^{13}C-detection in Bio-NMR: An Introduction

Advantages of ^{13}C-detection (vs. ^{1}H-detection)

+ shorter sequences, less relaxation

+ high chemical shift dispersion

+ detection of non-protonated carbons

Advantages of ^{13}C-detection (vs. ^{1}H-detection)

Examples for simple experiments

Overcoming the problem with ^{13}C, ^{13}C homonuclear coupling:

- ***Homonuclear decoupling***
- ***IPAP technique***

Assorted examples

When do we need X-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

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

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

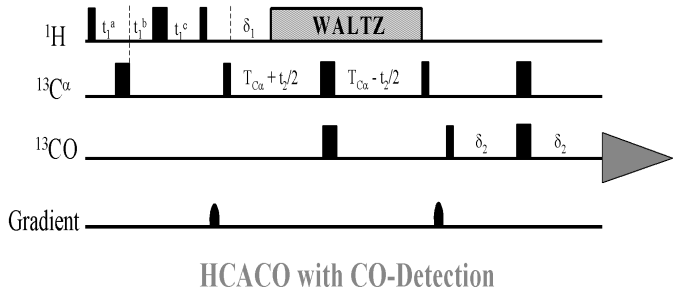
Strategies for backbone assignment

Dötsch et al.

Stern, *Serber, Richter, Moskau, Boehlen, Gerfin, Marek, Häberli, Baselgia, Laukien, Hoch & Dötsch, J. Am. Chem. Soc. 2000, 112, 3554.*

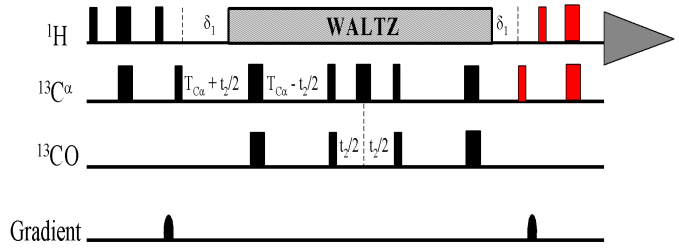
HACACO with ^{13}C-detection and HCC-TOCSY with ^{13}C-detection

Sequence 1

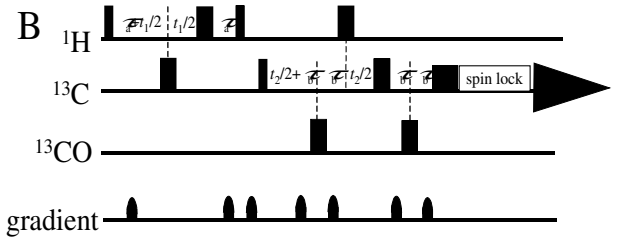


HCACO with CO-Detection

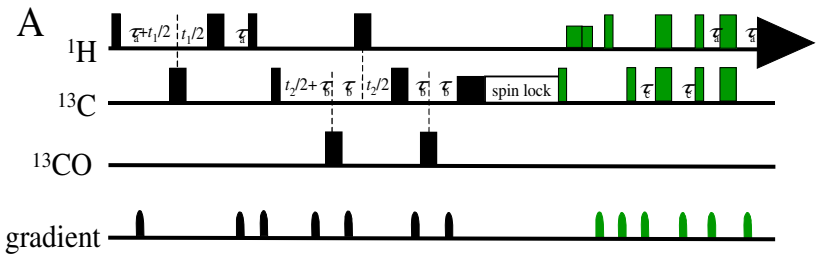
Sequence 2



HCACO



HCC-TOCSY



HCCH-TOCSY

Strategies for backbone assignment

Pervushin et al.

#1 *TROSY-HNCA, TROSY-HNCO and* ***MQ-HACACO (^{13}C-obs.)***

Pervushin & Eletsky, J. Biomol. NMR, 25 (2003) 147-152

#2 *TROSY-HNCO, TROSY-HN(CA)HA,* ***MQ-HACACO (^{13}C-obs.)***

Hu, Eletsky & Pervushin J. Biomol. NMR, 26 (2003) 69-77

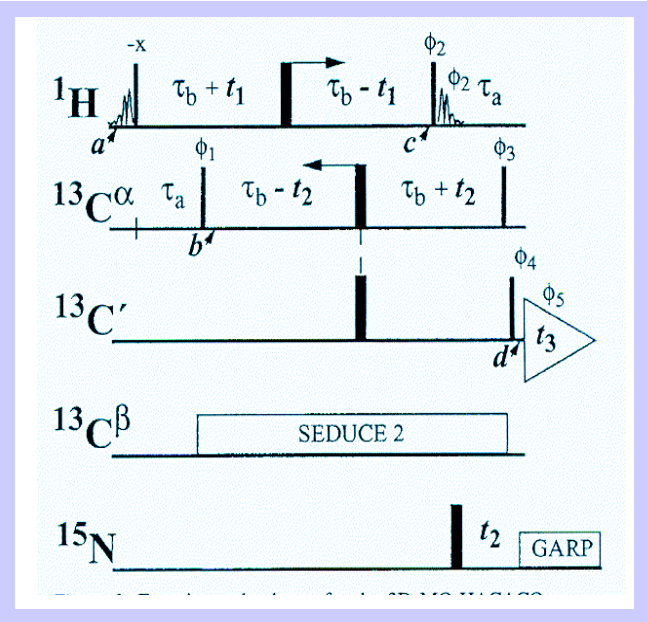
- **Set of experiments with both, ^{1}H and ^{13}C-detection**
- **Best sensitivity therefore required for both, ^{1}H and ^{13}C**

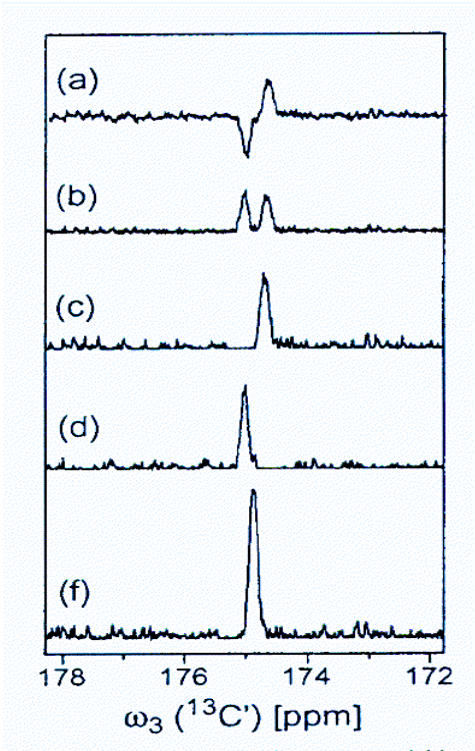
Strategies for backbone assignment

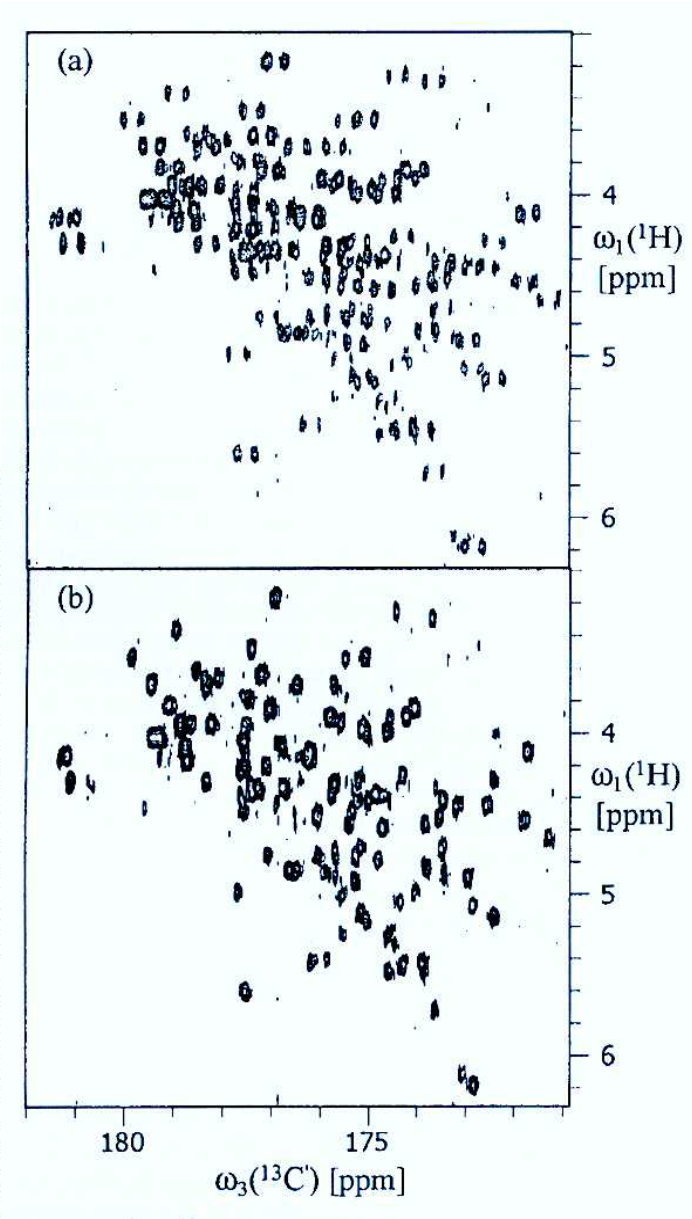
MQ-HACACO with direct ^{13}C-detection

Pervushin & Eletsky, J. Biomol. NMR, 25 (2003) 147-152

35%-^{2}H,^{13}C,^{15}N-labeled chorismate mutase 44 kDa







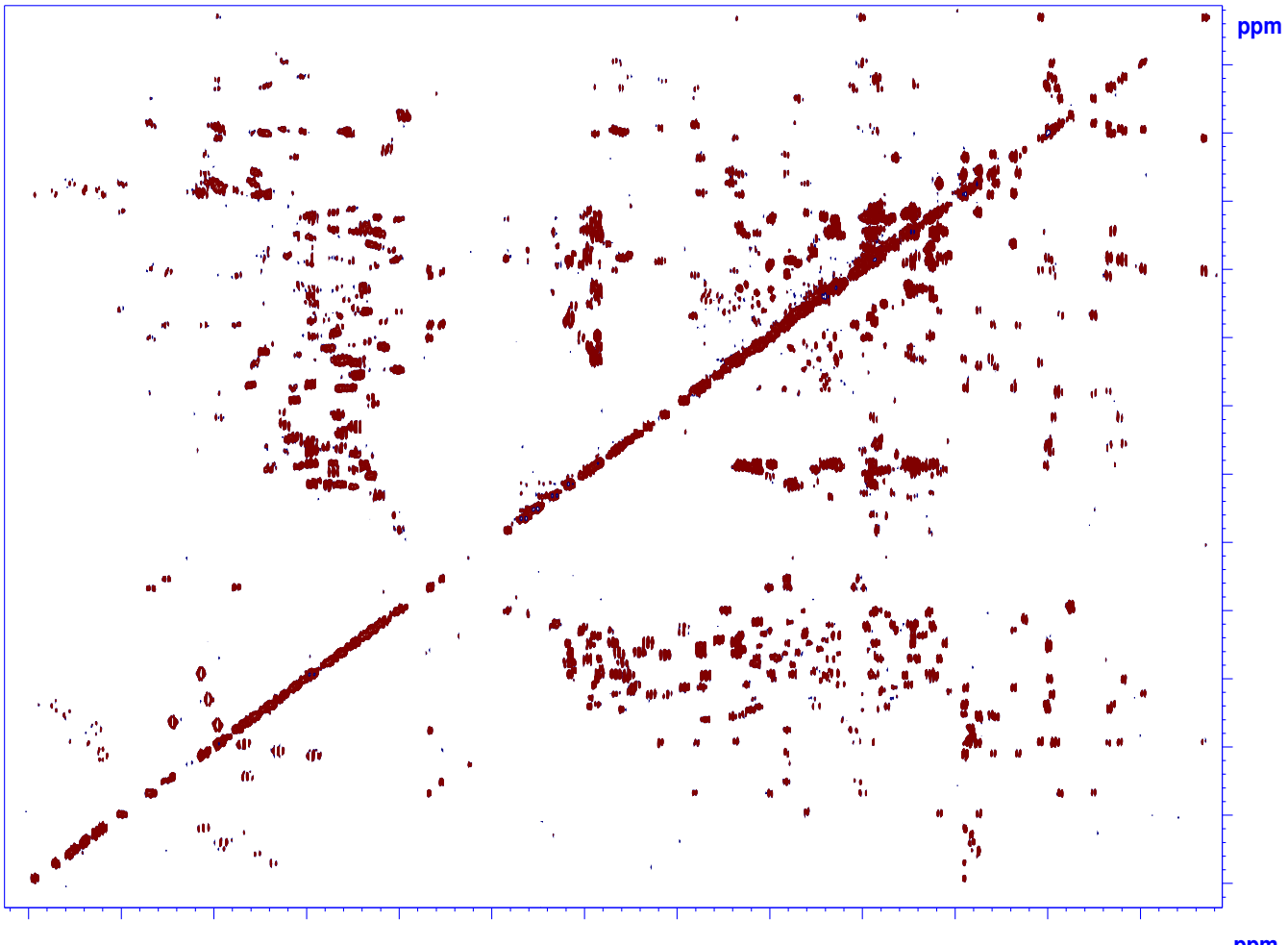
5mm TCI CryoProbe™ $^{1}H\{^{13}C, ^{15}N\}$ 800MHz

^{13}C-detection in biomolecular NMR spectroscopy

$^{13}C,^{13}C$-TOCSY

2mM $^{15}N,^{13}C$ labeled ubiquitin

Exp. time 11 min



Jüergen Schleucher,
Umea University

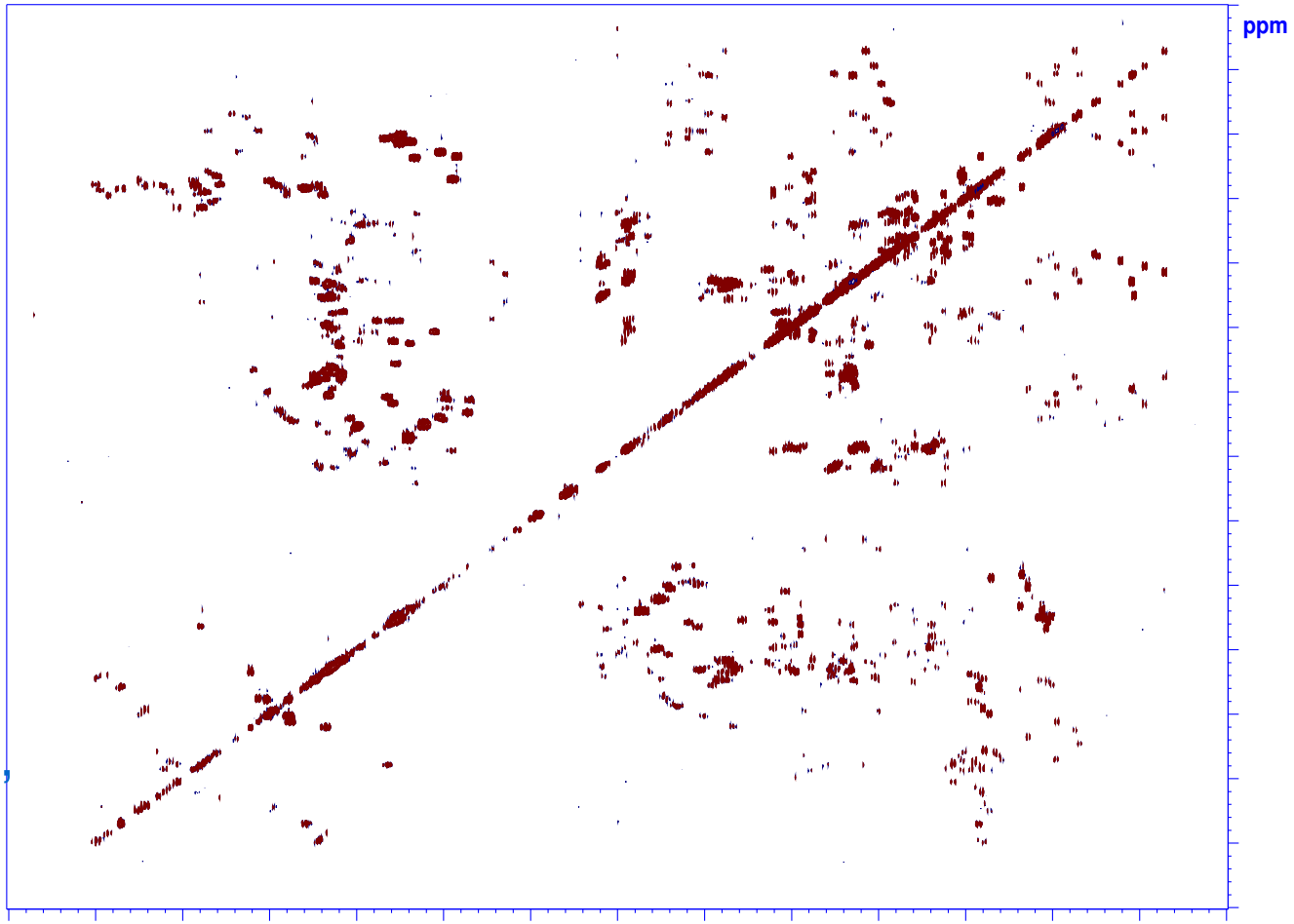
5mm TCI CryoProbe™ $^{1}H\{^{13}C, ^{15}N\}$ 800MHz

