Carbon-detection in biomolecular NMR: techniques and applications

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Literature: ^{13}C detected 2D/3D bio-NMR

Early publications

DQC	*	B.H. Oh, W.M. Westler, P. Darba & J.L. Markley, Science 240, 908-910 (1988)
HCC-TOCSY		Z. Serber et al., J. Am. Chem. Soc. 122, 3554-3555 (2000) Z. Serber, C. Richter & V. Dötsch, ChemBioChem 2, 247-251 (2001)
COSY	*	I. Bertini, Y.-M. Lee, C. Luchinat, M. Piccioli & L. Poggi, ChemBioChem. 2, 550-558 (2001)
ct-COSY	*	T.E. Machonkin, W.M. Westler & J.L. Markley, J. Am. Chem. Soc. 124, 3204-3205 (2002)
mq-CaCO **mq-CON**	*	M. Kostic, S.S. Pochapsky & T.C. Pochapsky, J.Am. Chem. Soc. 124, 9054-9055 (2002)
TOCSY	+	A. Eletsky, O. Moreira, H. Kovacs & K. Pervushin, J. Biomol. NMR 26, 167-179 (2003)

* paramagnetic protein + paramagnetic relaxation agent

Motivation for ^{13}C-detection

- shorter pulse sequences, less relaxation
- high chemical shift dispersion
- detection of non-protonated carbons
- But: Isn‘t the sensitivity of ^{13}C too small?

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- high chemical shift dispersion
- detection of non-protonated carbons
- But: Isn't the sensitivity of ^{13}C too small?

  Answer: not necessarily
- ^{13}C direct detection is a complementary tool

When do we need ^{13}C-Nuclei detection?

(1) When we have low resolution for ^{1}H

- (partially) unfolded proteins

(2) When we face problems with ^{1}H linewidth (relaxation)

- High Molecular Weight (perhaps)
- Exchange of NH (or Proline residues!)
- Paramagnetism: Paramagnetic relaxation rate enhancements

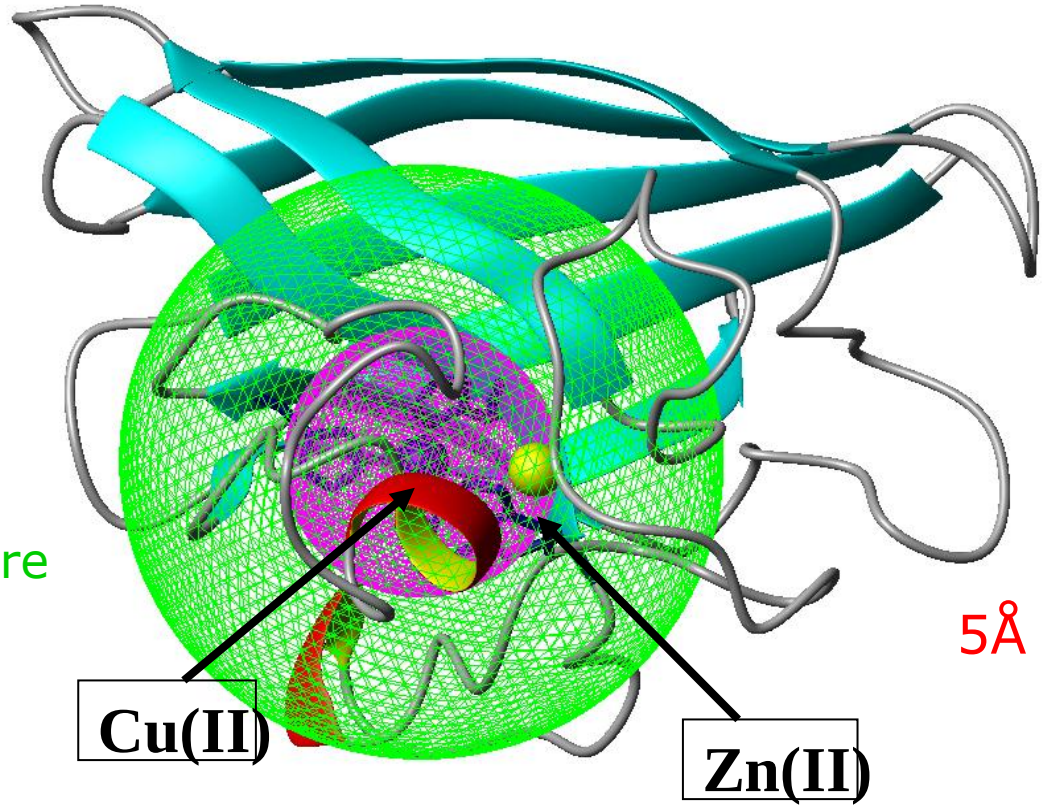
$$R^{I}_{(1,2,Curie)} \propto \gamma_I^2 \cdot \gamma_S^2 \cdot f(\tau_c, \omega)$$

$$(\gamma_{13C})^2 \sim (\gamma_{1H})^2/16$$

Protonless High Resolution Bio-NMR

Why no protons?

Visible in
^{1}H and ^{13}C



11Å radius sphere
Visible in ^{13}C

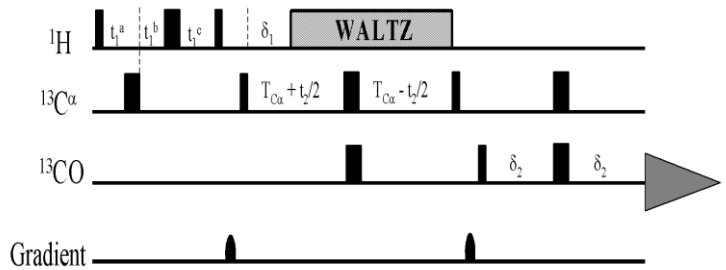
5Å radius sphere
Blind zone

Bermel, Bertini, Felli, Kümmerle, Pierattelli, *JACS*, 2003

Details: pulse sequences

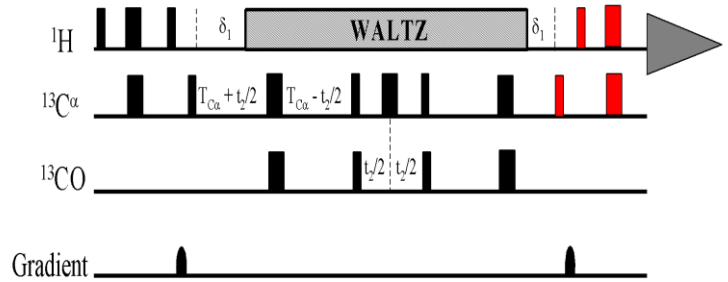
Dötsch et al. ***J. Am. Chem. Soc. 2000, 112, 3554.***

