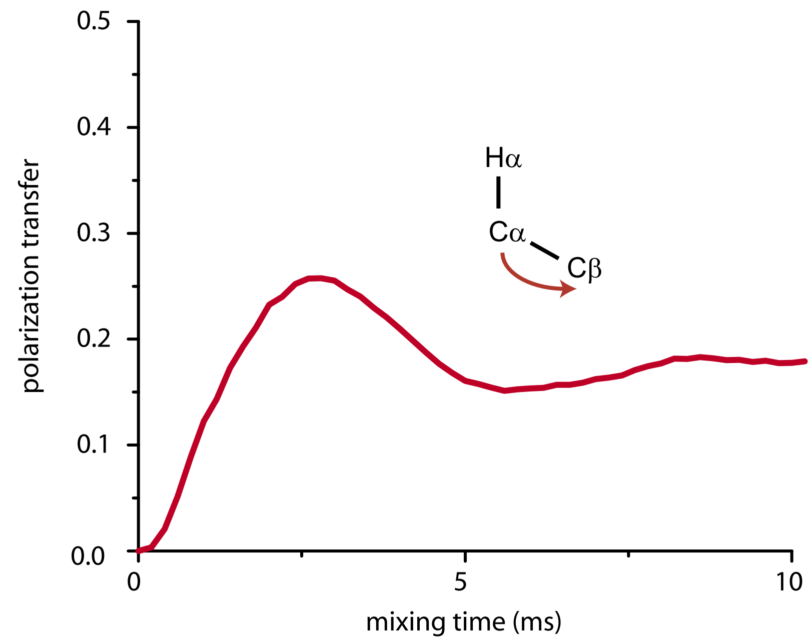
^{15}N-[^{1}H]-^{15}N PAR





[U-^{13}C,^{15}N]-N-*f*-MLF
30 kHz MAS
750 MHz B_0
^{15}N r.f. 3kHz, ^{1}H r.f. 44 kHz
20 ms mixing

Very high MAS (60 kHz) PAR recoupling



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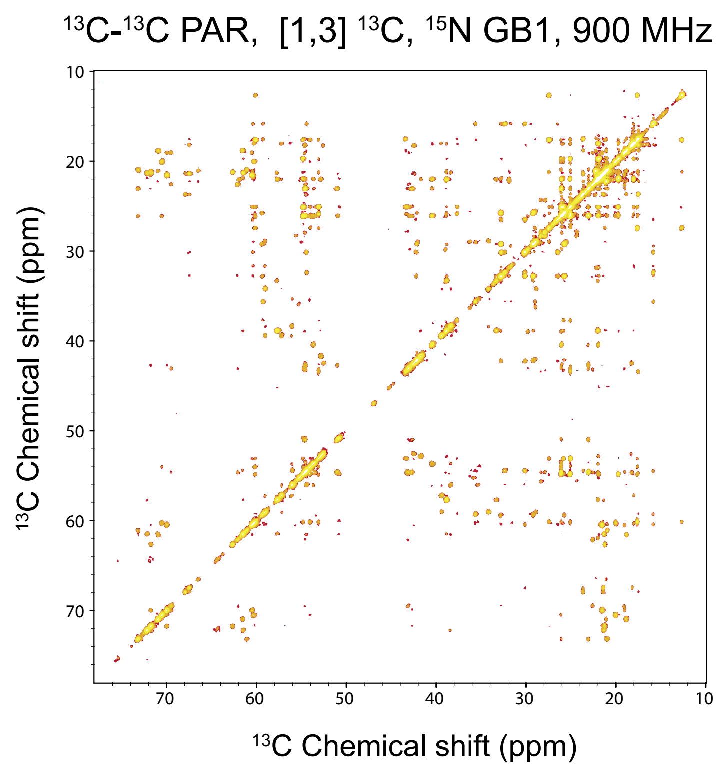
... and perspectives

• Application of the TSAR mechanism on selectively labeled systems

in order to study larger biomolecular systems



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Dr. Anja Böckmann

Massachusetts Institute of Technology

Institut de Biologie et Chimie des Protéines

Department of Health & Human Services
NATIONAL INSTITUTES OF HEALTH
National Institute of Biomedical Imaging and Bioengineering