^{13}C-detection in biomolecular NMR spectroscopy

$^{13}C,^{13}C$-TOCSY

100uM $^{15}N,^{13}C$ labeled calmodulin

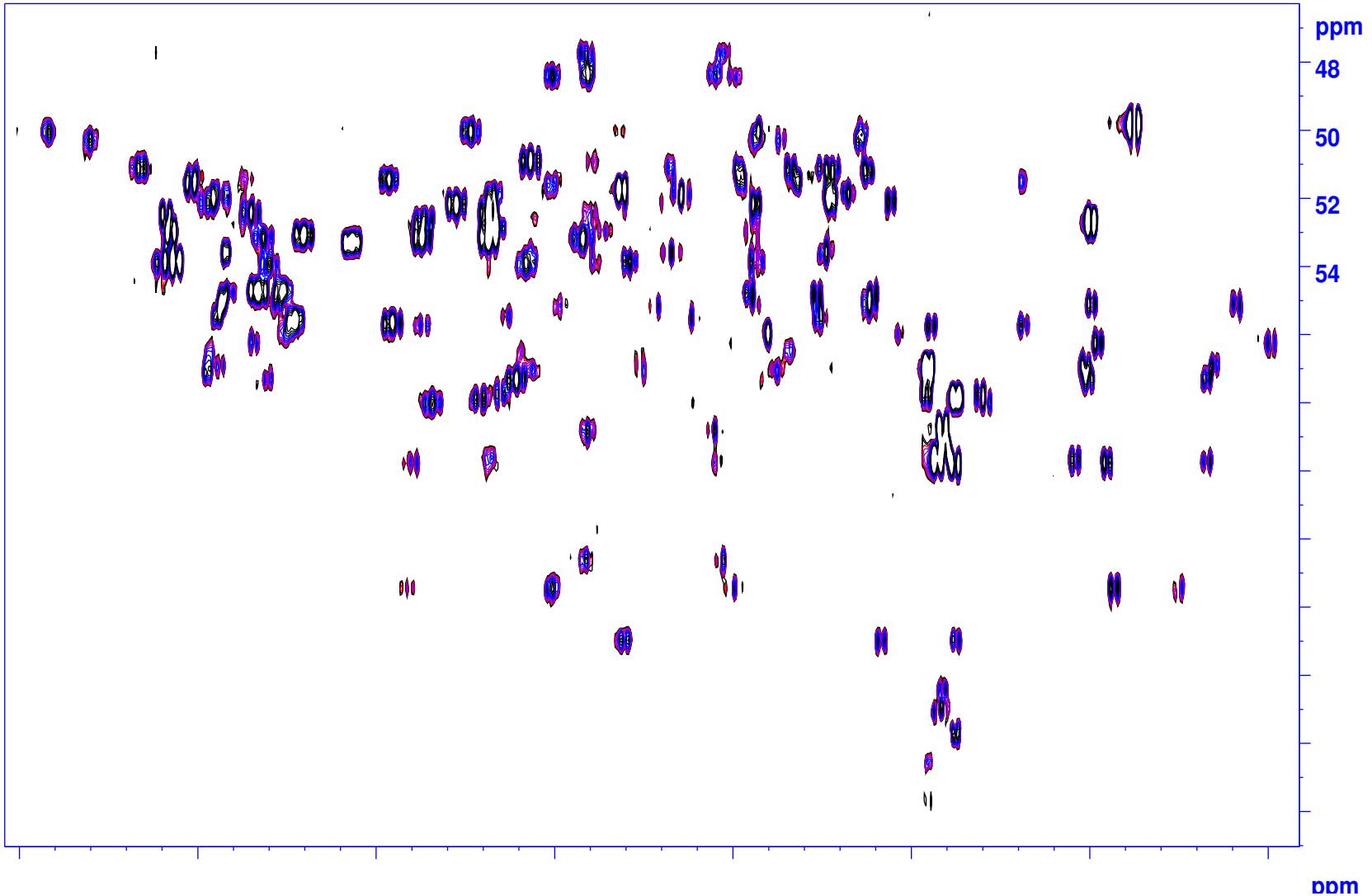
Exp. time 11 h



Raphael Brüschweiler,
Clark University

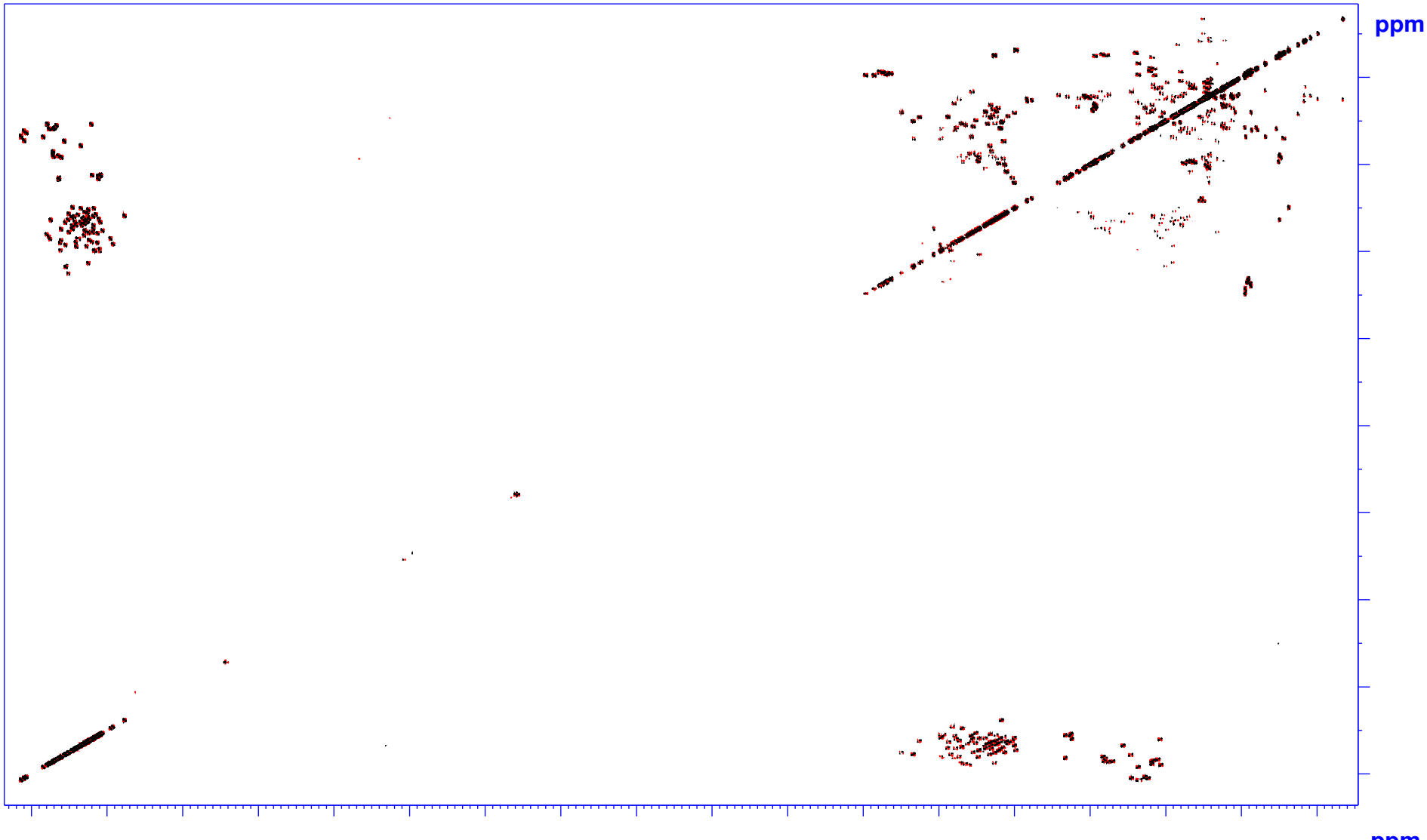
CryoProbe TCI 800MHz

AV800, TCI800 CryoProbe
1mM Ubiqitin, 13C{1H, 15N}-TOCSY
NS=32 exp time 7h 30min



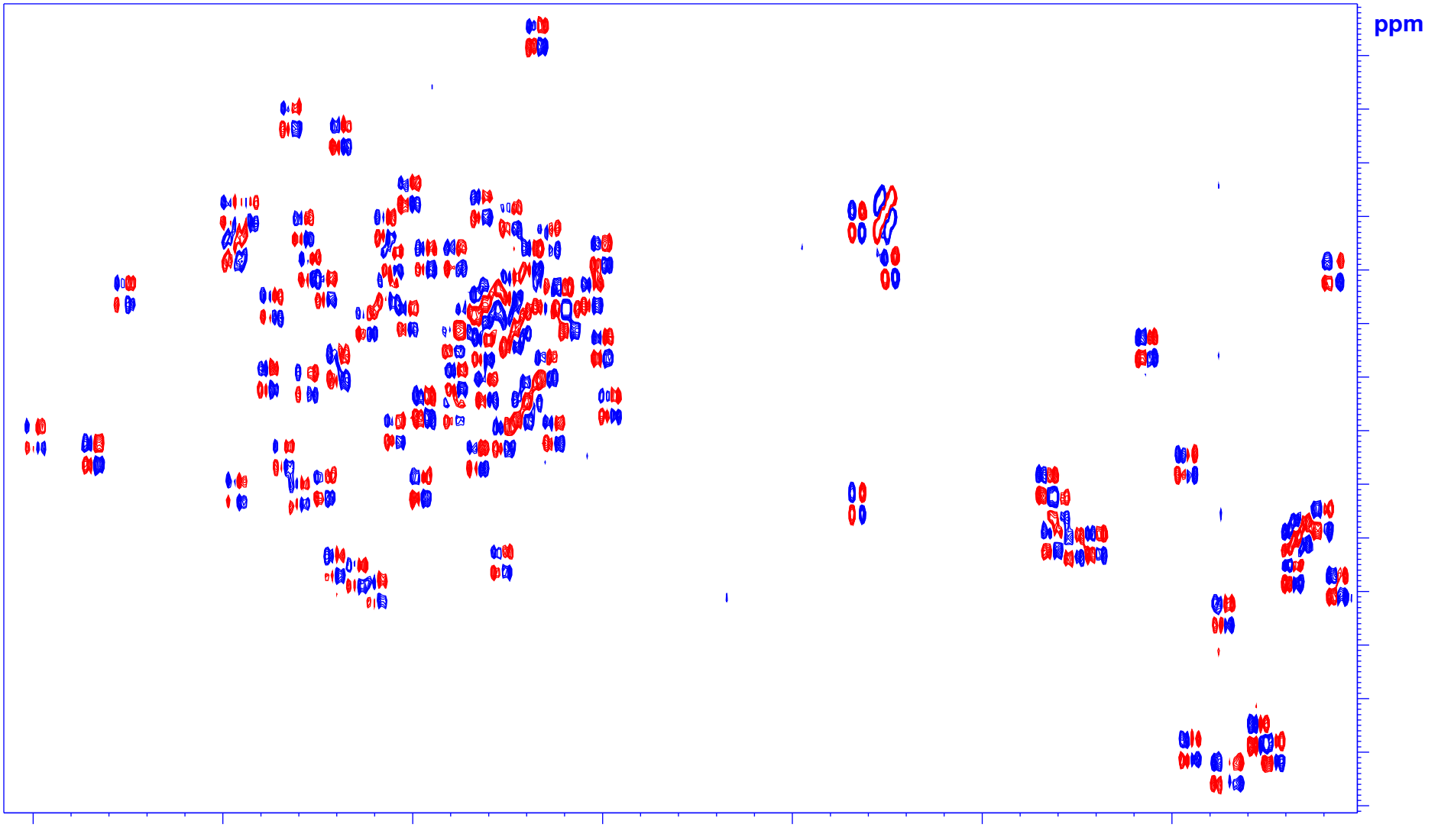
CryoProbe TCI 800MHz

AV800. TCI800 CI



CryoProbe TCI 800MHz

AV800. TCI800 CI



The problem with ^{13}C-^{13}C couplings

$^{1}J_{C\alpha C'}$ 55 Hz

$^{1}J_{C\alpha Cb}$ 35 Hz

$^{2}J_{C'C\beta} < 2$ Hz

$^{3}J_{C'C'} < 3$ Hz

$^{3}J_{C\alpha C\alpha} < 2$ Hz

$^{3}J_{C'C\gamma} < 5$ Hz

$^{3}J_{C\alpha C\delta} < 5$ Hz

Bystrov, Progress in NMR spectroscopy, 1976

^{13}C-^{13}C coupling removal: literature

maximum entropy:

Z. Serber et al., J. Am. Chem. Soc. 122, 3554-3555 (2000)

N. Shimba, A.S. Stern, C.S. Craik, J.C. Hoch & V. Dötsch, J. Am. Chem. Soc. 125, 2382-2383 (2003)

virtual decoupling

K. Pervushin & A. Eletsky, J. Biomol. NMR 25, 147-152 (2003)

L. Duma, S. Hediger, A. Lesage & L. Emsley, J. Magn. Reson. 164, 187-195 (2003)

I. Bertini, I.C. Felli, R. Kümmerle, C. Luchinat & R. Pierattelli, J. Biomol. NMR 30, 245-251 (2004)

W. Bermel, I. Bertini, L. Duma, I.C. Felli, L. Emsley, R. Pierattelli & P.R. Vasos, Angew. Chem. Int. Ed. 44, 2-5 (2005)

bandselective homodecoupling

W. Bermel, I. Bertini, I.C. Felli, R. Kümmerle & R. Pierattelli, J. Am. Chem. Soc. 125, 16423-16429 (2003)

‘Decoupling’ homonuclear C,C couplings

‚Decoupling‘ ^{13}C, ^{13}C scalar couplings

Homonuclear decoupling:

real decoupling

IPAP and double IPAP method:

virtual decoupling

‘Decoupling’ homonuclear C,C couplings

‚Decoupling‘ ^{13}C, ^{13}C scalar couplings

Homonuclear decoupling:

real decoupling

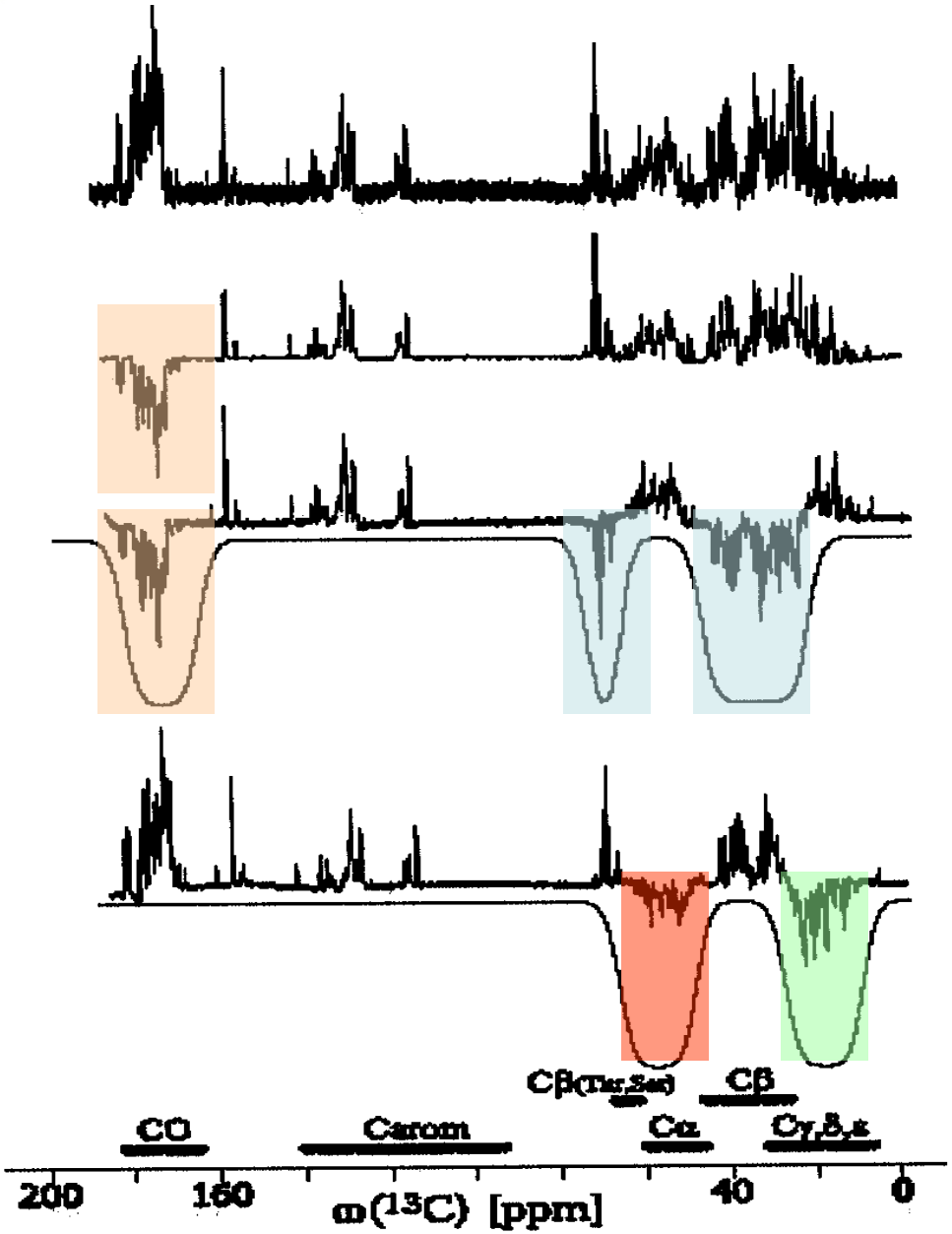
IPAP and double IPAP method:

virtual decoupling

^{13}C-Homodecoupling

Vögeli, Kovacs, Pervushin J. Biomol. NMR 31 (2005) 1-9

^{13}C-homodecoupling profiles - protein



^{13}C spectrum of ubiquitin

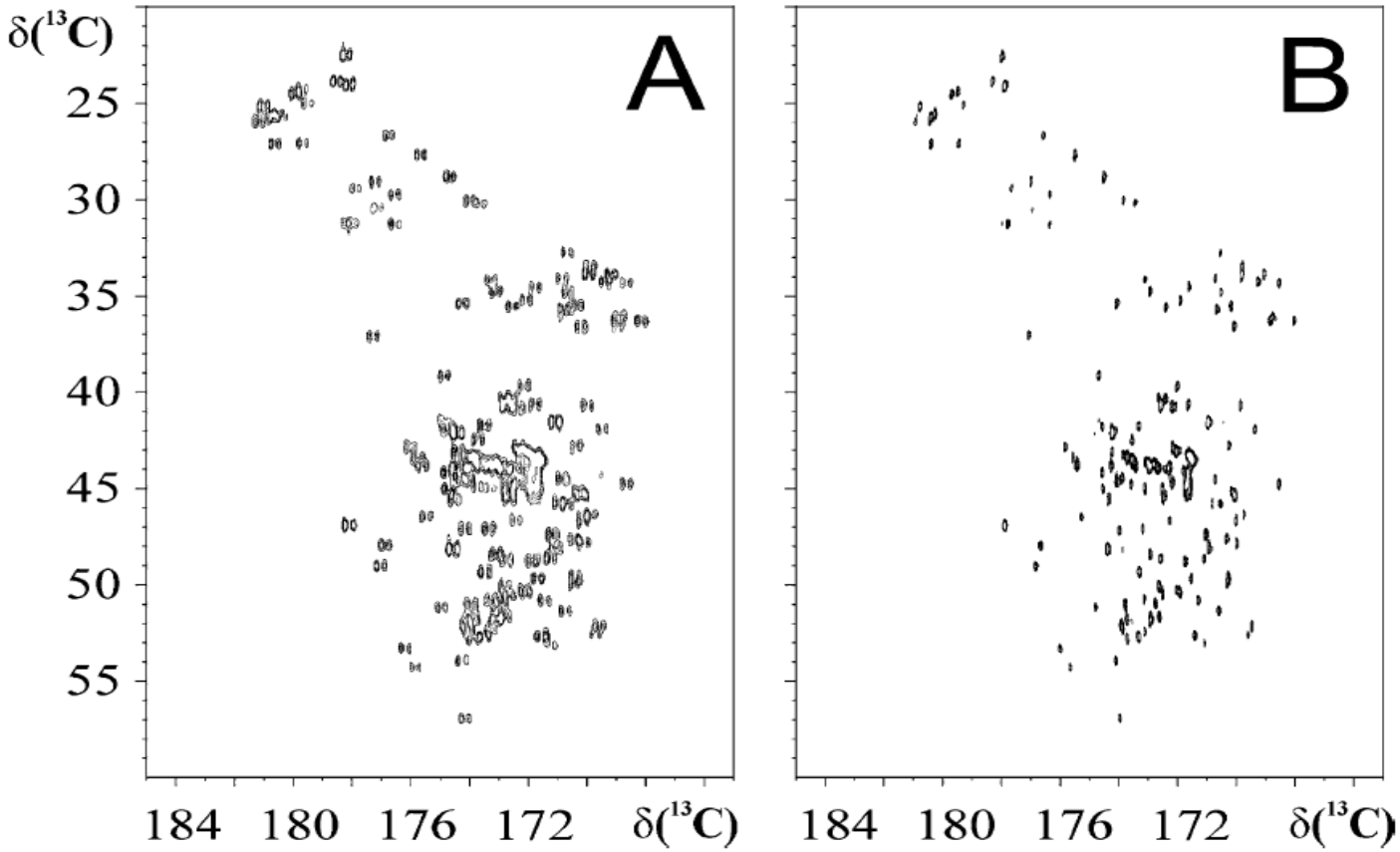
carbonyl

rbonyl and C-beta

alpha and C-gamma

Bandselective ^{13}C homodecoupling

COCA-MQ without (A) and with (B) bandselective homodecoupling



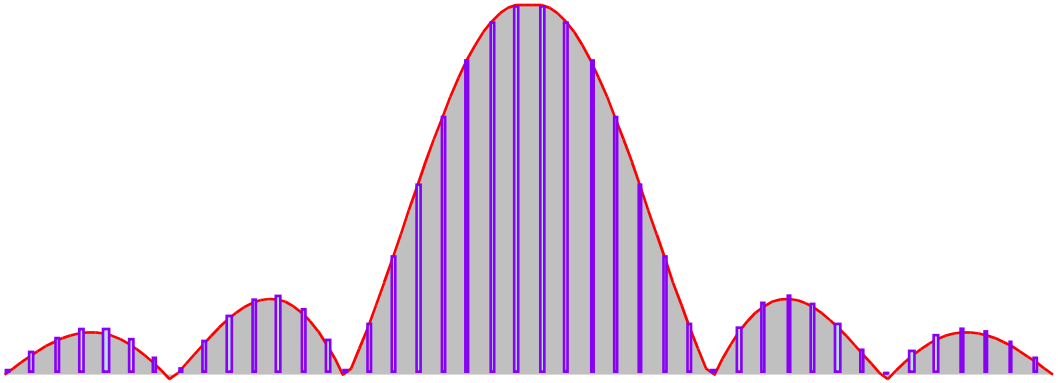
CrpXX,10,25.1

XX: 20% more than decoupled region

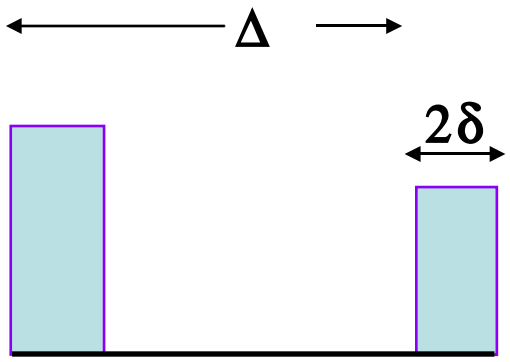
Bandselective Homodecoupling: Details

Decoupling harmonics during HD

shape:



zoomed region of shape:



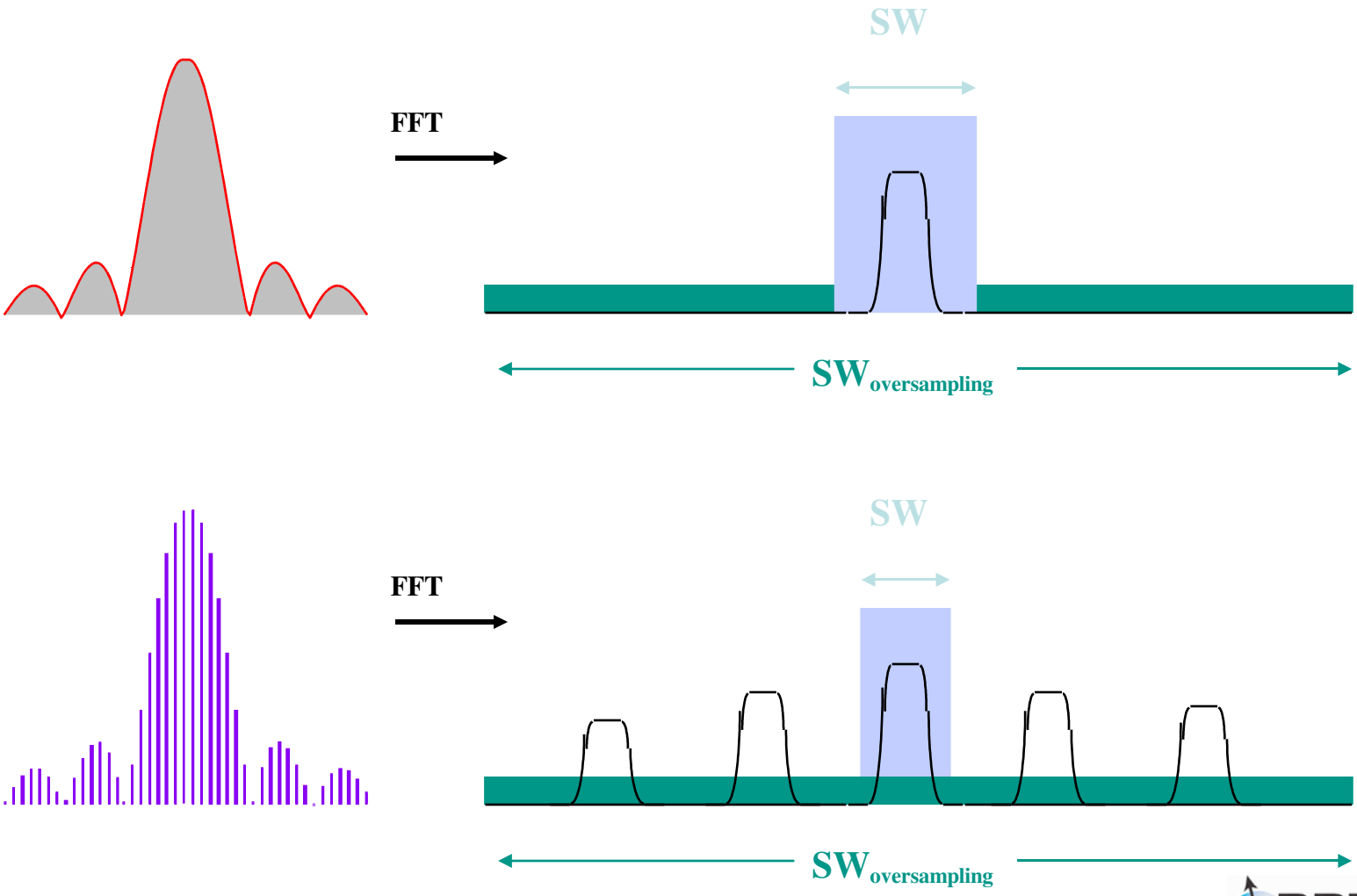
Position and Intensity of harmonics is given by Fourier Series

for equal intensity and sign of each element:

$$\delta/\Delta + 2/\pi * [-\sin(\pi\delta/\Delta) * \cos(\pi x/\Delta) + 1/2 * \sin(2\pi\delta/\Delta) * \cos(2\pi x/\Delta) - 1/3 * \sin(3\pi\delta/\Delta) * \cos(3\pi x/\Delta) + \ldots\ldots]$$

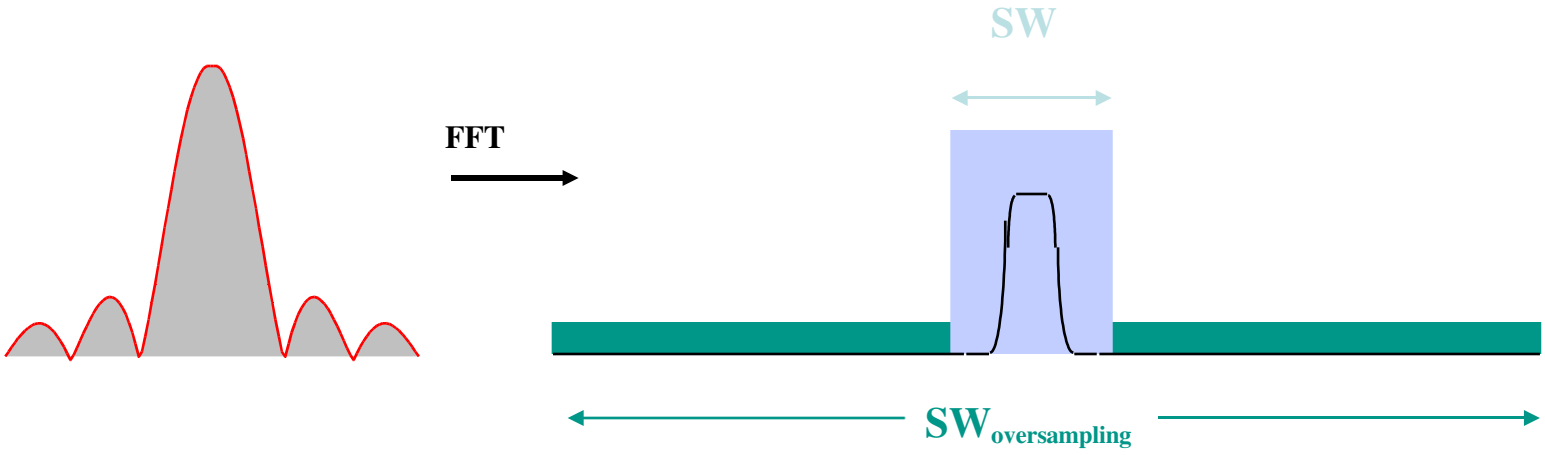
Bandselective Homodecoupling: Details

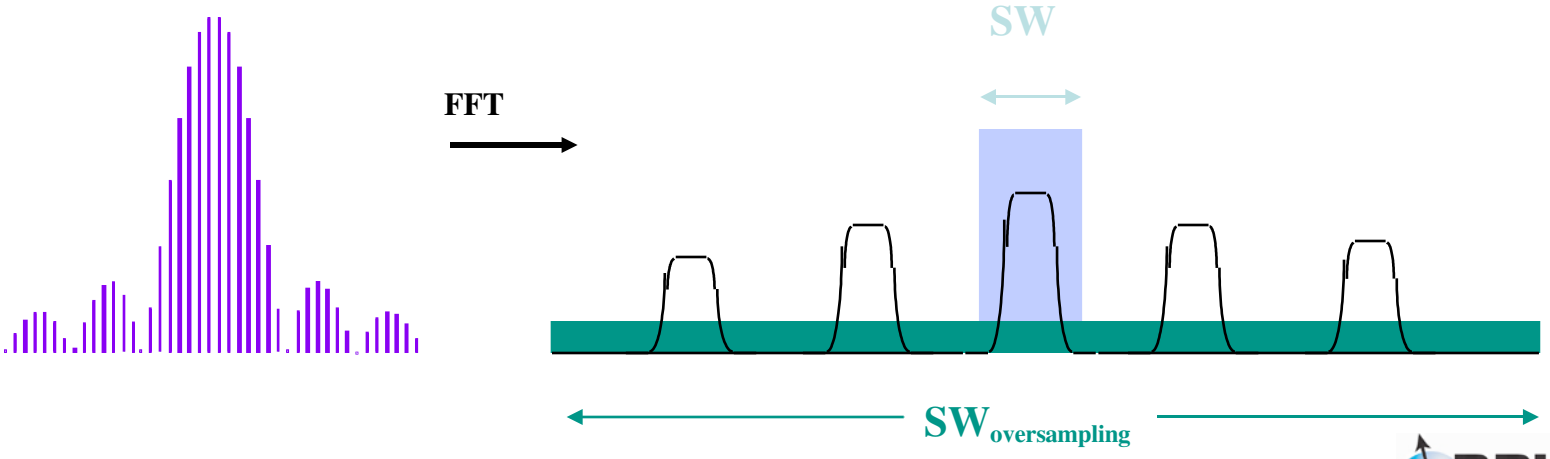
Decoupling harmonics during HD



Bandselective Homodecoupling: Details

Can we make use of harmonic sidebands?



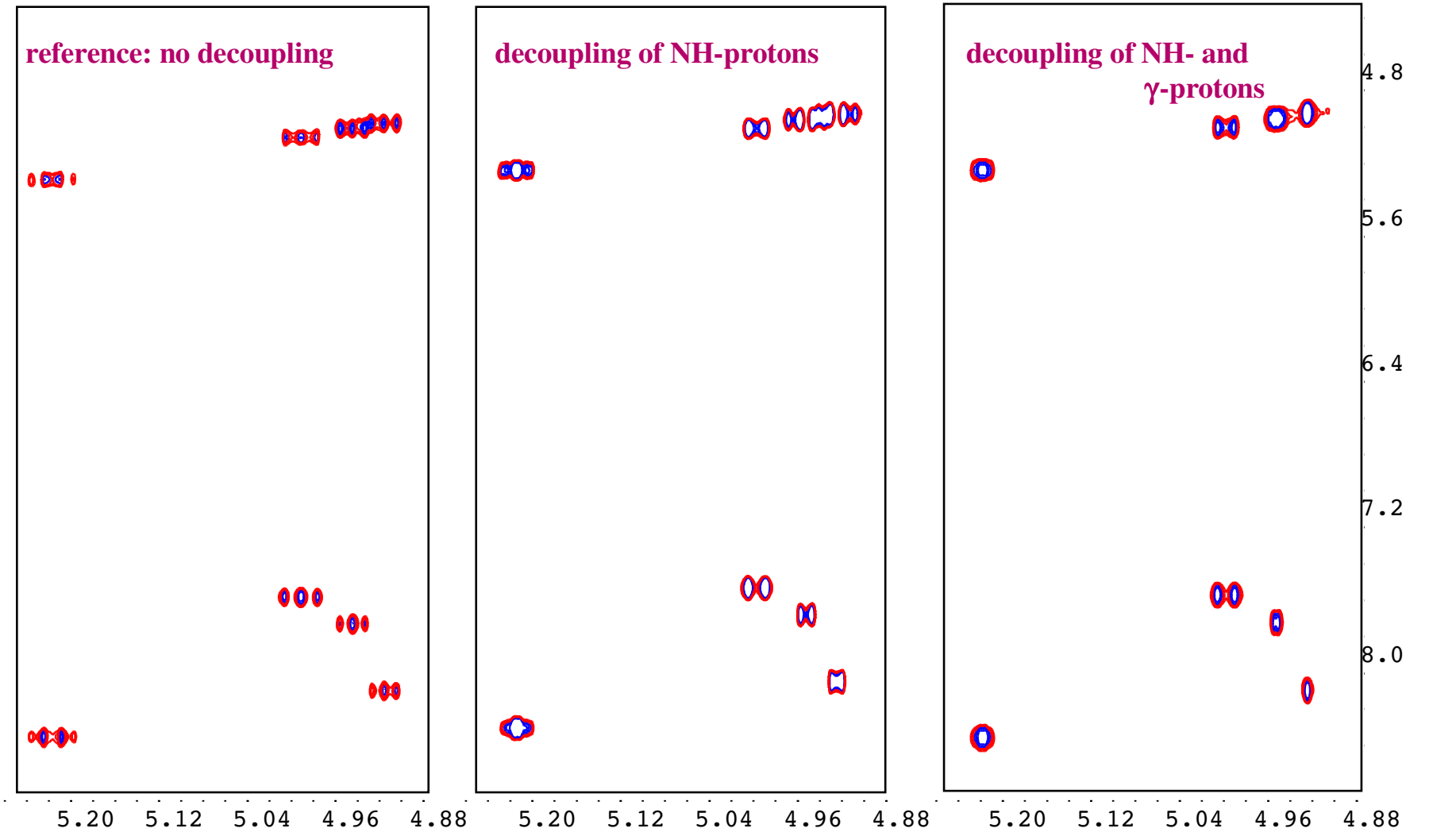


Bandselective Homodecoupling: Details

Can we make use of harmonic sidebands?

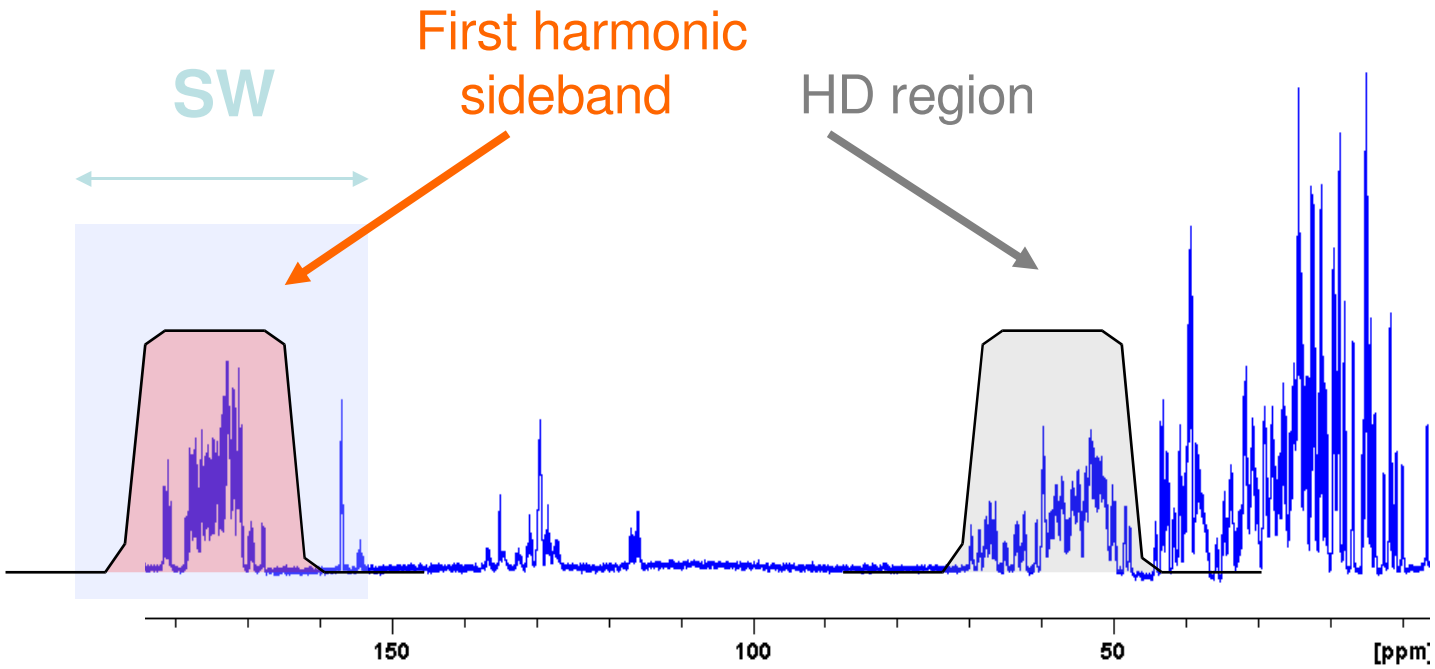
band selective decoupling of NH- and γ -protons with G3 pulse

25mM cyclosporin A in C6D6, region of α-protons selected with digital filter, DQD



Bandselective Homodecoupling: Details

Take care of position of harmonic sidebands!