Sequence 1



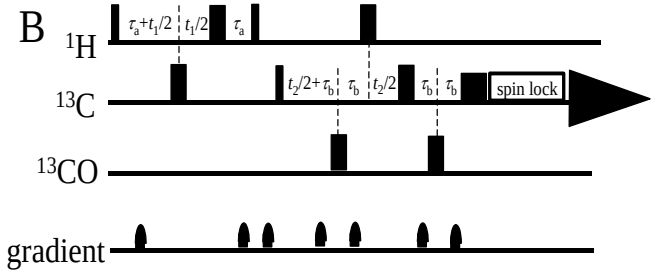
HCACO with CO-Detection

Sequence 2



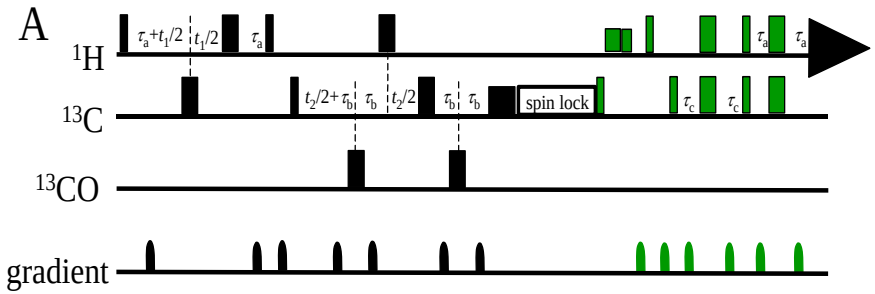
HCACO

B



HCC-TOCSY

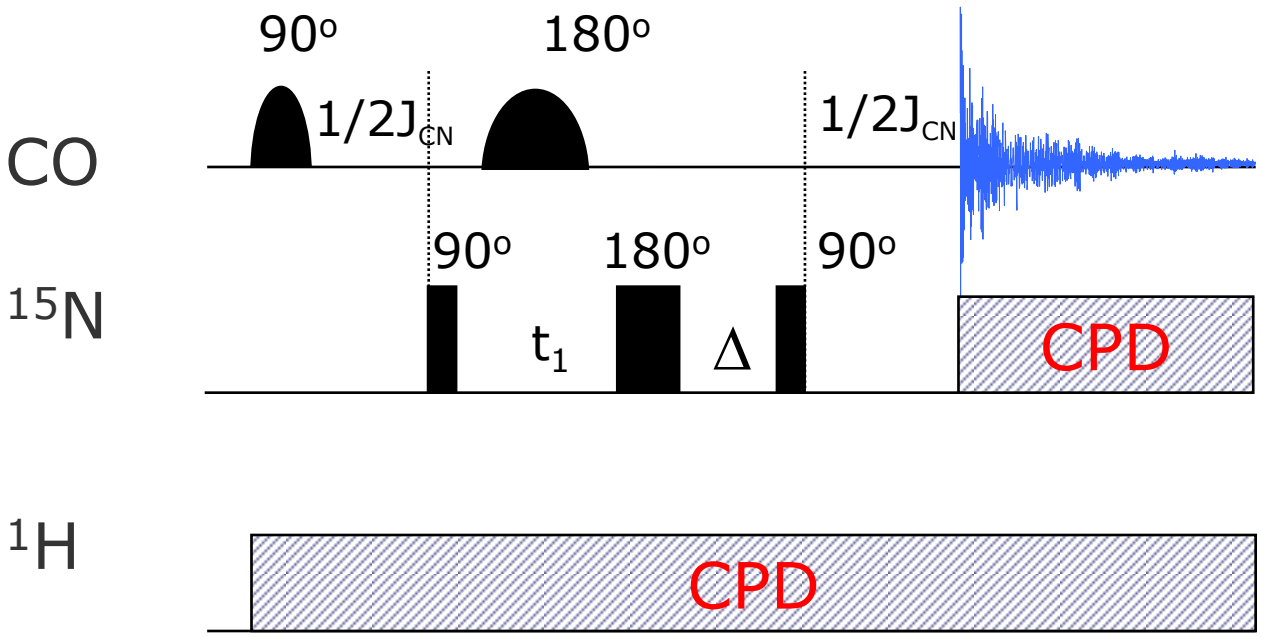
A



HCCH-TOCSY

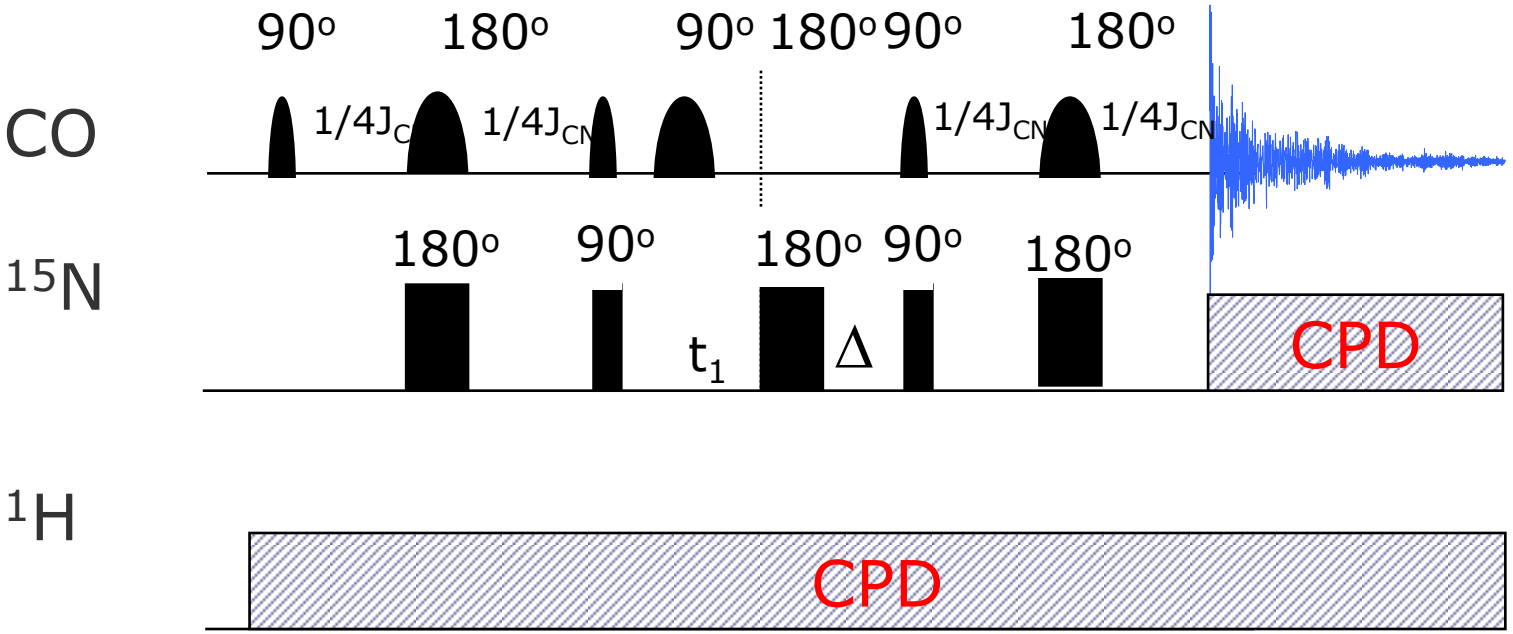
Details: pulse sequences

CON, multiple quantum ‚HMQC'