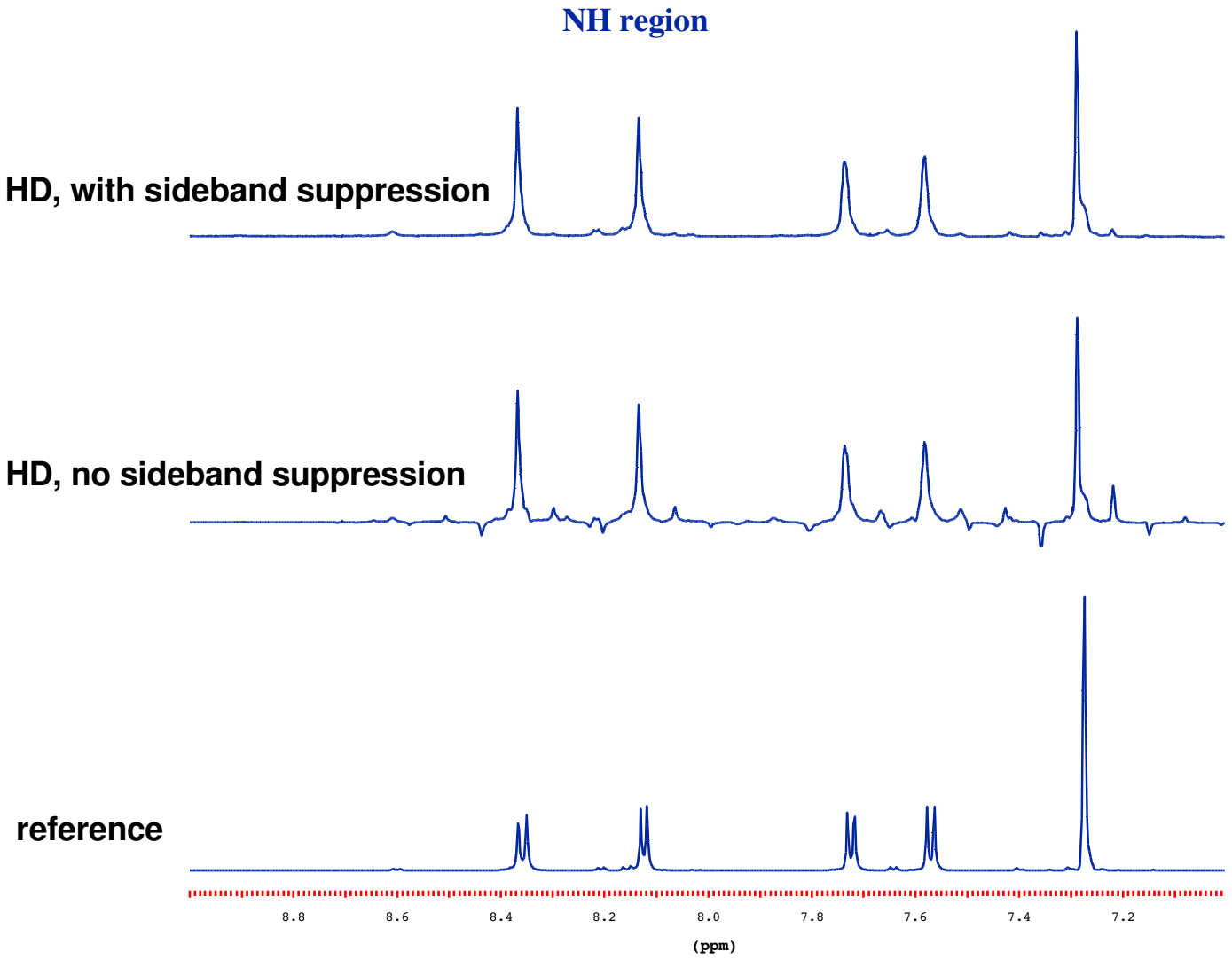
Example: HD on C-α, observation of CO, 800 MHz:

SW: 60 ppm, DW = 41.2 usec:

- *sideband at 1 / 41.2us = 24 kHz = 120 ppm*
- *^{13}C HD offset: 54 ppm*
- *First harmonic at 54+120 = 174 ppm*

Bandselective Homodecoupling: Details

Artifacts: decoupling sidebands



Bandselective Homodecoupling: Details

Bandselective decoupling: pulse programming
Example with sideband suppression: **zghc.2**

```
1 ze
  d12 pl24:f2
2 d11 do:f2
  3m
3 3m
4 d1
  d20 cpdngs2:f2        controlled delay of start position of HD
                        CPD decoupling sequence using shaped pulses,
                        amplifier blanking is closed, no pulse
  p1 ph1
  go=2 ph31 hd:f2       amplifier gating created by HD-command
  d11 do:f2 wr #0
  3m id20 zd
  lo to 3 times 4
  3m rd20
  lo to 4 times l5
```

‘Decoupling’ homonuclear C,C couplings

‚Decoupling‘ ^{13}C, ^{13}C scalar couplings

Homonuclear decoupling:
real decoupling

IPAP and double IPAP method:
virtual decoupling

‘Decoupling’ homonuclear C,C couplings

IPAP = In-Phase Anti-Phase

1.Two datasets are recorded:

CC multiplets are In-Phase

CC multiplets are Anti-Phase

2. Linear combination of those two datasets

Double IPAP:

IPAP scheme applied to two coupling constants

Four datasets are recorded

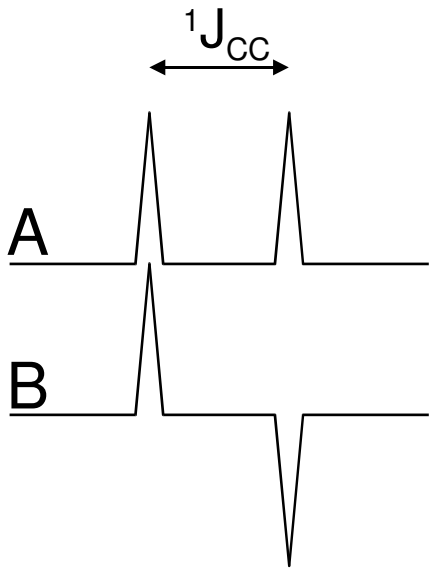
Note:

Identical coupling constants assumed

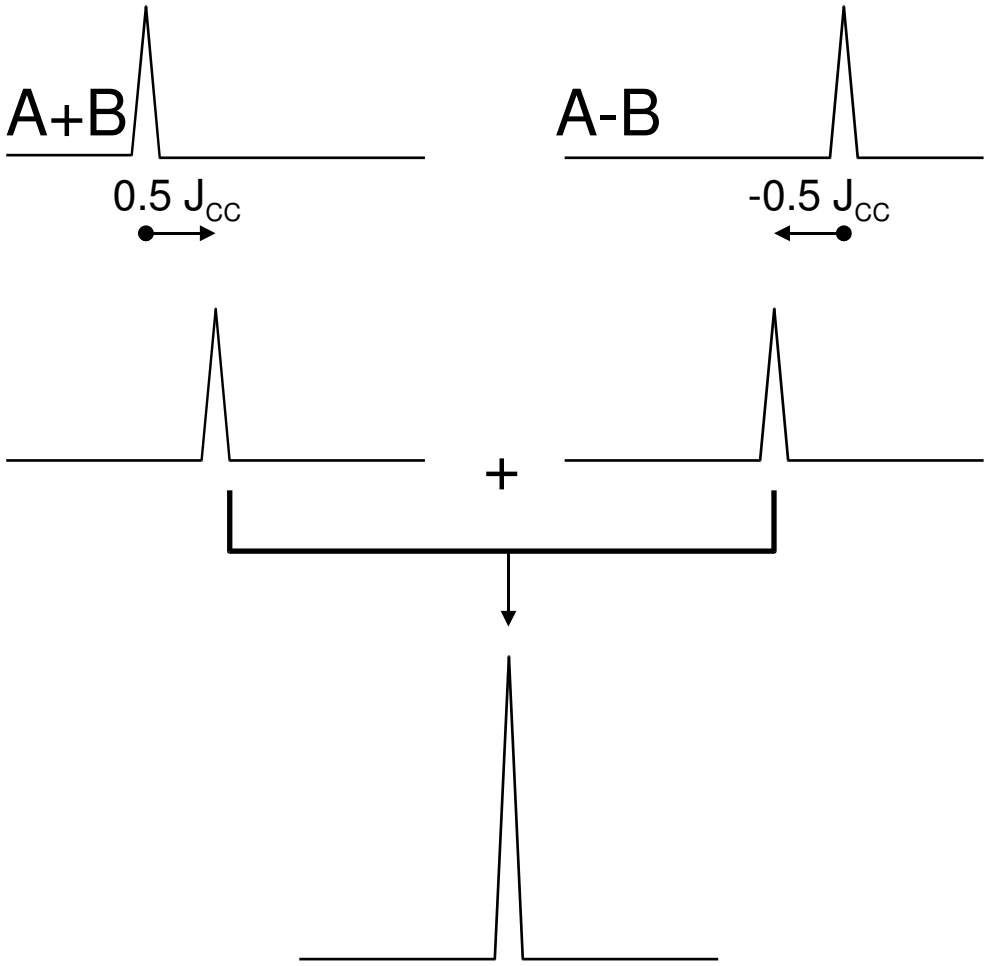
longer pulse sequence, reduced sensitivity due to relaxation

IPAP

Acquisition

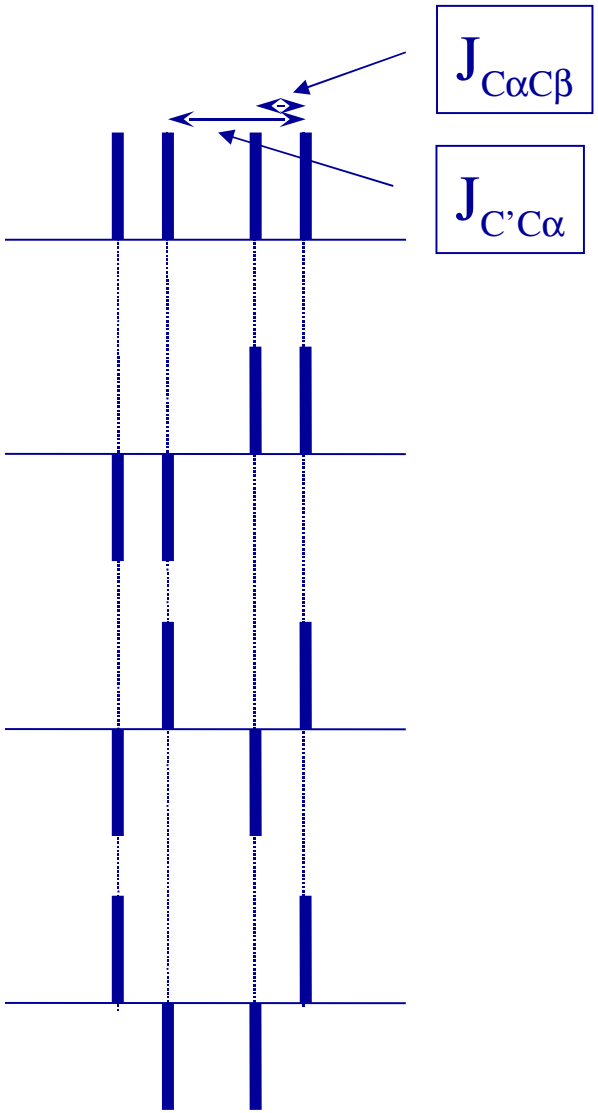


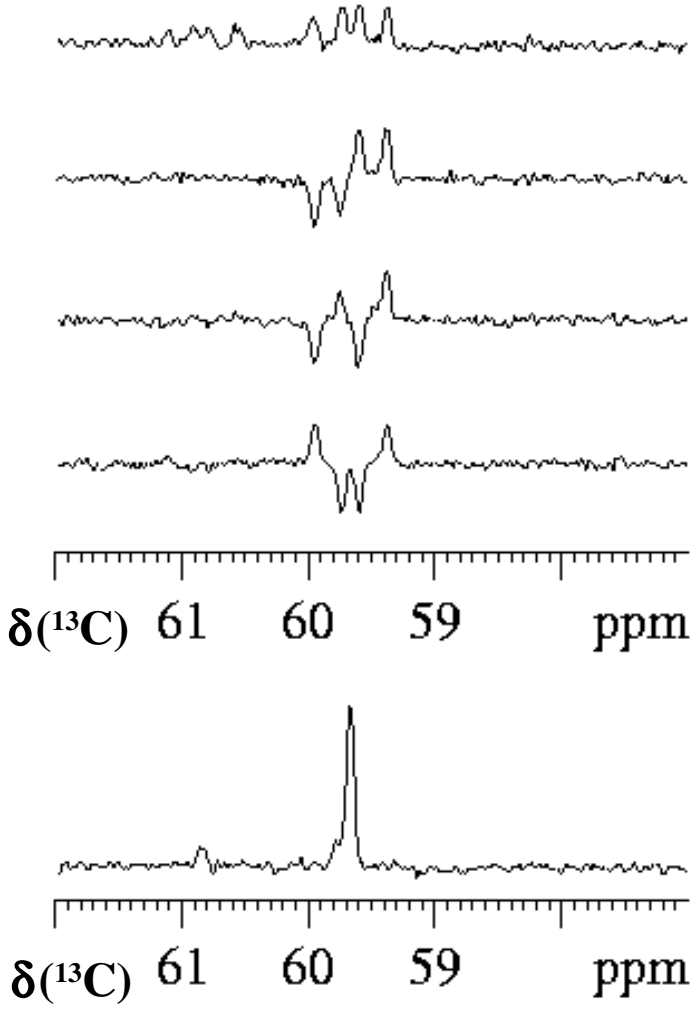
Linear combination



The DIPAP approach

Set-up on ^{13}C-^{15}N labeled Alanine





Bermel W., Bertini I., Duma L., Felli I.C., Emsley L., Pierattelli R., Vasos P.R., Angew. Chem., 2005, 44, 3089- 3092

The IPAP / DIPAP approach

In fully labeled proteins:

IPAP approach allows CO-detected experiments (1/2J)

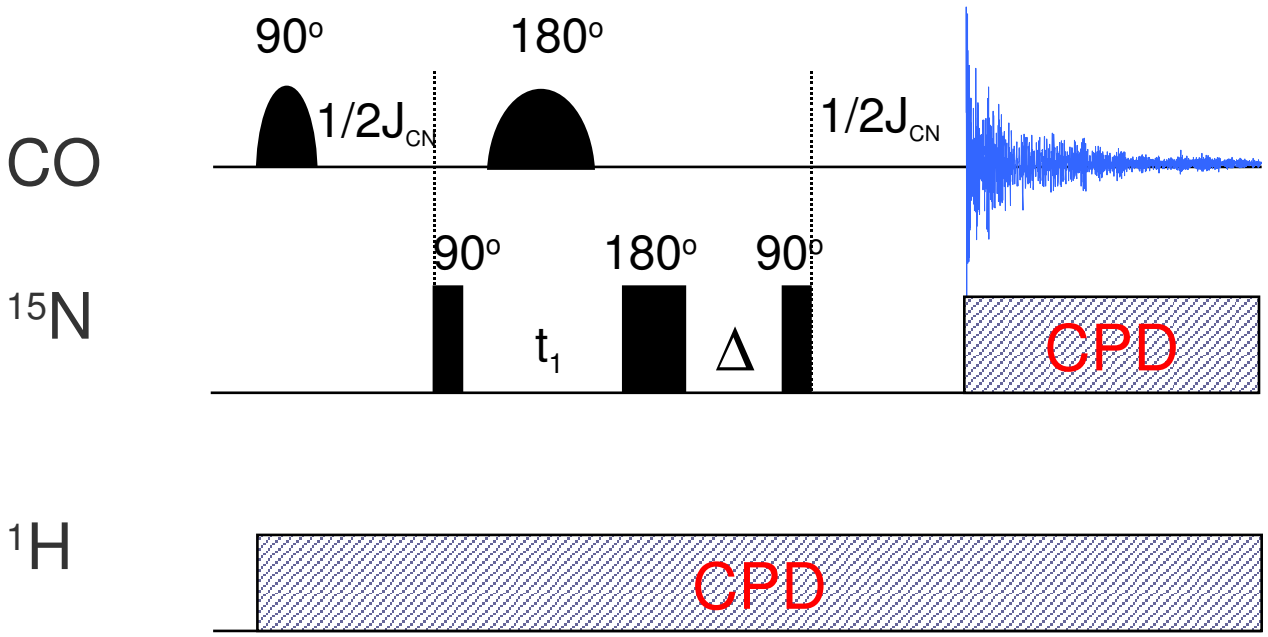
S3E likewise IPAP for CO-detected experiments (1/4J)

DIPAP approach allows Cα detected experiments

Details: pulse sequences

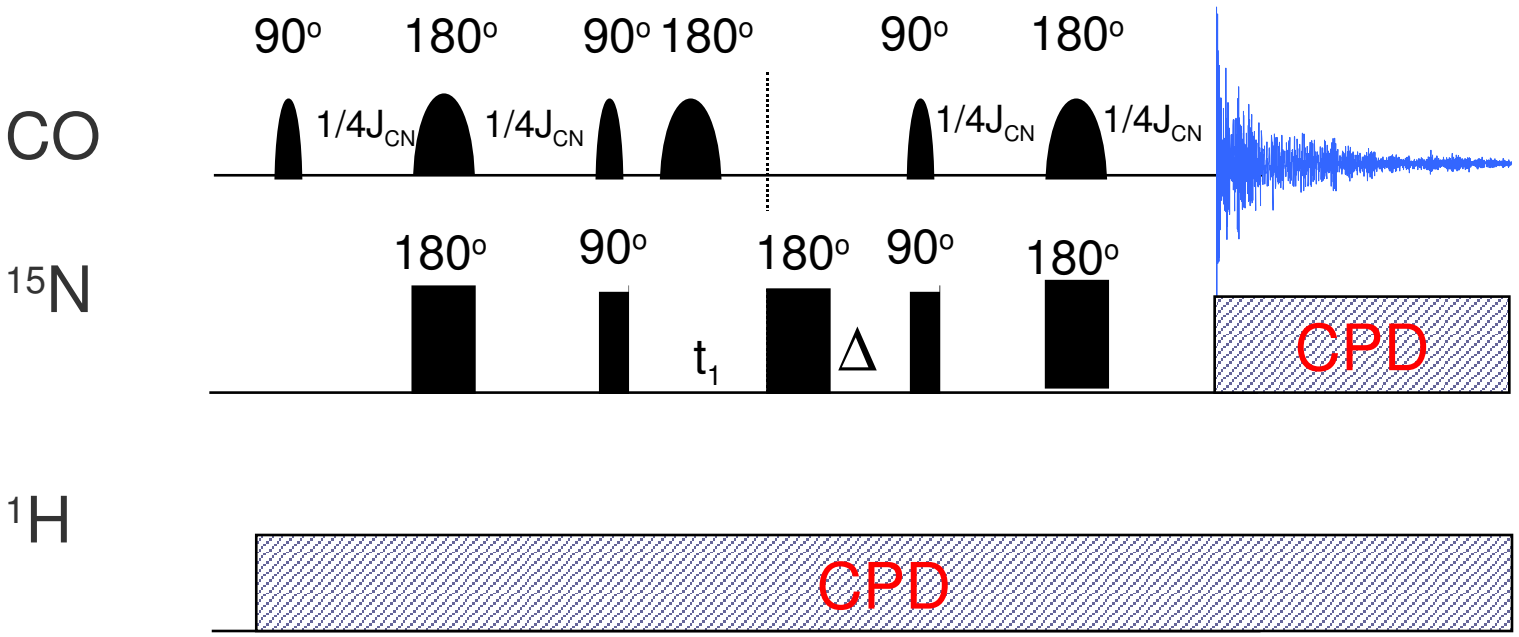
CON, multiple quantum

‚HMQC‘



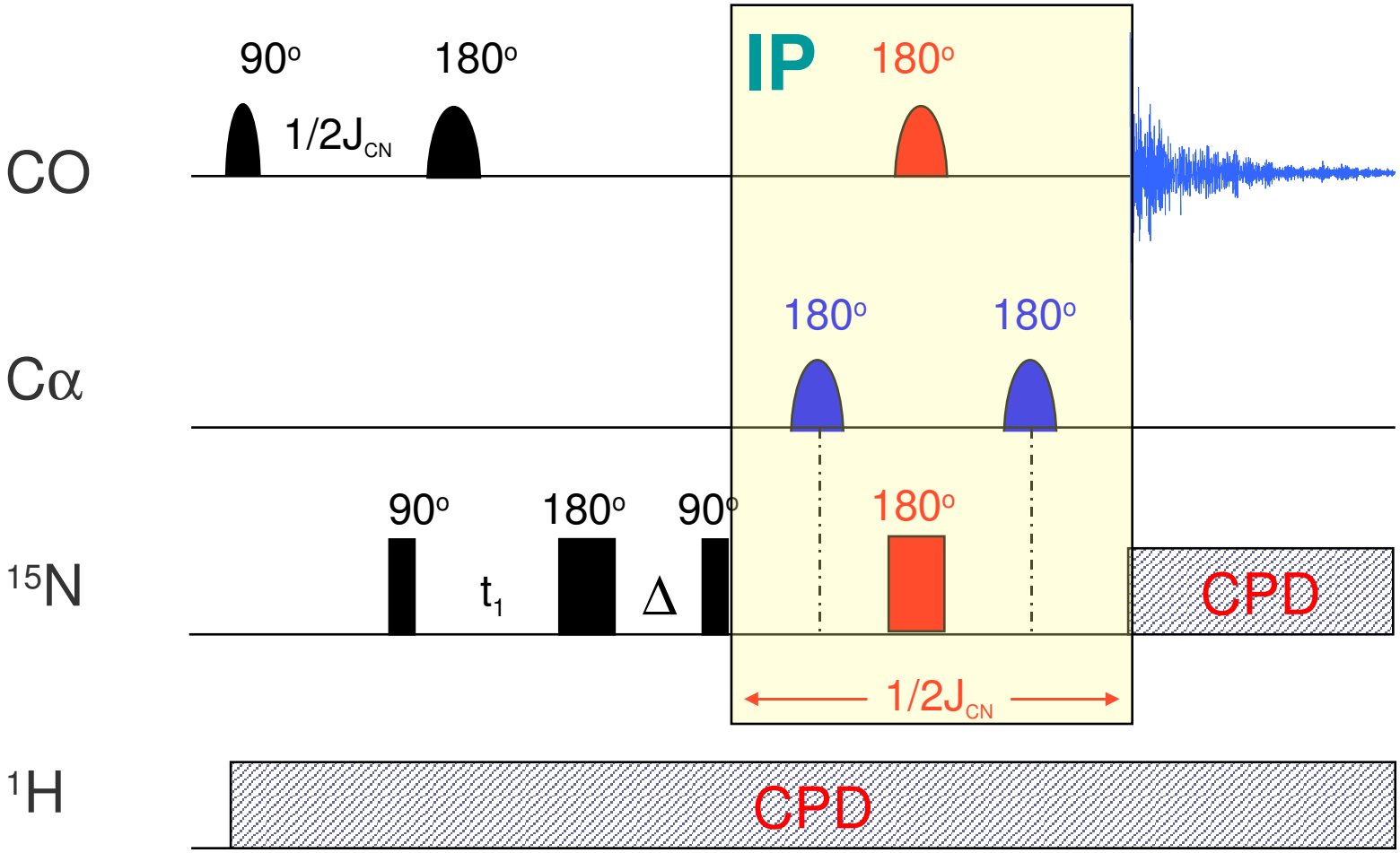
Details: pulse sequences

CON, single quantum ***‚HSQC‘***



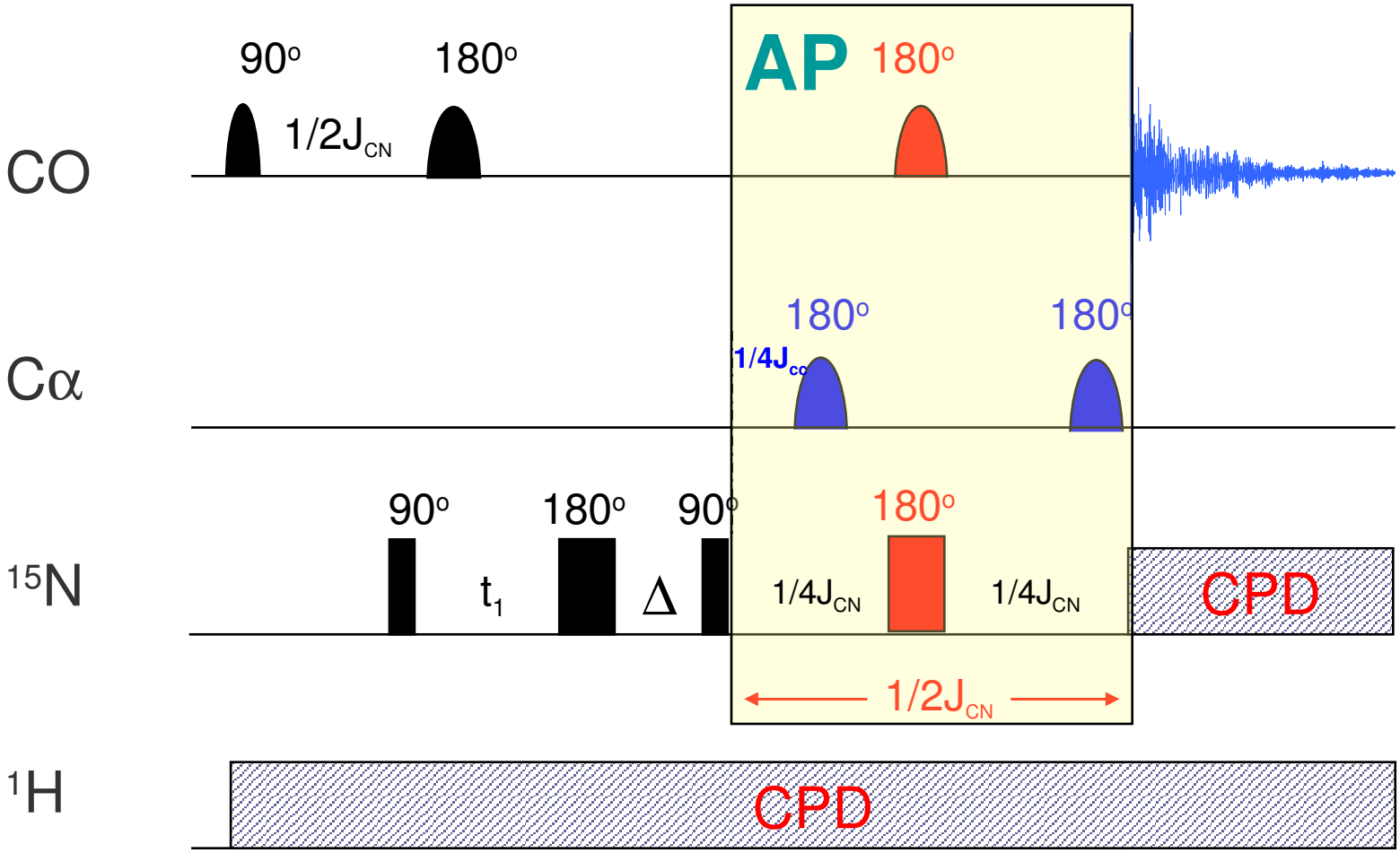
Details: pulse sequences

CON, multiple quantum, IPAP for decoupling of $J_{C'C\alpha}$



Details: pulse sequences

CON, multiple quantum, IPAP for decoupling of $J_{C'C\alpha}$



Details: pulse sequences

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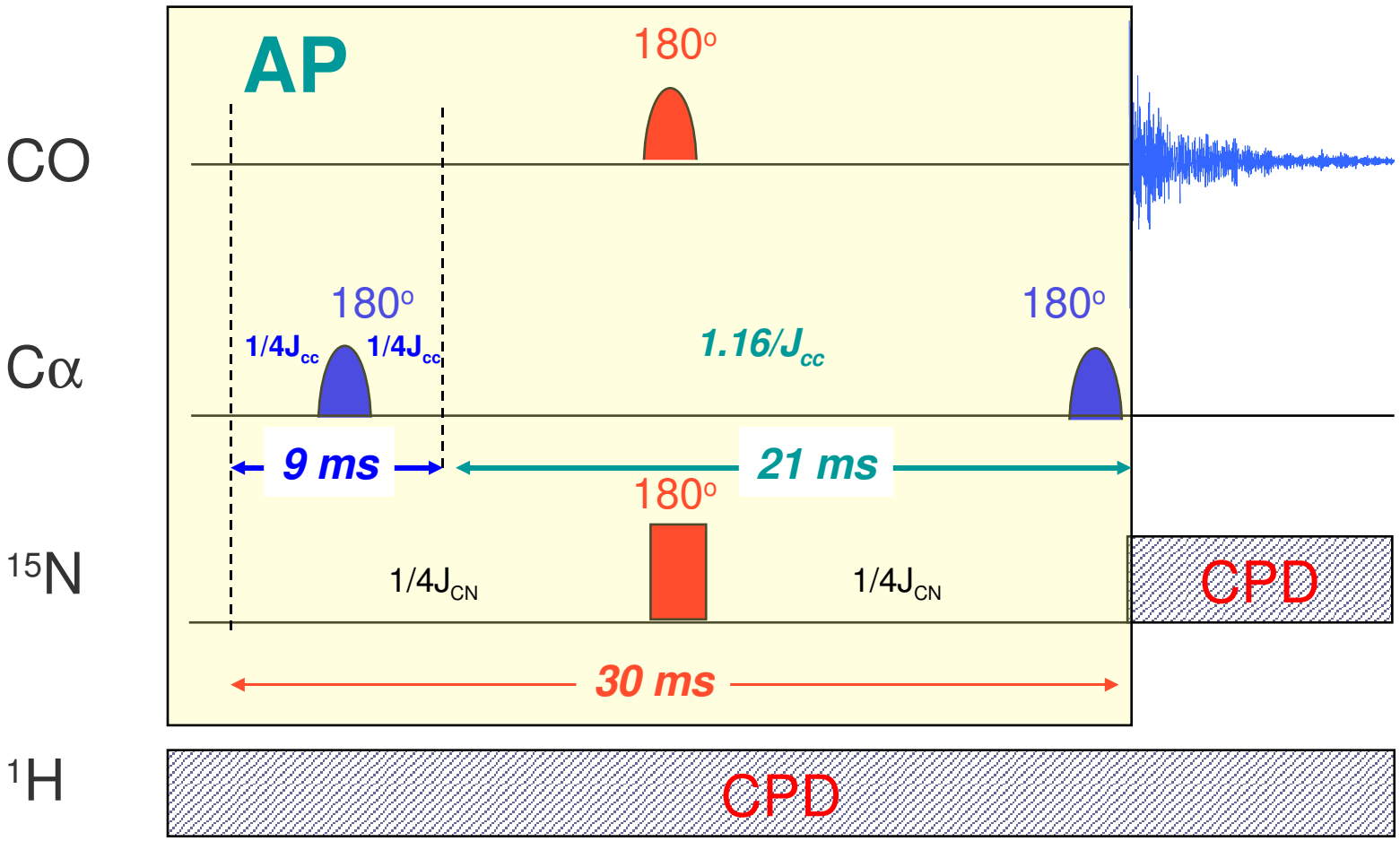
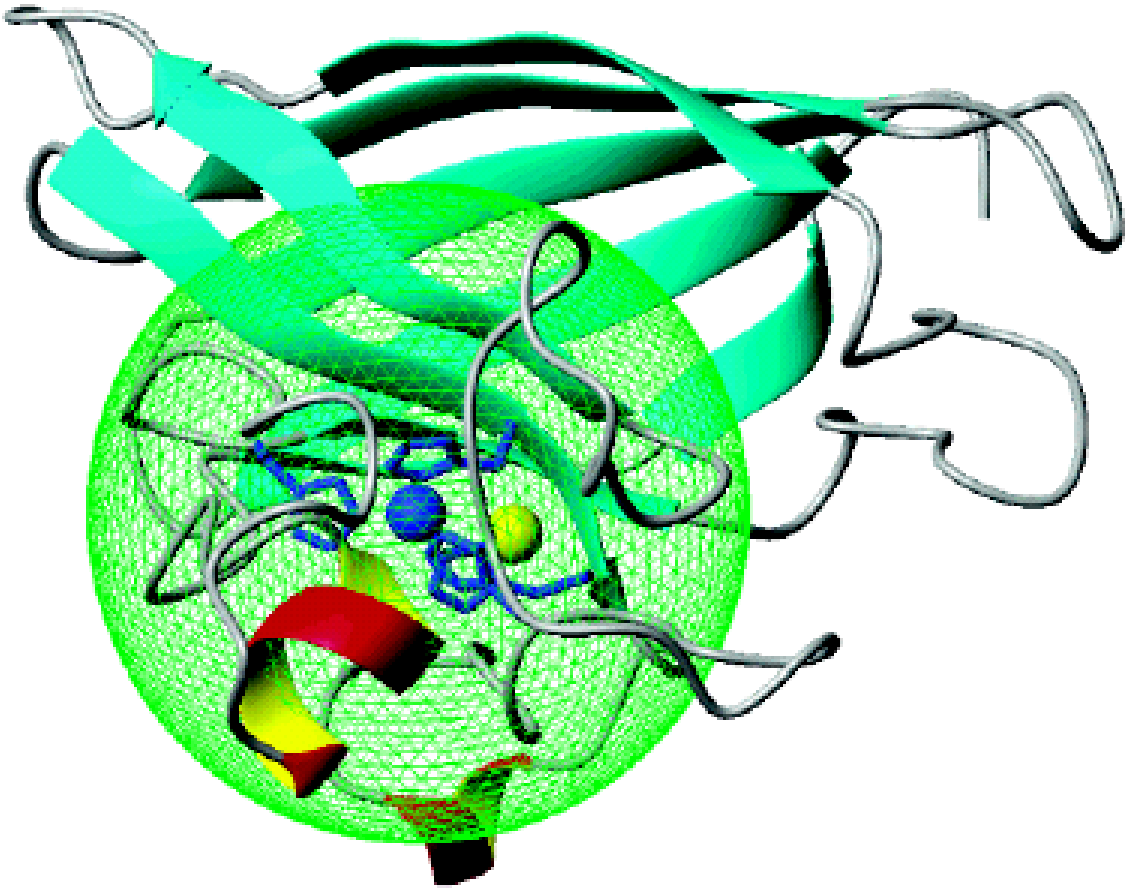


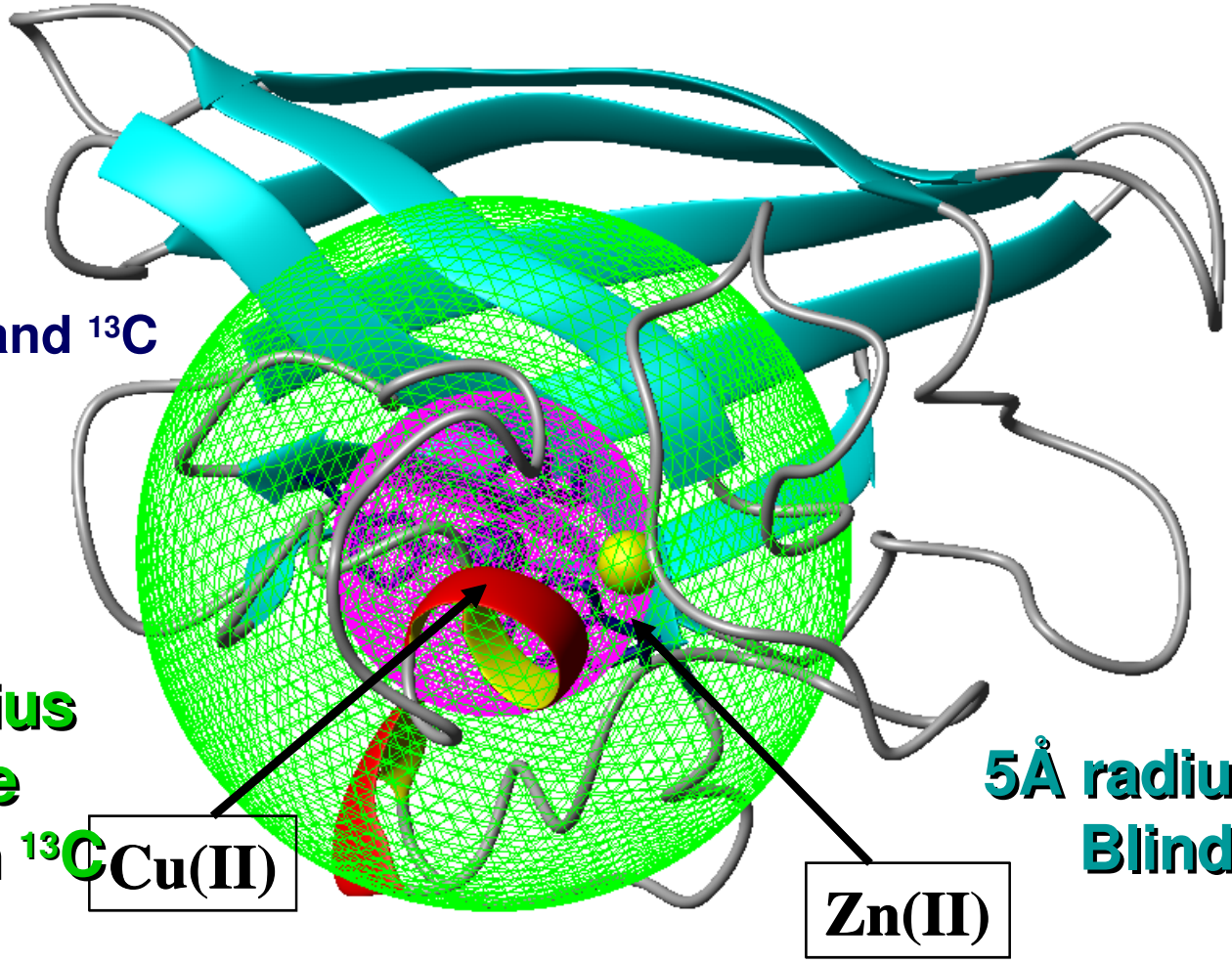
Why no protons?

Monomeric SOD:
152 amino acids



For Cu(II) no NH signals detectable within 11Å radius sphere around the metal!

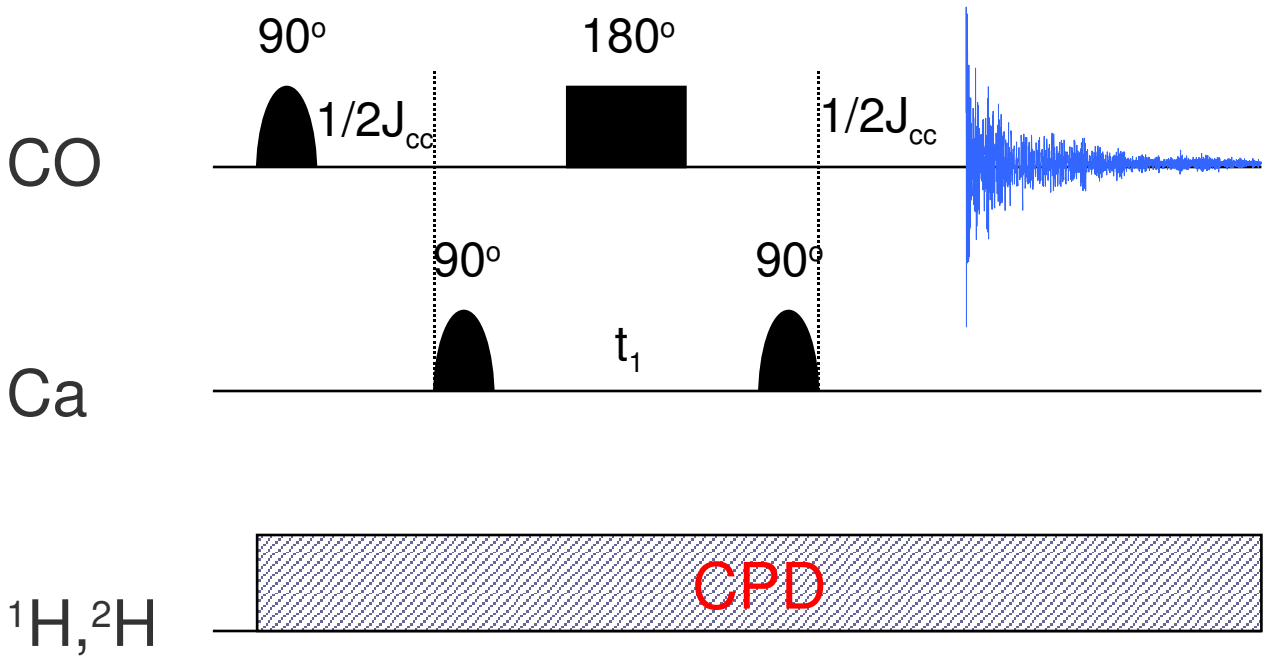
Why no protons?