Details: pulse sequences

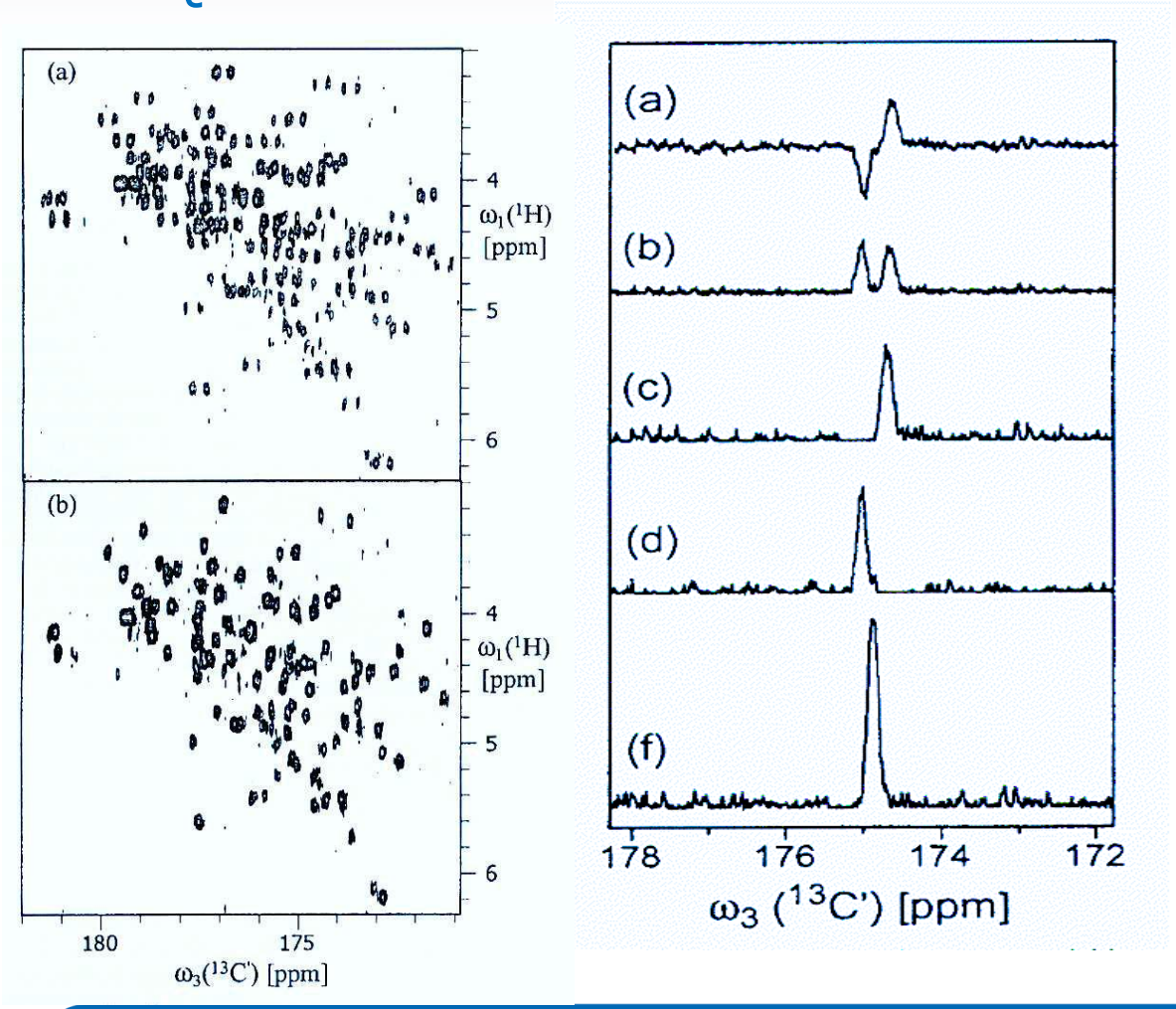
CON, single quantum ‚HSQC'