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

Applications with ^{13}C-Detection

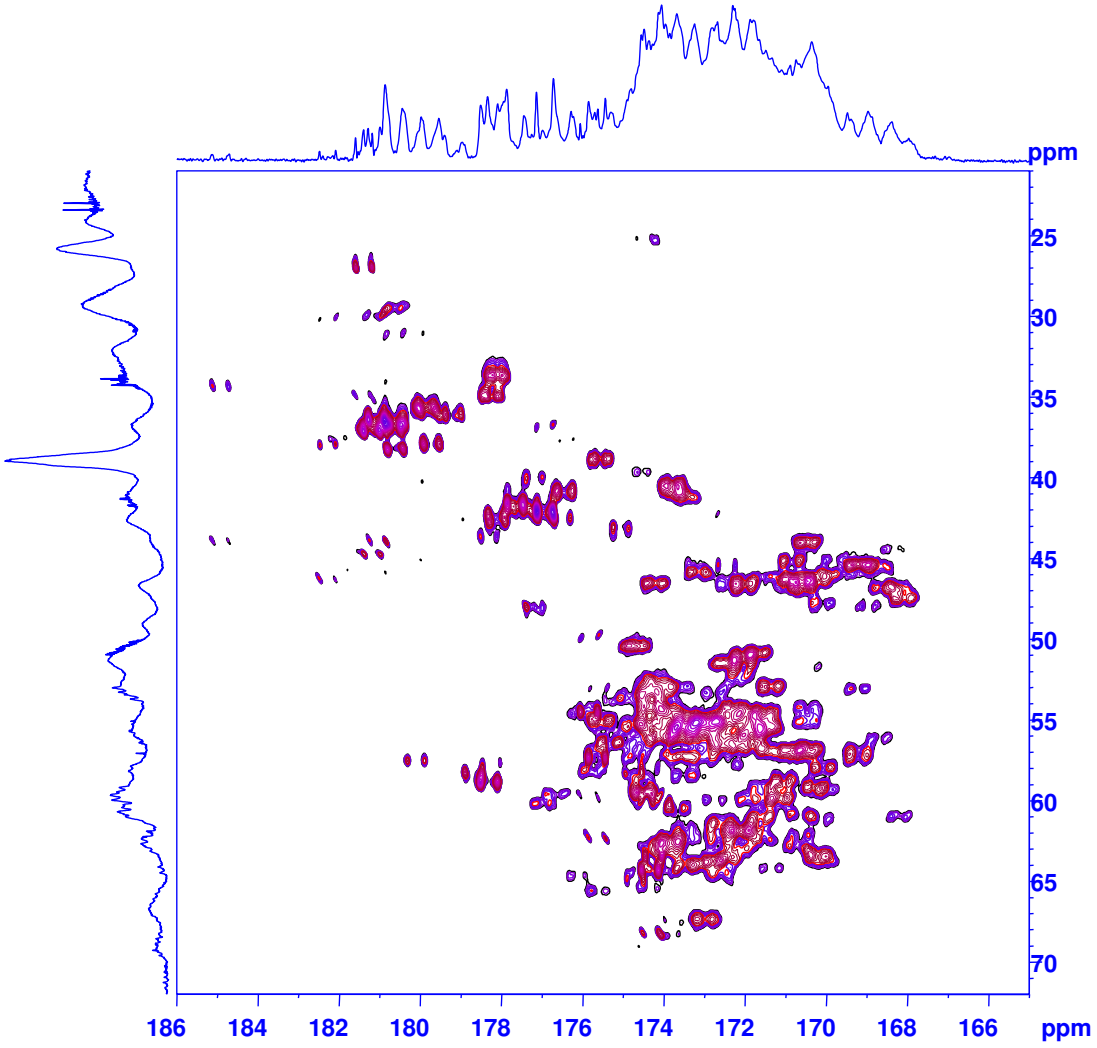
COCa MQ experiment



In collaboration with
I. Bertini,
I.C. Felli,
R. Pierattelli,
CERM, Florence, Italy

Applications with ^{13}C-Detection

2D COCa MQ correlation experiment on 2.5mM ^{2}H,^{13}C,^{15}N,-labelled SOD Dimer in reduced state, measured on a *Bruker 500MHz Dual ^{13}C{^{1}H} CryoProbe™*.



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CERM, Florence, Italy

Applications with ^{13}C-Detection

Any further advantages?

- **Increased molecular size:**
  - **transverse relaxation increased**
  - **longitudinal relaxation decreased**

➡ **CC transfer through NOE will become competitive with transfer through scalar couplings**

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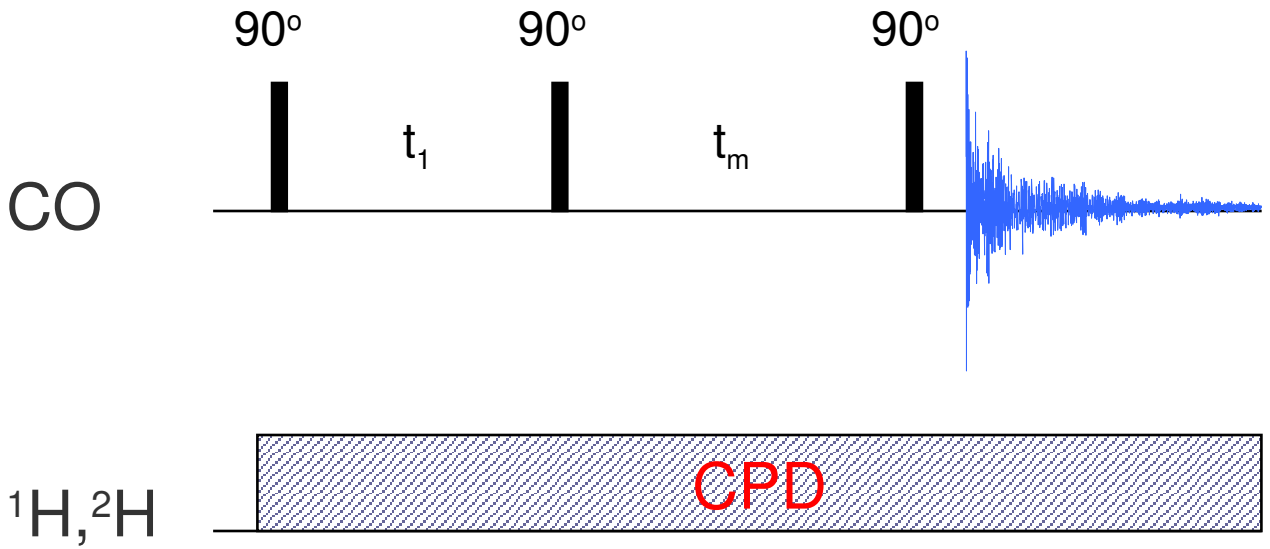
R. Pierattelli,
CERM, Florence, Italy

Applications with ^{13}C-Detection

CC NOESY experiment



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I. Bertini,
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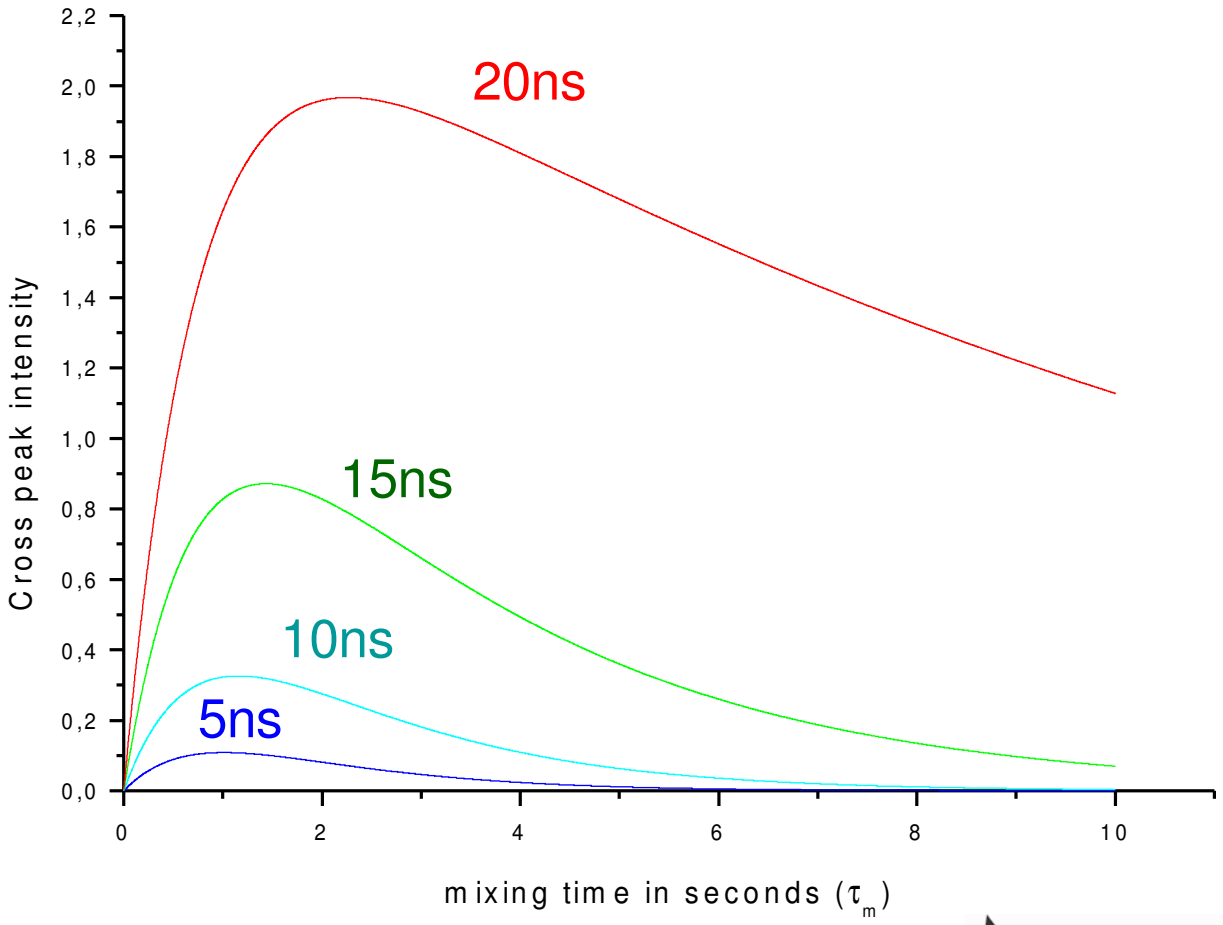
R. Pierattelli,
CERM, Florence, Italy

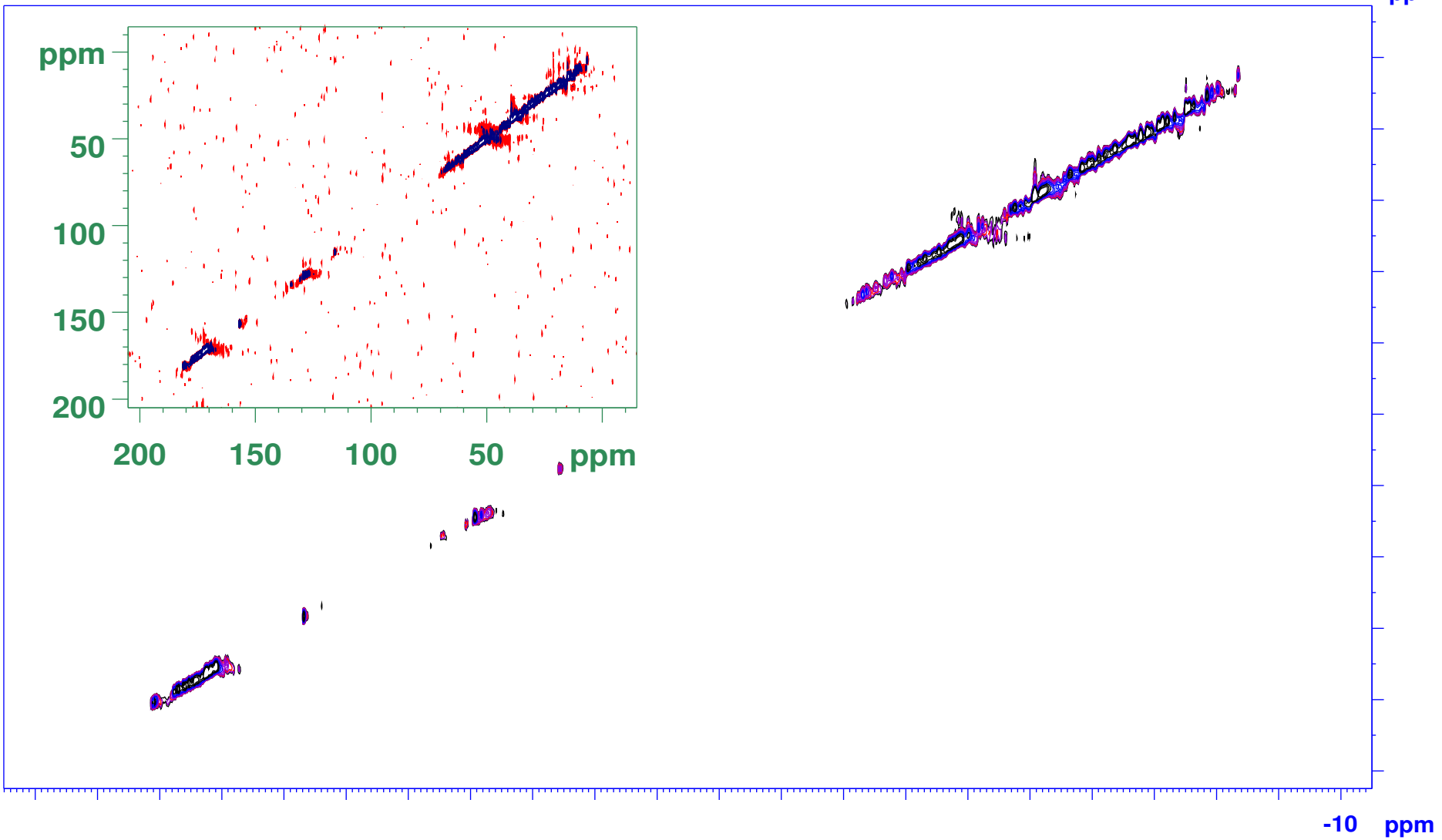
Applications with ^{13}C-Detection

Buildup of cross peak intensity in NOESY spectra simulation for correlation times of 5, 10, 15, 20 ns



In collaboration with
I. Bertini,
I.C. Felli,
R. Pierattelli,
CERM, Florence, Italy

RT TXI 700MHz



^{13}C-NOESY experiment

- ^{13}C-NOESY data published for large proteins only

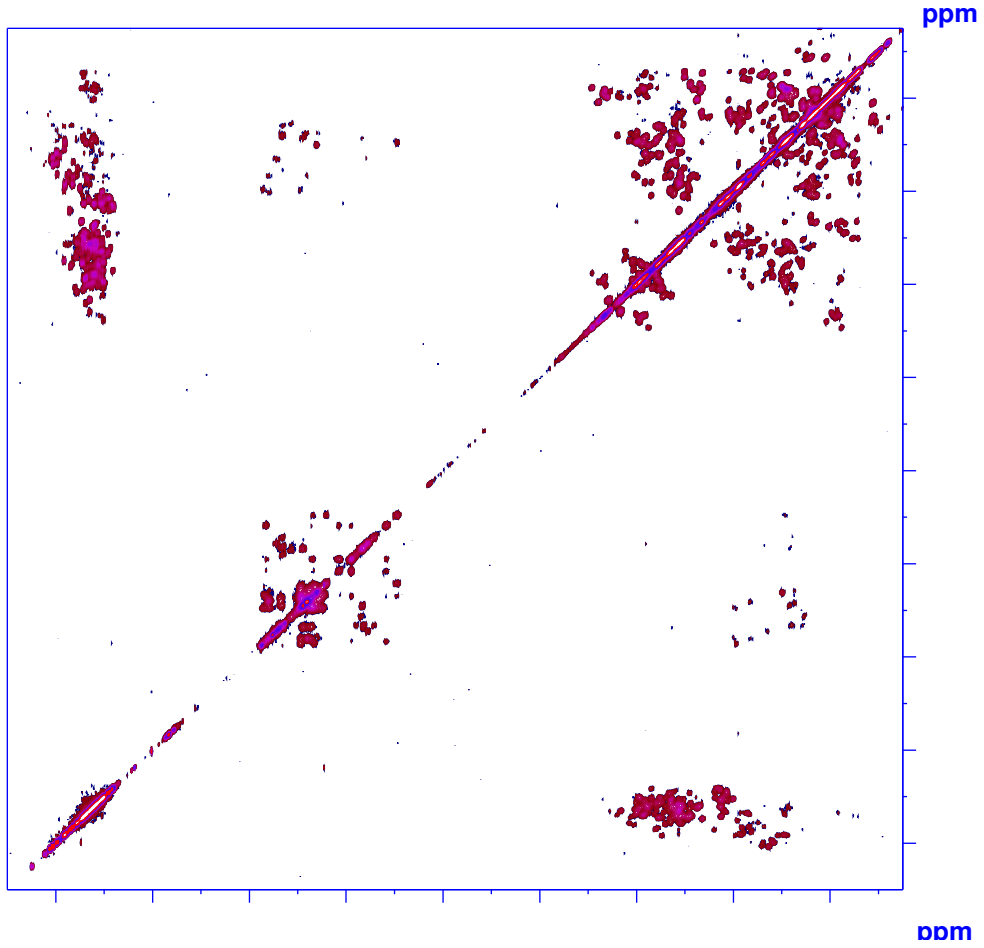
- No correlation peaks were observed on ubiquitin samples, neither for ^{1}H- or ^{2}H labeled samples (H. Kovacs, K. Pervushin et. al., unpublished results)

Applications with ^{13}C-Detection

13C-NOESY

experiment on 2.5mM ^{2}H,^{13}C,^{15}N,-labelled SOD Dimer in reduced state, measured on a *Bruker 500MHz Dual ^{13}C{^{1}H} CryoProbe™*.