Details: pulse sequences

Virtual homonuclear decoupling: IPAP

MQ-HACACO with direct ^{13}C-detection

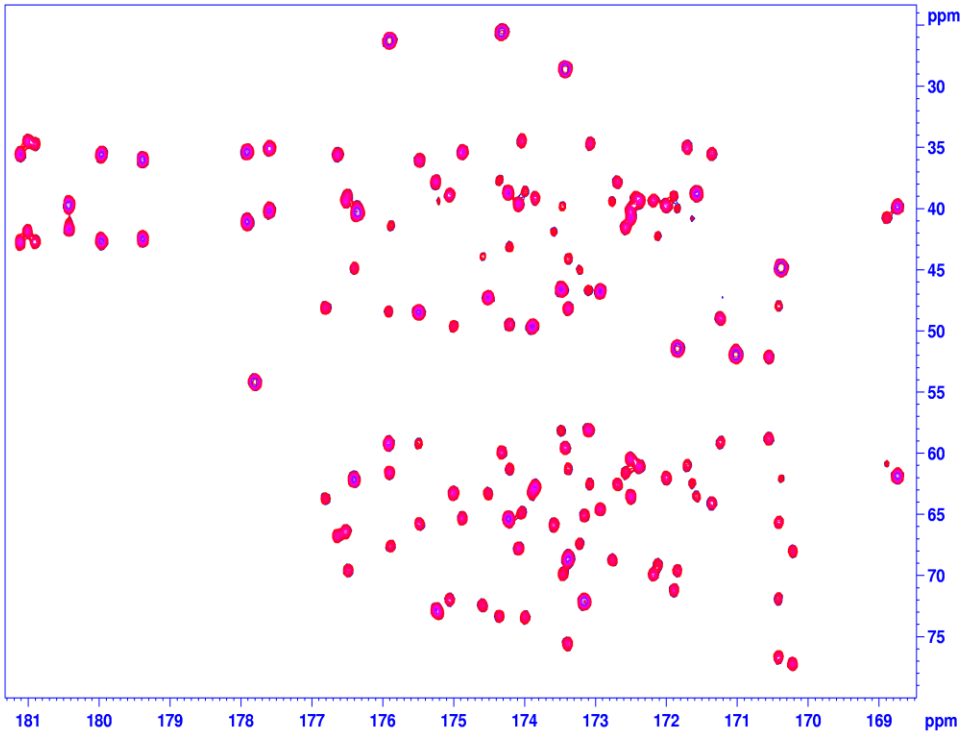


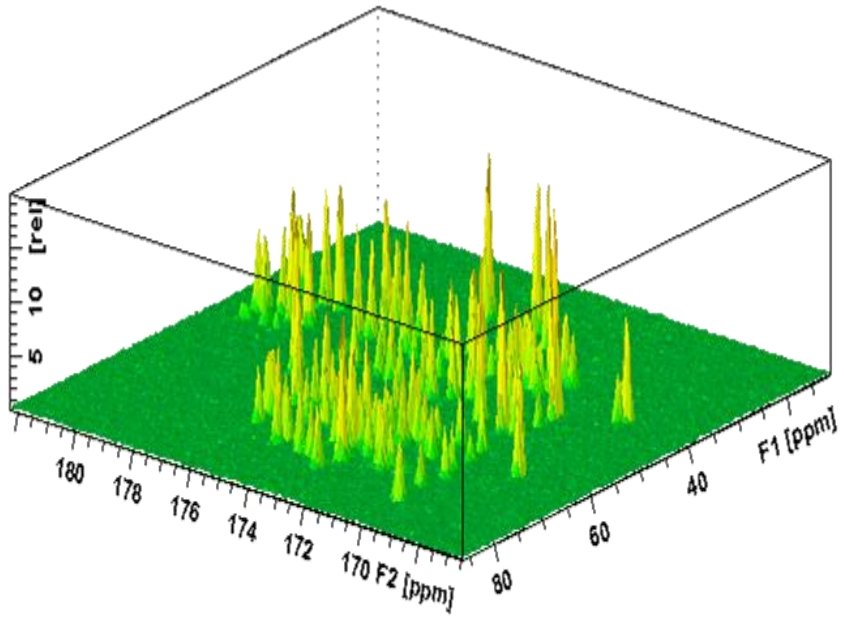
5mm TCI CryoProbe™ $^{1}H\{^{13}C, ^{15}N\}$ 800MHz

CBCACO IPAP spectrum of a 0.5 mM protein sample

CO, CαCβ projection of 3D

Experiment time: 8 h 30 min





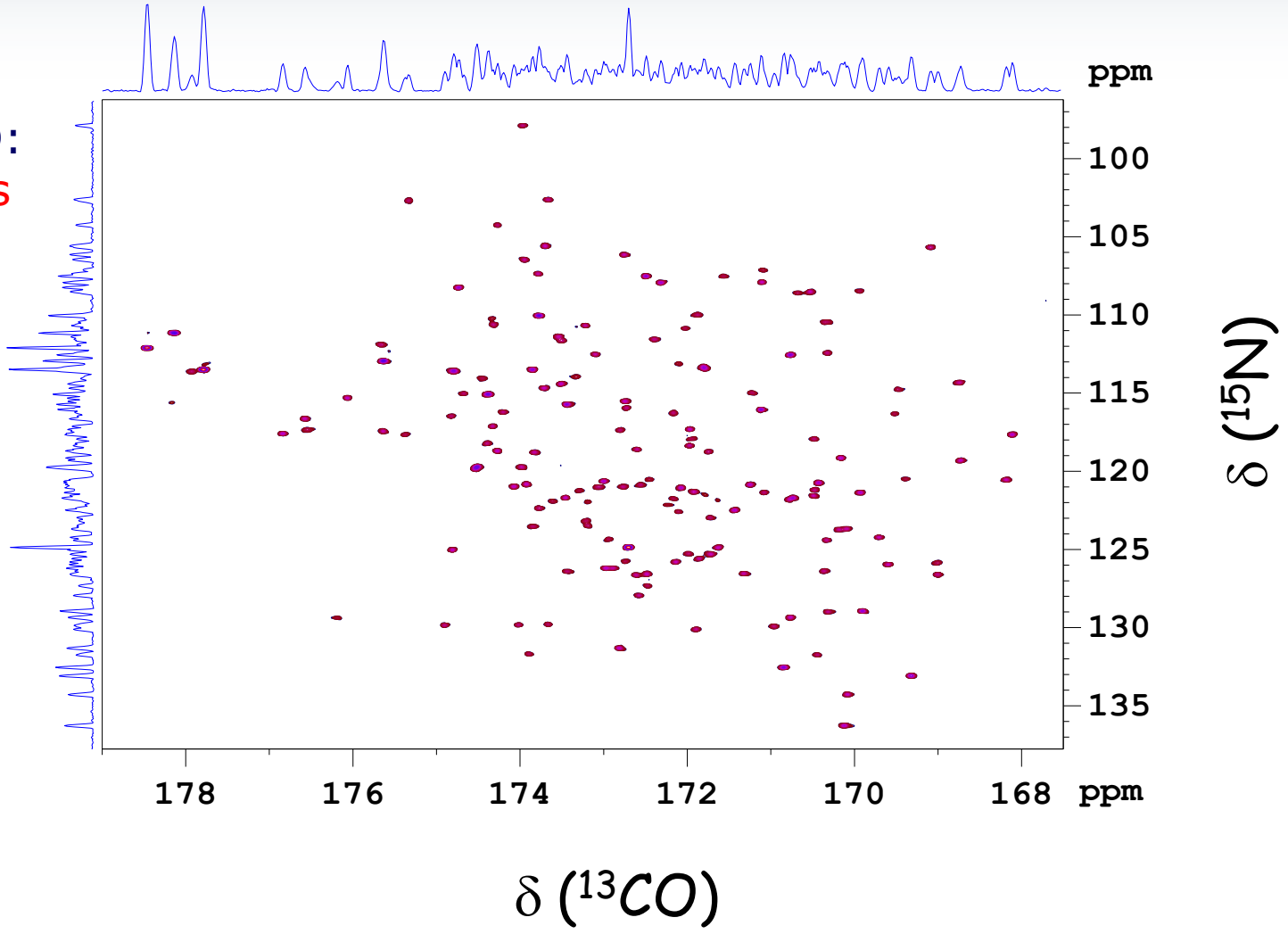
0.5 mM $^{13}C/^{15}N$ Chymotrypsin Inhibitor 2 (CI2) pH 4.2, 1% D2O

Courtesy by Flemming M. Poulsen

Protonless High Resolution Bio-NMR

^{13}C-^{15}N heteronuclear correlation spectrum

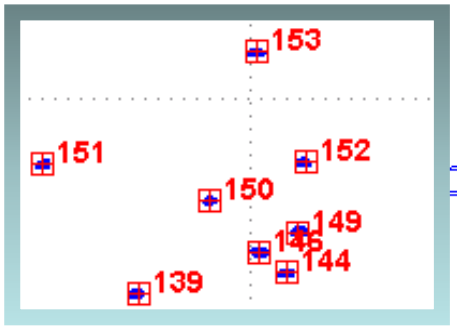
Monomeric SOD:
152 amino acids

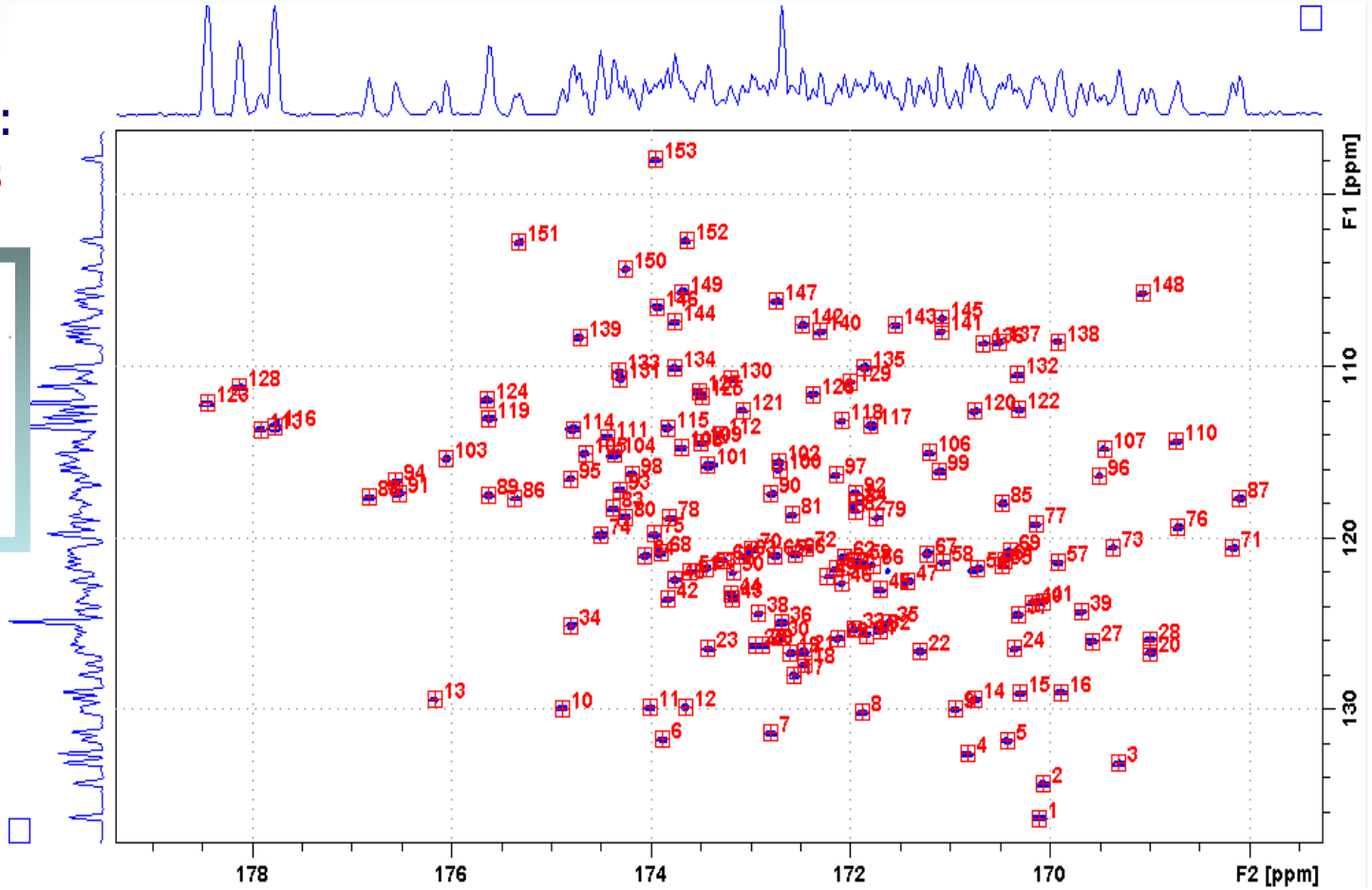


Protonless High Resolution Bio-NMR

^{13}C-^{15}N heteronuclear correlation spectrum

Monomeric SOD:
152 amino acids





Applications with ^{13}C-Detection

Reducing Experiment time

- Protonless?

  ^{1}H start – ^{13}C detect!

- Longitudinal relaxation enhancement

  HN-transfer: BEST approach

  HC-transfer: H-flip approach

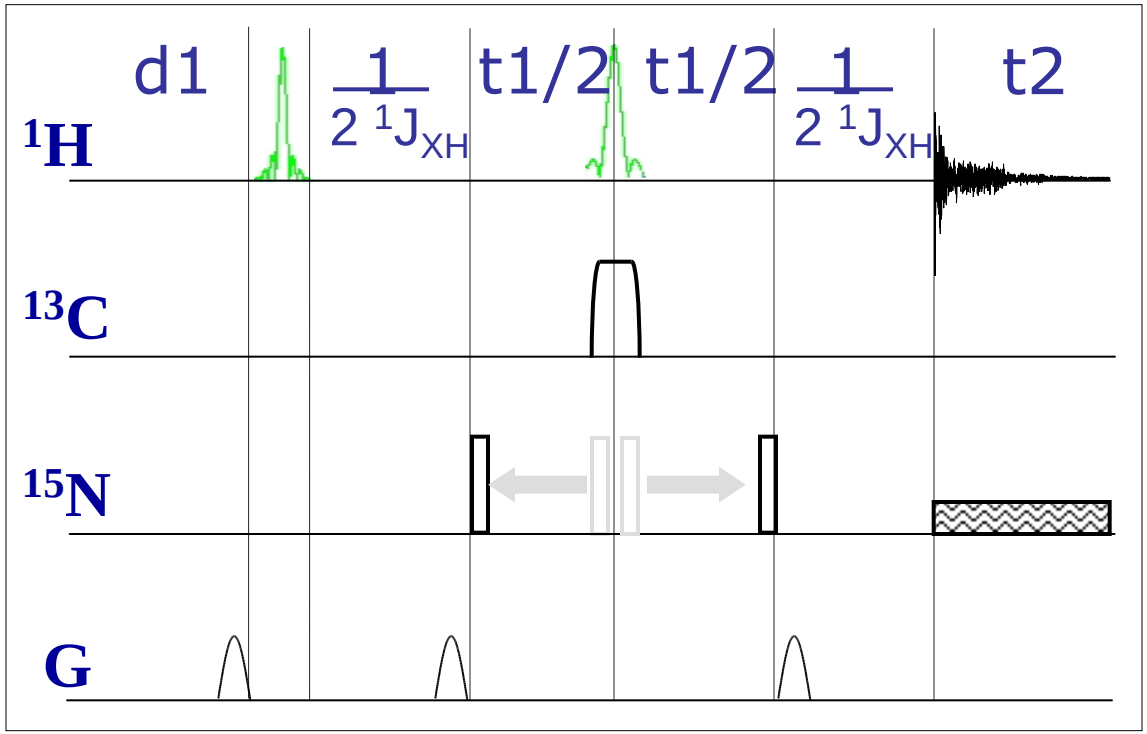
- Combine with other fast acquisition methods:

  Projection reconstruction, non-uniform sampling, …

^{13}C direct detection – speeding up

BEST approach

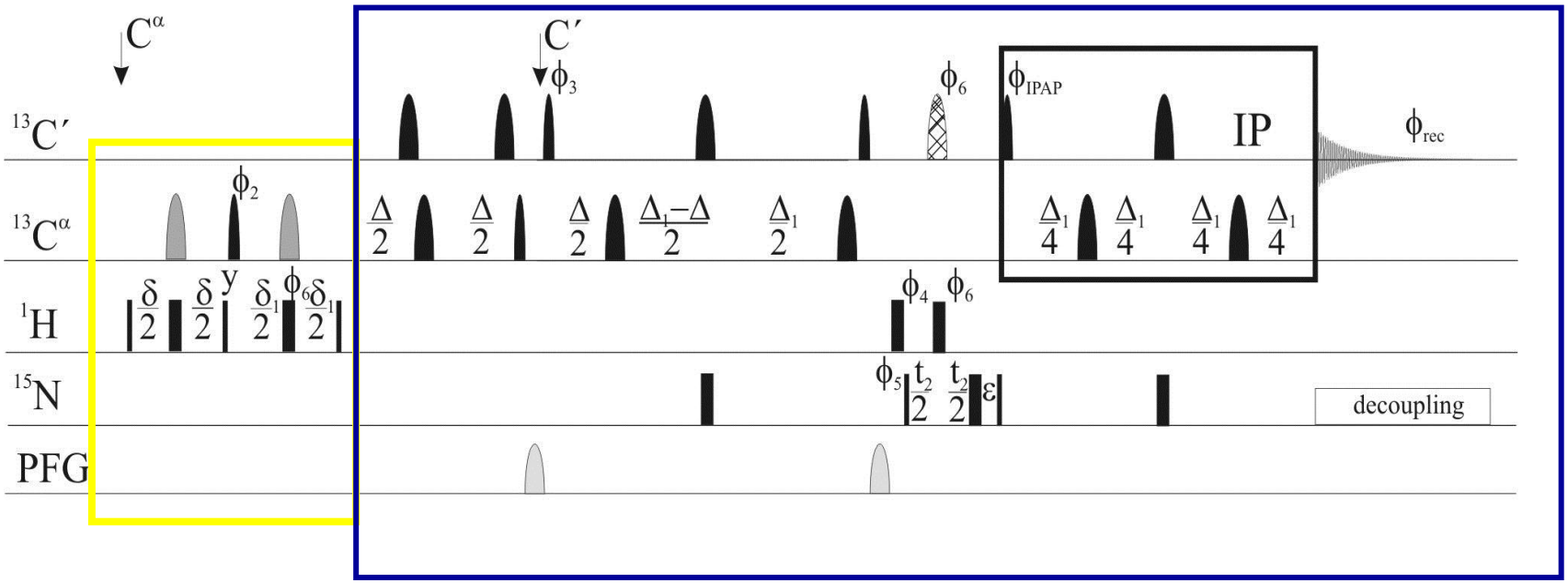
- Selective ^{1}H-excitation of NH protons only
  - Aliphatic protons act as pool for relaxation



^{13}C direct detection – speeding up

H-flip approach

- Non-selective ^{1}H-excitation of all protons.
- Selectively flip-back protons not required to Z-axis
  - ➢ pool for relaxation



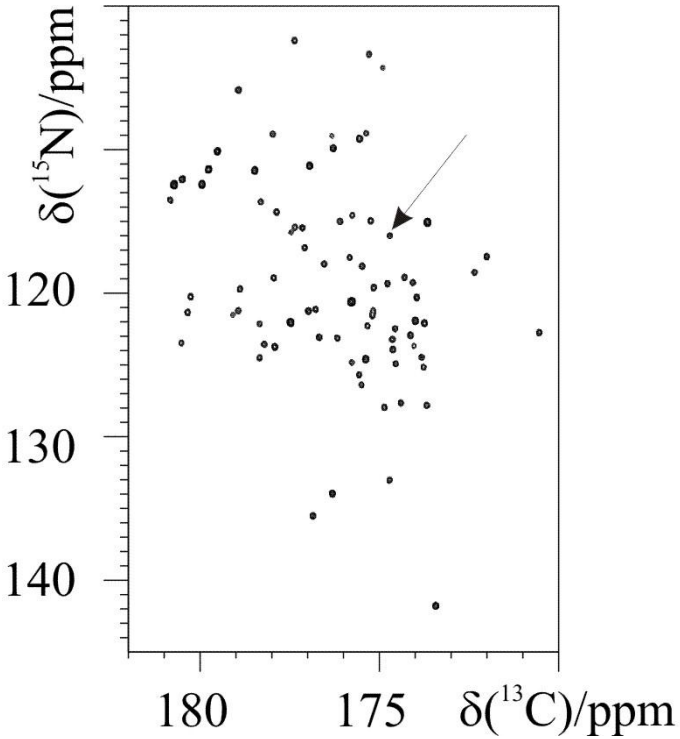
^{13}C direct detection – speeding up

Definitions

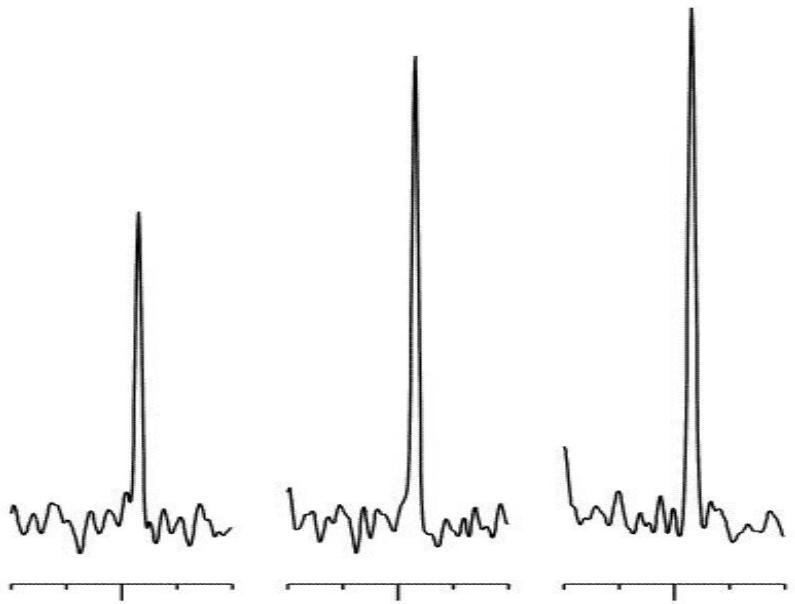
Term	initial excitation
^{13}C-start	^{13}C
$^{1}H^{\alpha}$-start	^{1}H non-selective
$^{1}H^{\alpha}$-flip	$^{1}H^{\alpha}$ selective