NOESY mixing time: 800ms



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Applications with ^{13}C-Detection

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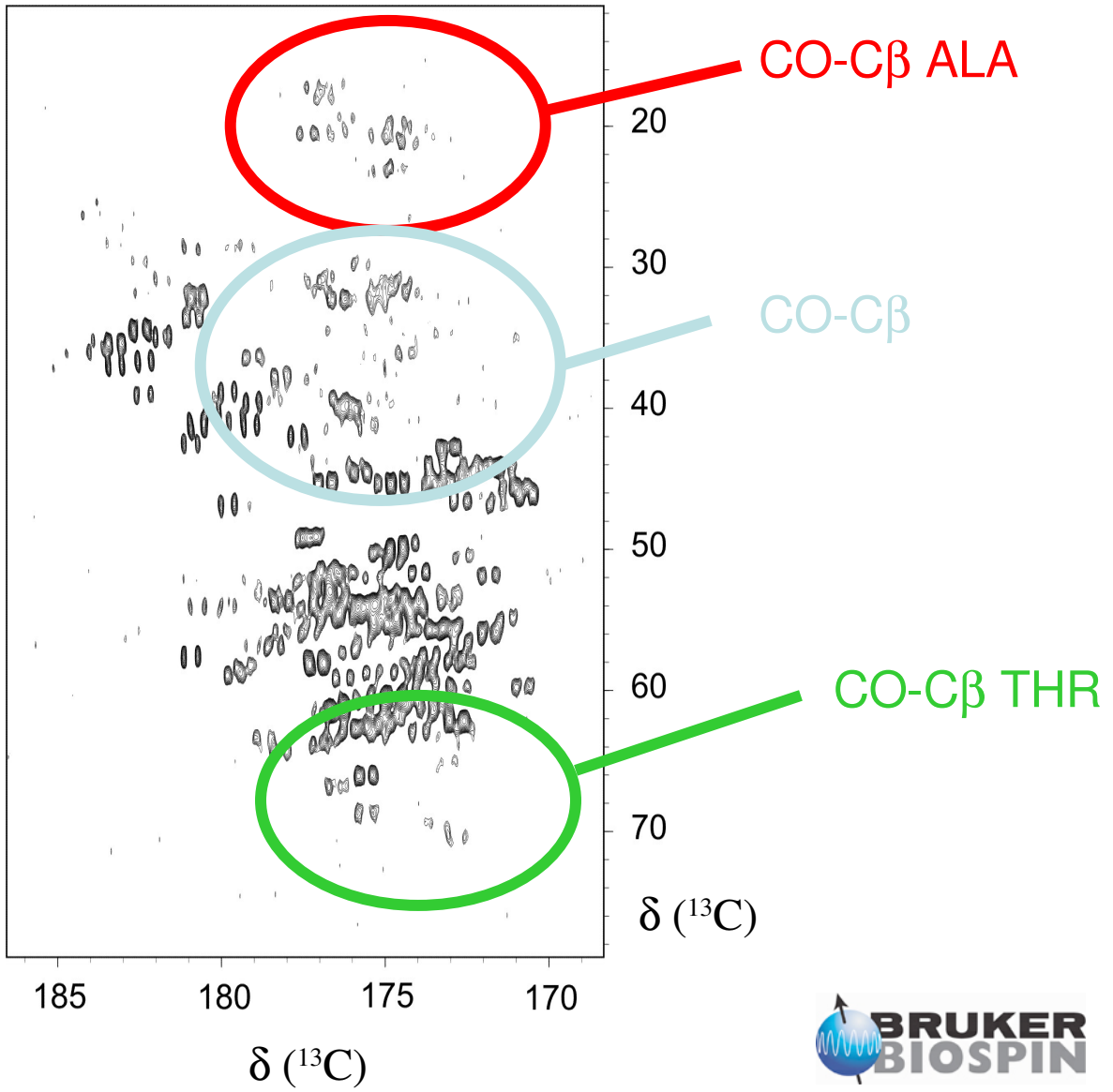
NOESY mixing time: 800ms



In collaboration with
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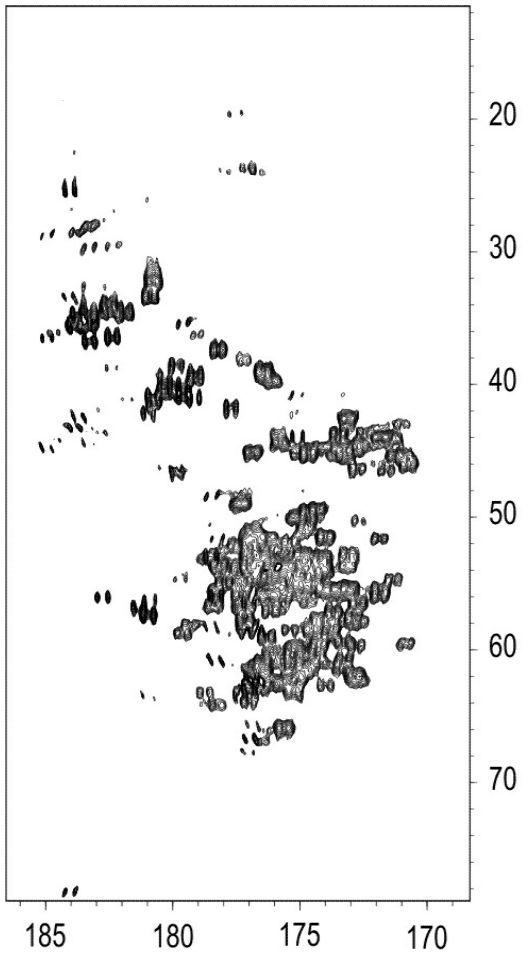
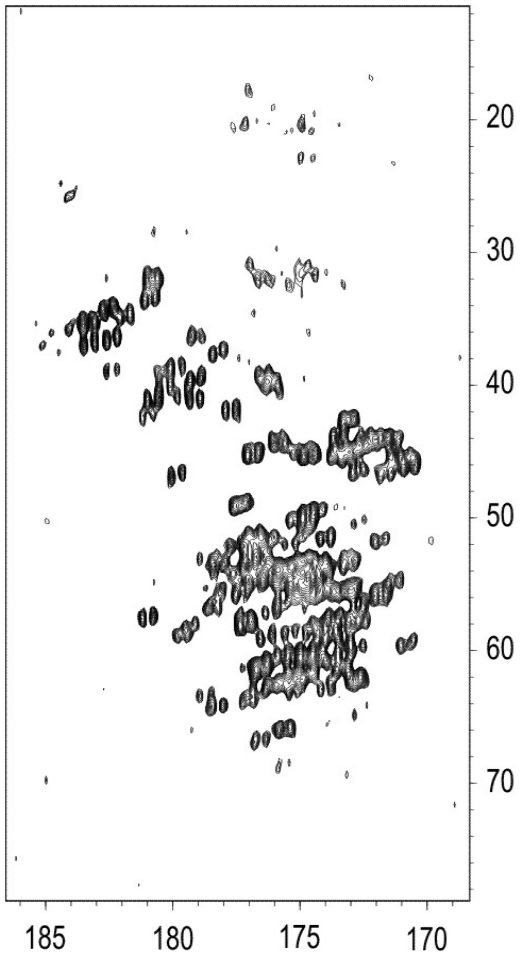
I.C. Felli,
R. Pierattelli,
CERM, Florence, Italy

Applications with ^{13}C-Detection

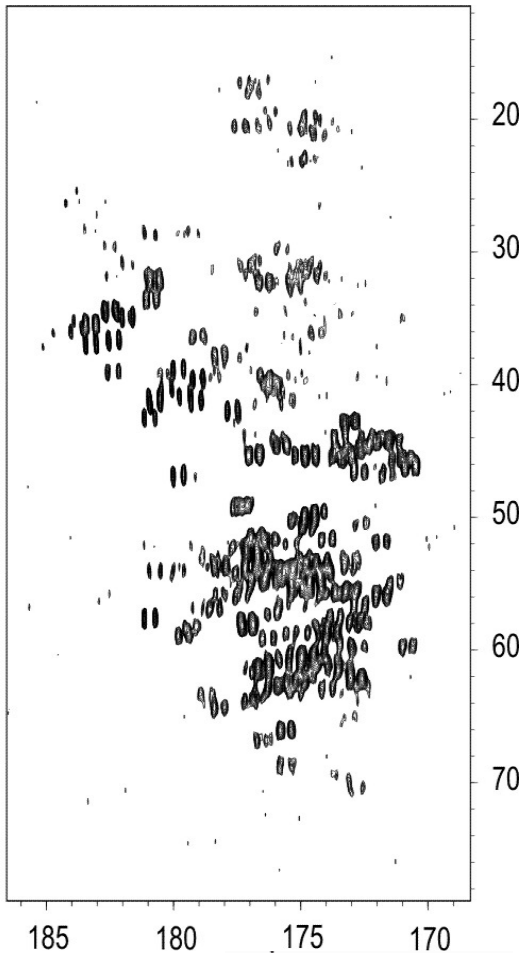
COCAMQ



NOESY 300ms



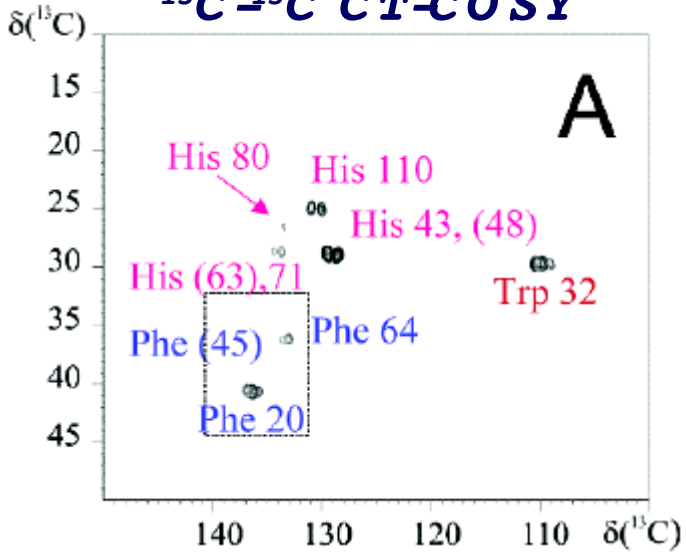
NOESY 800ms



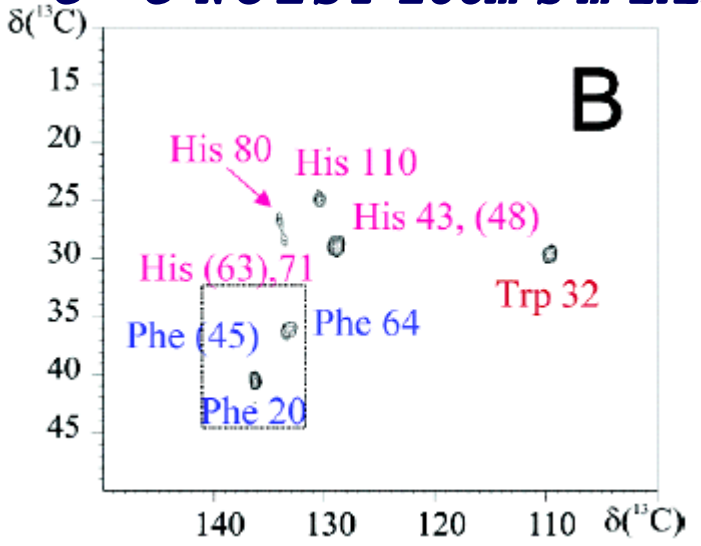
Protonless High Resolution Bio-NMR

BRUKER

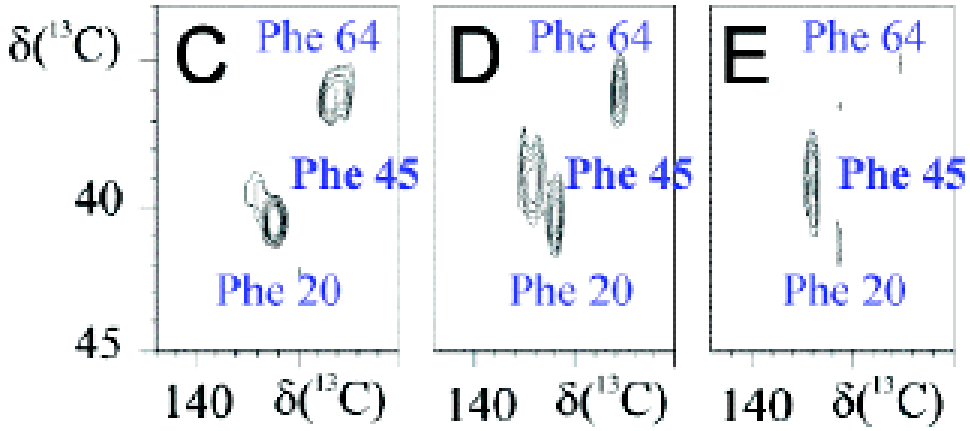
^{13}C–^{13}C CT-COSY



^{13}C–^{13}C NOESY 200m s mixing



^{13}C–^{13}C NOESY zoom

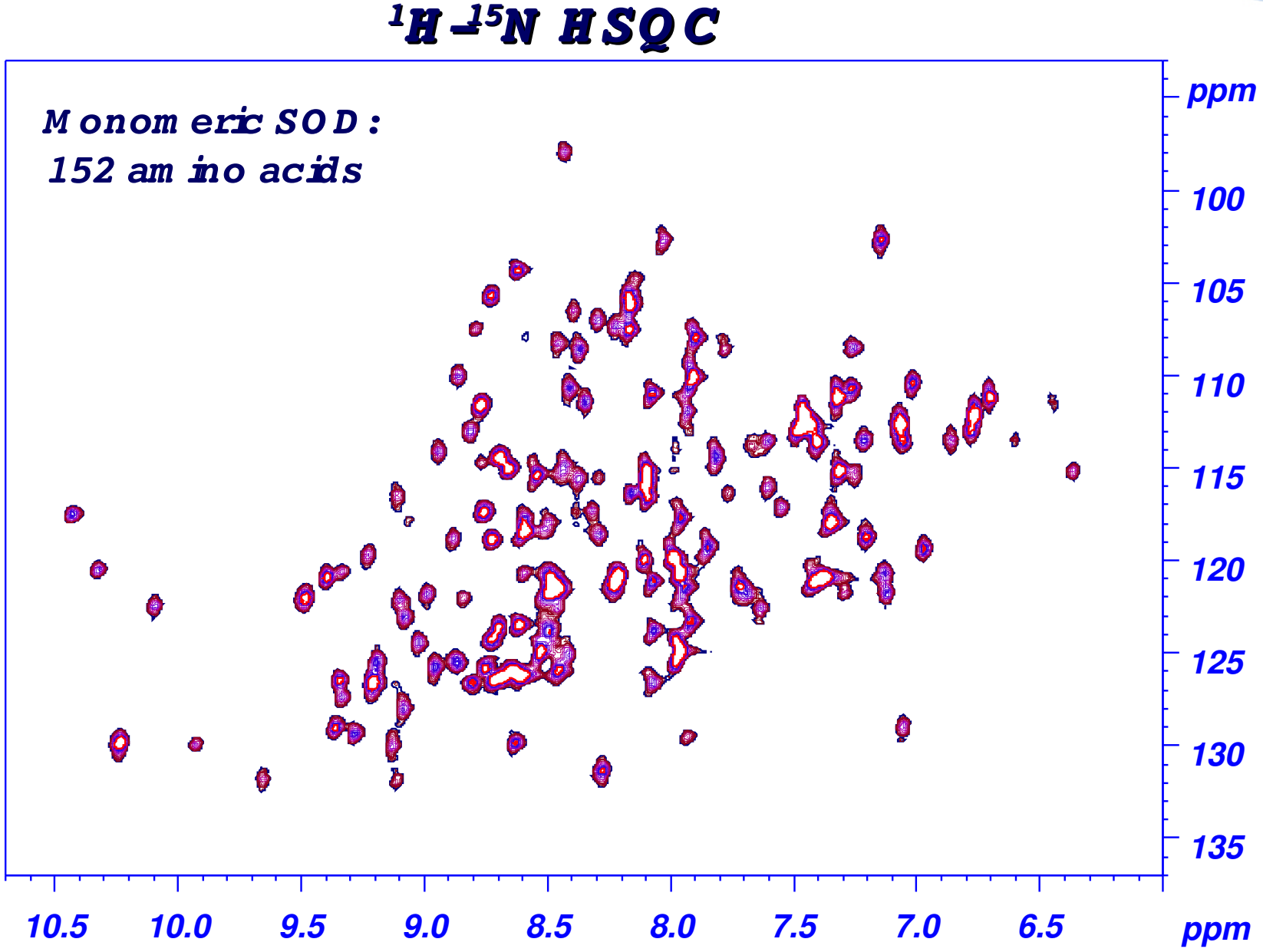


200m s 50m s 25m s

Table 1. Residues for Which the C′−Cα Connectivity Was Identified in the ^{13}C Observed 2D Experiments[a]

	residue no.	C′–Cu(II) (Å)	Cα–Cu(II) (Å)		residue no.	C′–Cu(II) (Å)	Cα–Cu(II) (Å)
	44	5.3−6.0	6.0−6.6		121	9.8−10.2	9.5−9.8
	45	4.6−5.7	4.9−6.4		123	8.7−9.7	9.5−10.5
	46	4.3−5.1	4.0−4.7		124	9.1−10.2	7.7−8.8
	47	5.6−6.1	5.9−6.6		133	9.6−11.4	10.3−12.3
	48[c]	7.3−7.6	5.8−6.1		134	9.5−11.3	9.9−11.6
	62	5.7−7.0	6−0−7.8	*b*	136	6.4−8.4	7.9−99
	64	8.8−10.0	8.2−9.2	*b*	137	5.2−6.8	5.2−6.9
	72	10.2−11.5	10.5−11.9		139[c]	8.8−10.0	9.1−10.2
	80	10.2−11.9	9.8−11.4	*b*	141	7.8−8.6	8.5−9.5
	83	8.2−8.9	7.4−8.2	*b*	142	7.4−8.4	8.2−9.3
	85	8.0−8.8	8.6−9.5	*b*	143	7.9−9.1	6.6−7.8
	86	10.0−11.0	8.9−9.8		144	9.7−11.2	9.5−10.8
	116	7.6−9.0	8.9−10.2		145[c]	8.2−9.9	9.2−10.9
	118	4.2−5.9	4.7−6.0		146[c]	9.6−11.2	8.4−10.0
b	119	5.2−6.8	5.2−7.2		147	10.6−12.4	10.6−12.3
b	120	7.4−7.8	5.9−6.4				