^{13}C direct detection – speeding up

(H-flip)CON-IPAP



^{13}C-start ^{1}H$^{\alpha}$-start ^{1}H$^{\alpha}$-flip



2.5 s 1.5s 1.5s

Bermel, W., Bertini I., Felli I.C., Pierattelli, R., *J. Am. Chem. Soc.*, **2009**, *131*, 15339-15345

Felli I.C., Brutscher B., ChemPhysChem **2009**, *10*, 1356-1368

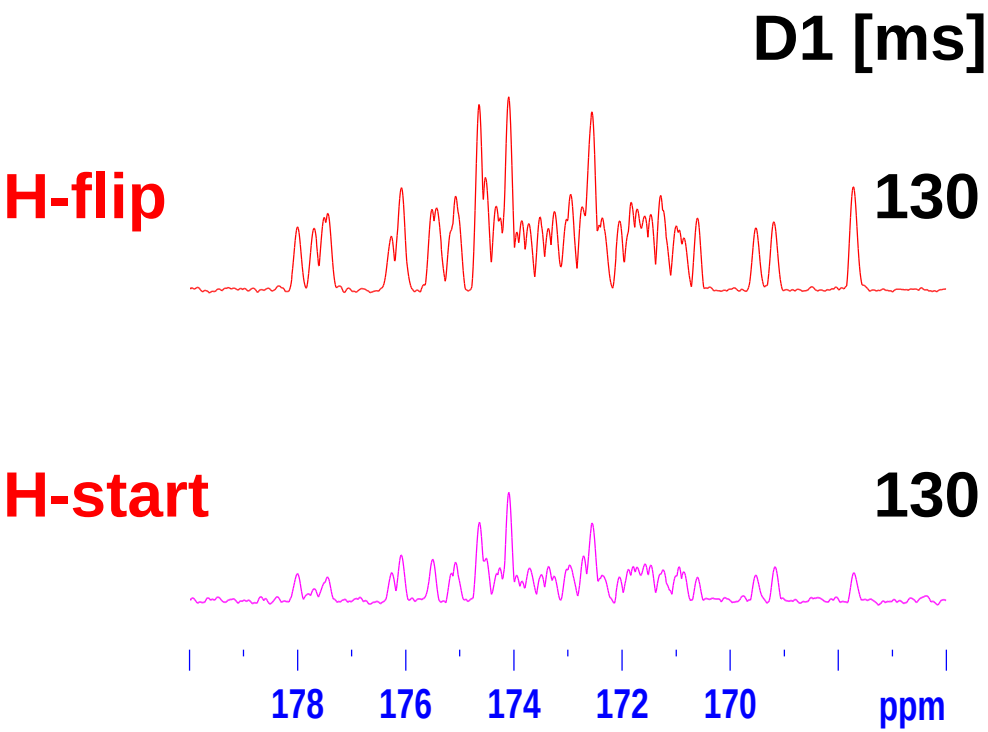
Relaxation-optimized CT-(H)CACO-IPAP

CT-(H)CACO IPAP

ns = 1
td 512 x 360

C/N labeled 1mM ubiquitin

700 MHz CP-TXO



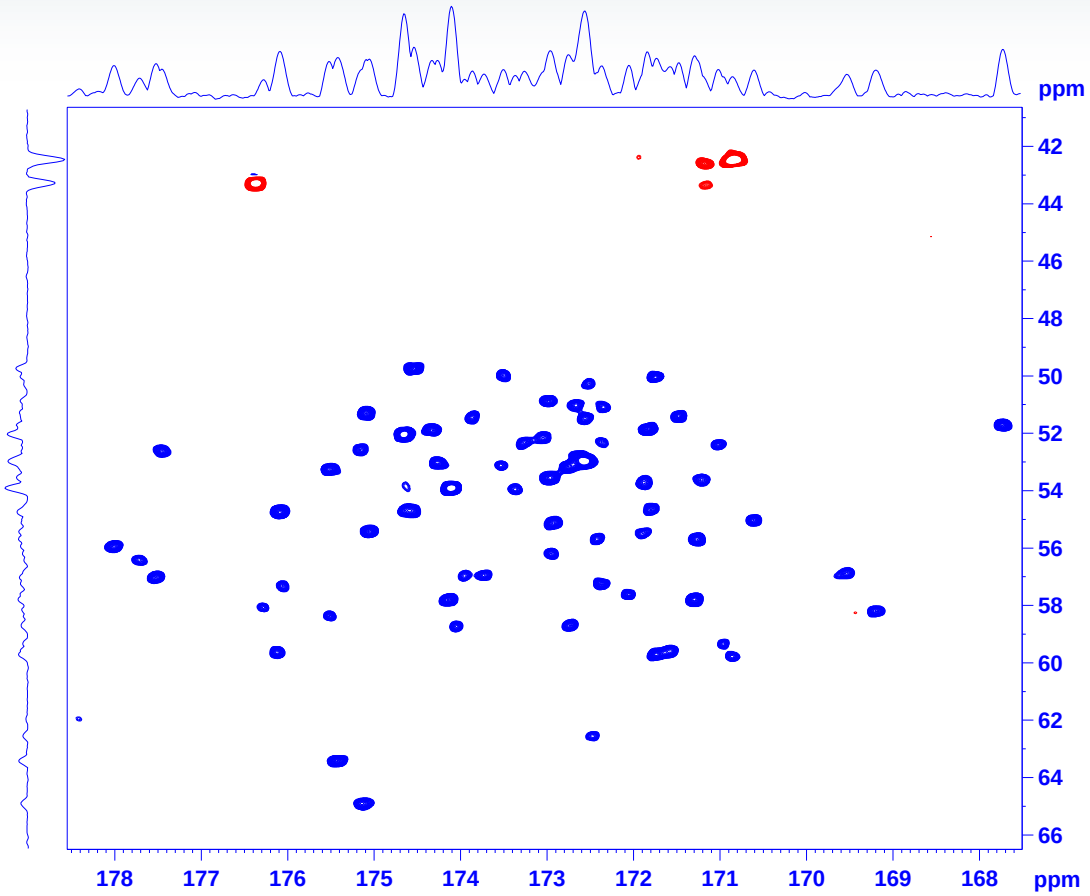
Relaxation-optimized CT-(H)CAC)-IPAP

CT-(H)CACO IPAP

ns = 1
td 512 x 360
D1=1ms
exp time: 55sec!

C/N labeled 1mM ubiquitin

700 MHz CP-TXO



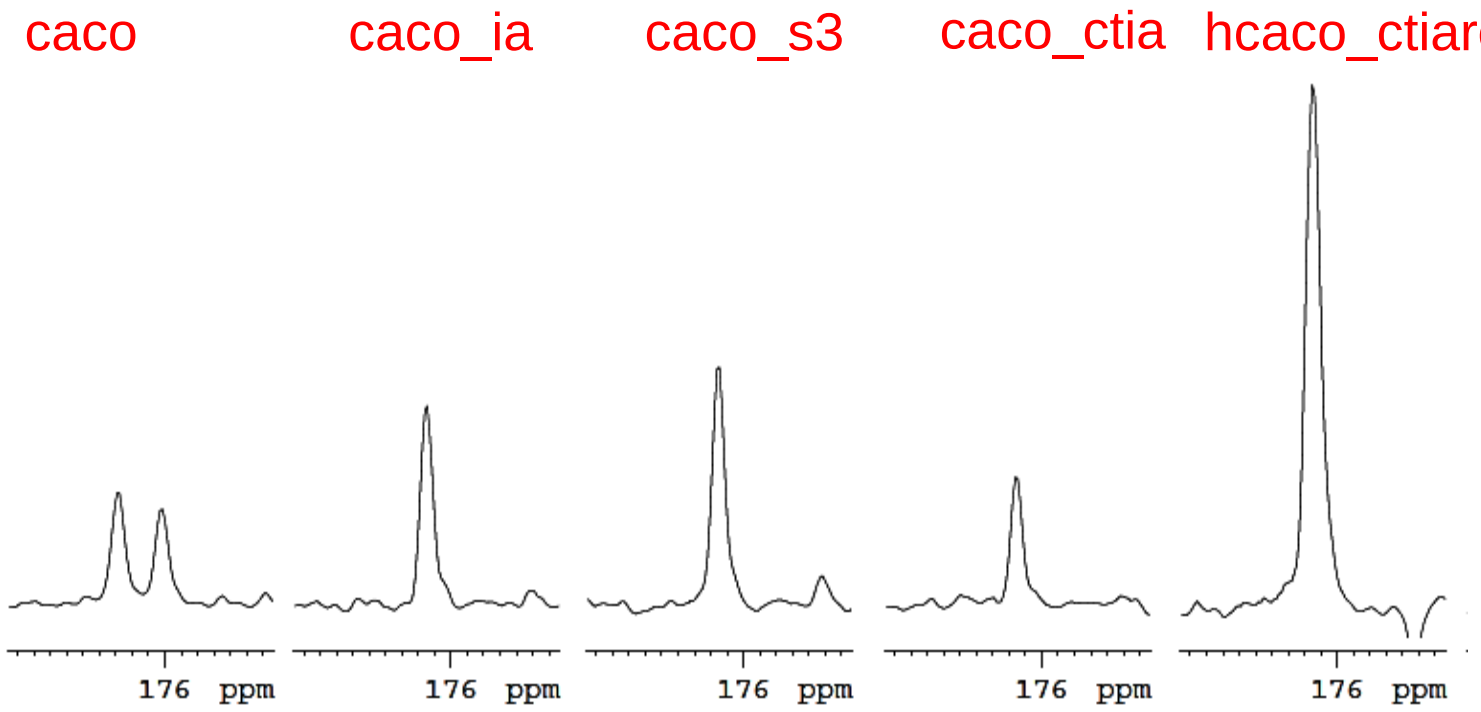
700 MHz CP-TXO

Variants of experiments

Different implementations for same experiment are available

- Standard — no virtual decoupling (no IPAP etc.)
- IPAP — virtual decoupling via IPAP
- S3 — virtual decoupling via S3 spin state selection
- CT — constant time, avoids evolution of homonuclear couplings during t_1 evolution
- RE — Relaxation optimized (H-flip)
- RC — Determination of $^{1}J(XY)$

Sensitivity of CACO pulse sequences



Pulse programs Topspin

examples

c_caco
c_can_mq
c_con_sq
c_canco_3d
c_ccflopsy16
c_ccflopsy16_ct
c_ccnoesy
c_cosy
c_cosy_ct

c_hcaco_ 3d
c_hccflopsy16_3d
c_hcacon_ iare
c_hcacon_ iare
c_hcacon_iarc_nc

h:	H-start (^{1}H-^{13}C INEPT)
mq:	HMQC version (multiple quantum)
sq:	HSQC version (single quantum)
ct:	contant time evolution
ia:	IPAP
re:	H-flip (relaxation enhanced)
rc:	determination of coupling constants
nc:	^{1}J(NC' coupling)

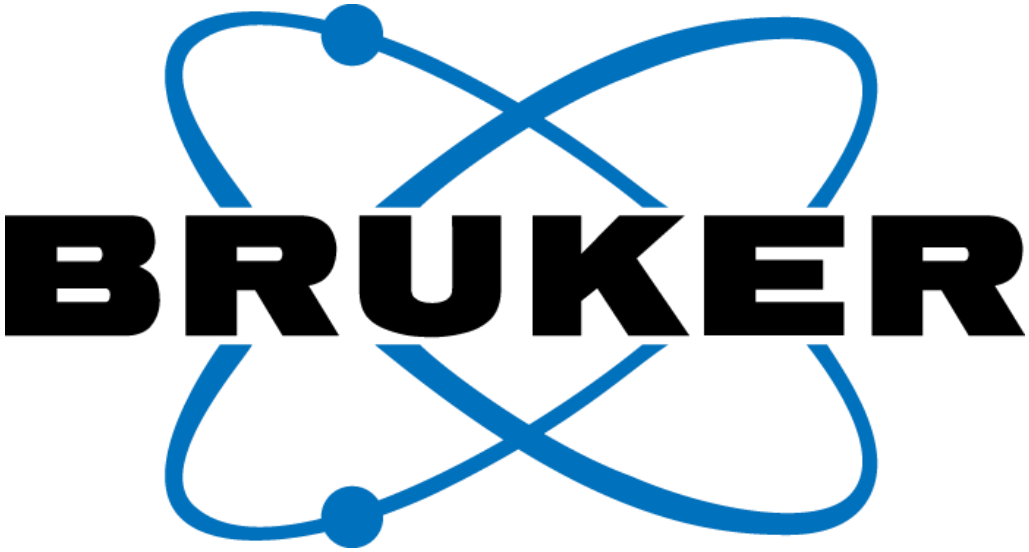
Thank you very much…

CERM

Ivano Bertini, Roberta Pierattelli & others

Bruker Application Department:

Helena Kovacs, Rainer Kümmerle, Wolfgang Bermel, Sergio Gil-Caballero



Innovation with Integrity

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