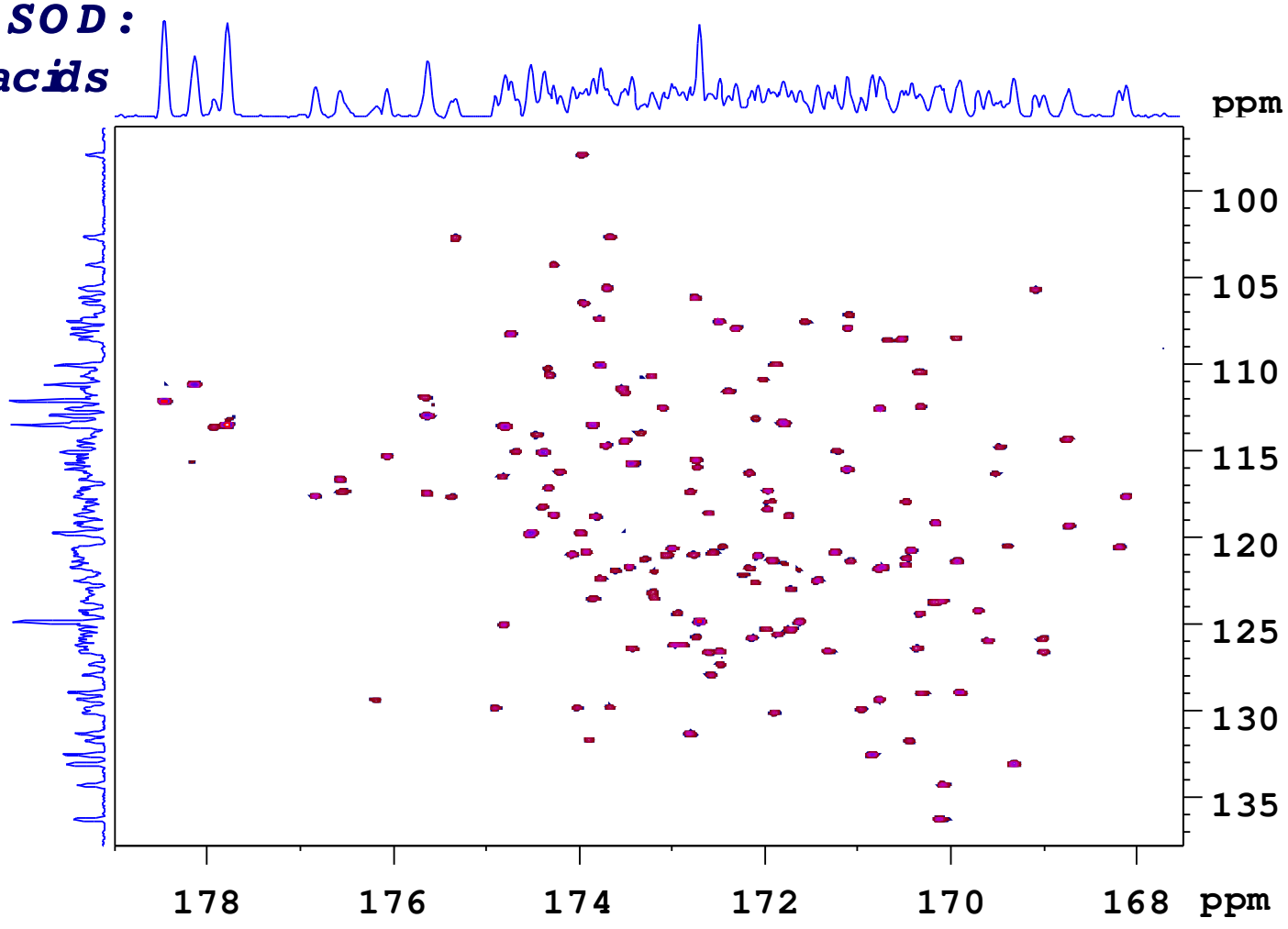
Protonless High Resolution Bio-NMR



Protonless High Resolution Bio-NMR

$^{13}C-^{15}N$ heteronucelar correlation spectrum

Monomeric SOD:
152 amino acids
ns = 16
3 hours
expt. time



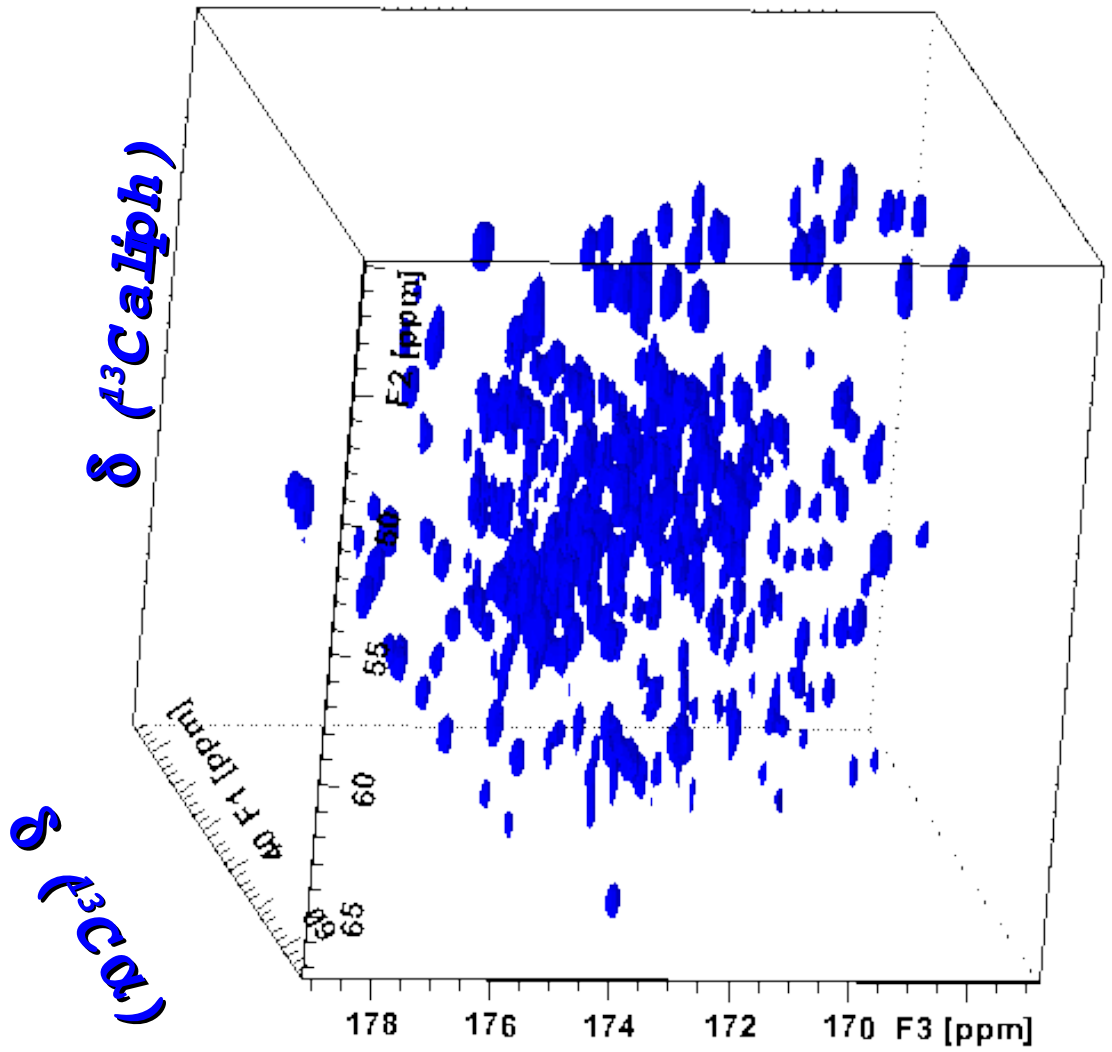
Protonless High Resolution Bio-NMR

ns= 8

TD :

800 x 32 x 128

21.5 hours



3D CBCACO-IPAP

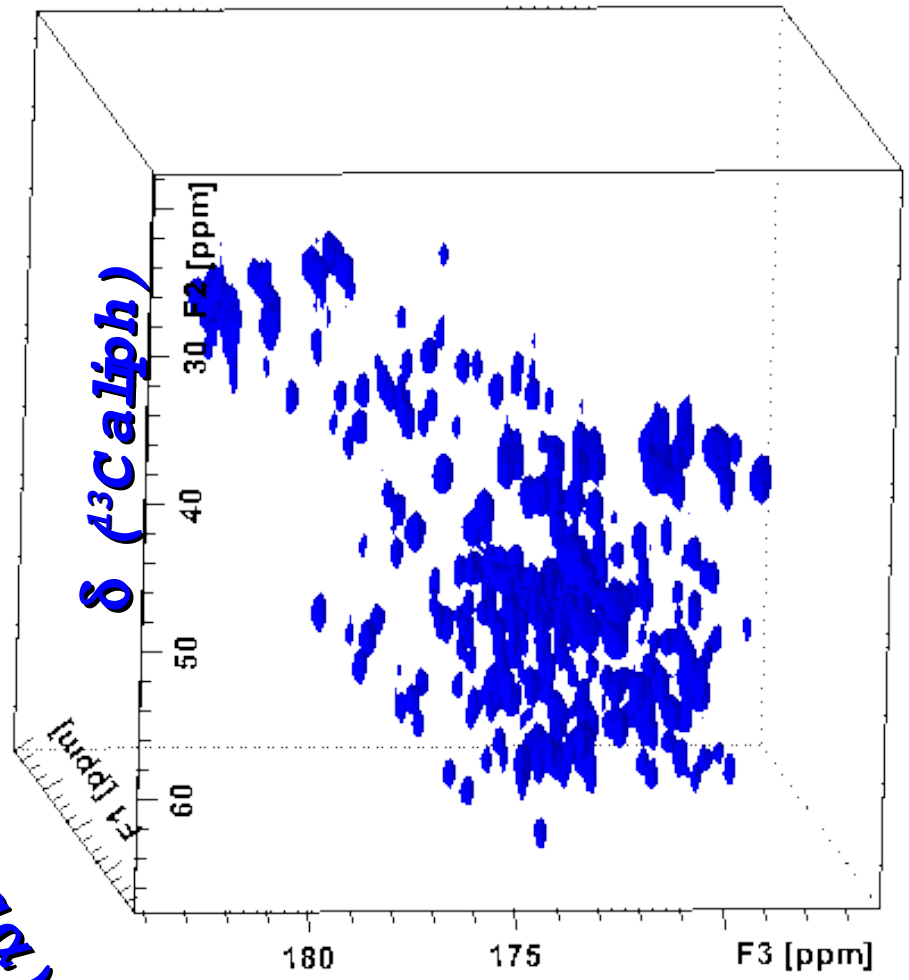
Protonless High Resolution Bio-NMR

ns= 32

TD :

800 x 32 x 128

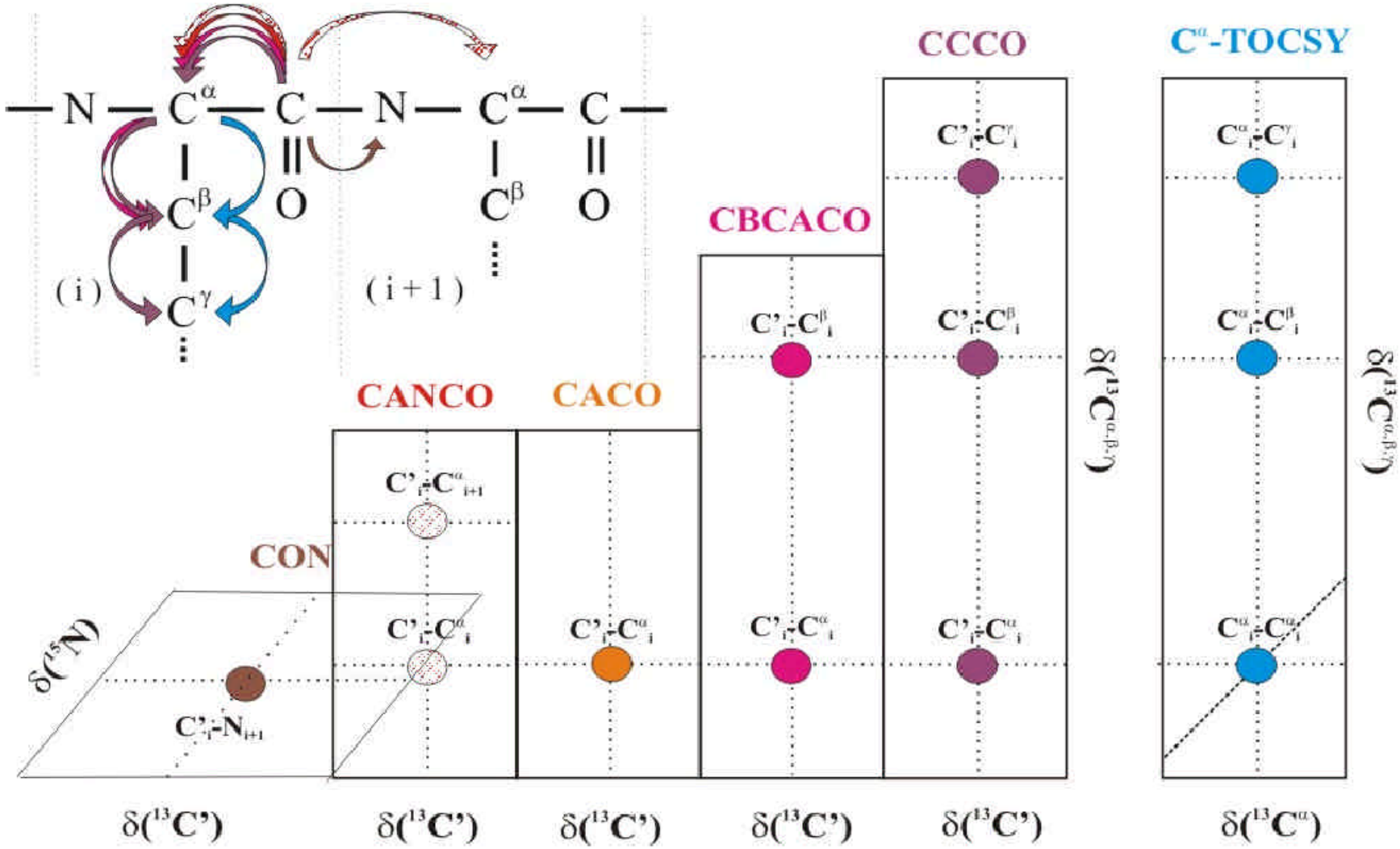
2 days 8 hours



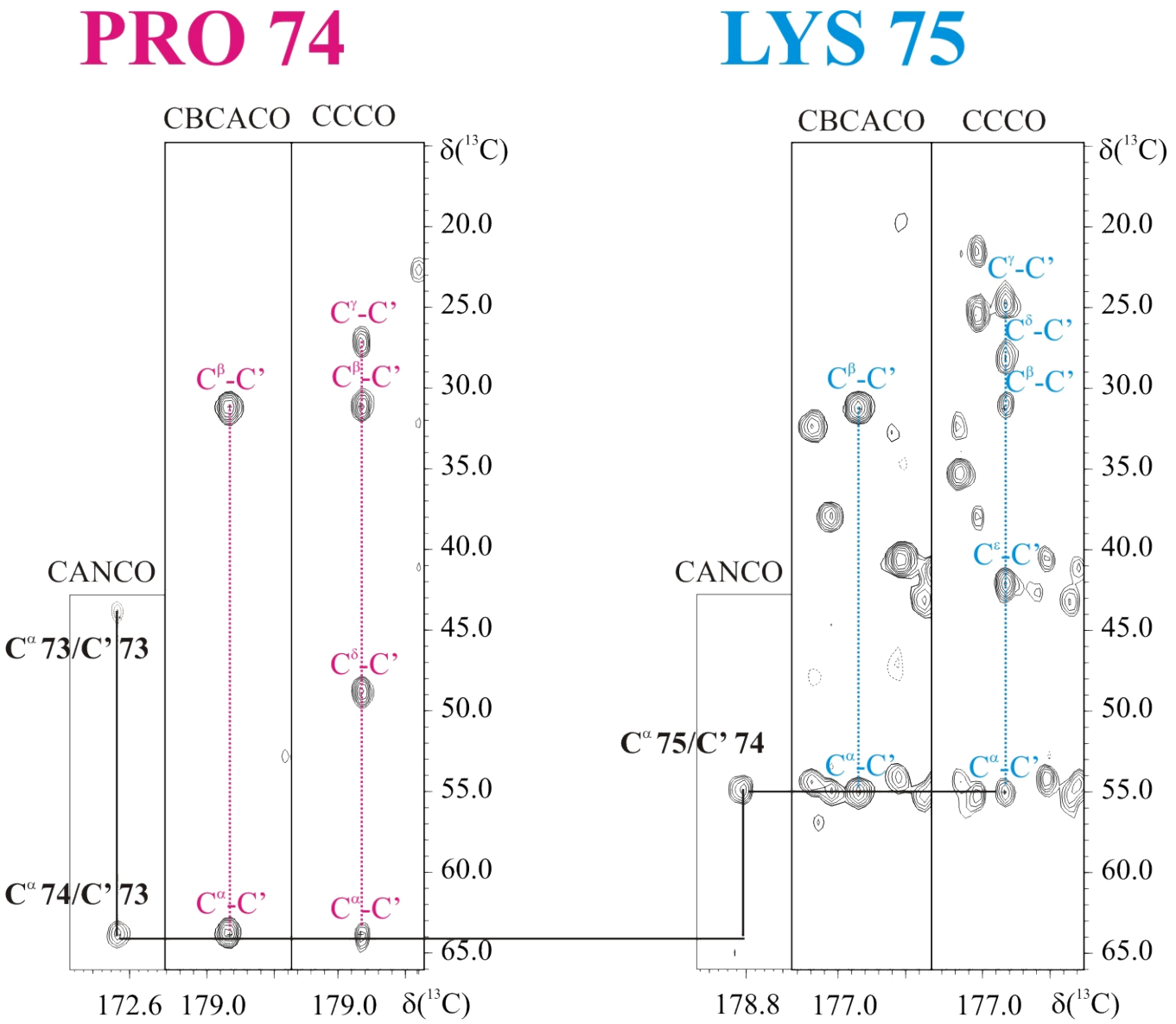
3D CCCO-IPAP

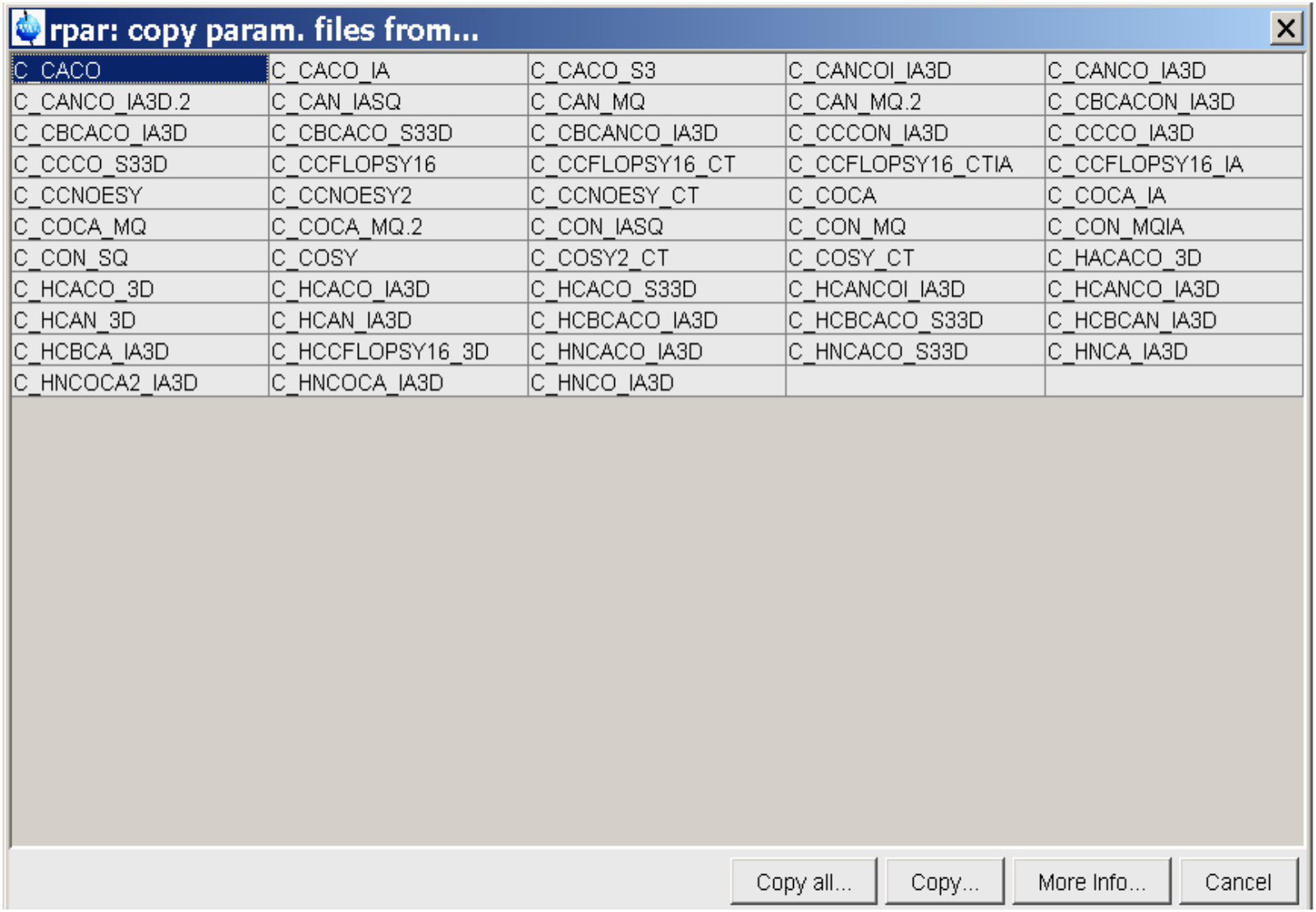
^{13}C detection: assignment strategy

Schematic assignment & walkthrough



Protonless resonance assignment strategy

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C_CACO_IA
C_CACO_S3
C_CANCOI_IA3D
C_CANCO_IA3D
C_CANCO_IA3D.2
C_CAN_IASQ
C_CAN_MQ
C_CAN_MQ.2
C_CBCACON_IA3D
C_CBCACO_IA3D
C_CBCACO_S33D
C_CBCANCO_IA3D
C_CCCON_IA3D
C_CCCO_IA3D
C_CCCO_S33D
C_CCFLOPSY16
C_CCFLOPSY16_CT
C_CCFLOPSY16_CTIA
C_CCFLOPSY16_IA
C_CCNOESY
C_CCNOESY2
C_CCNOESY_CT
C_COCA
C_COCA_IA
C_COCA_MQ
C_COCA_MQ.2
C_CON_IASQ
C_CON_MQ
C_CON_MQIA
C_CON_SQ
C_COSY
C_COSY2_CT
C_COSY_CT
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Acknowledgement

Helena Kovacs

Rainer Kümmerle