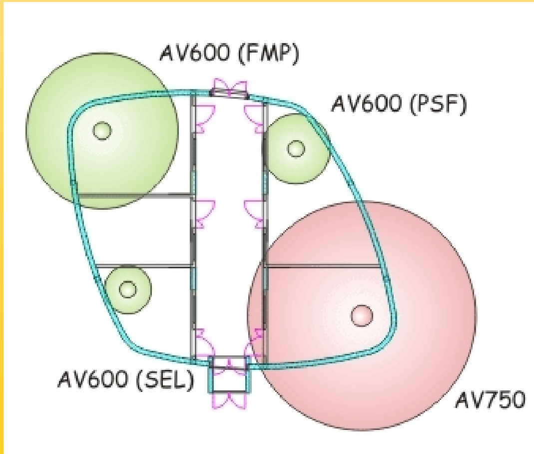
Remarks on methyl protonation in large molecules

Peter Schmieder



NMR on large proteins

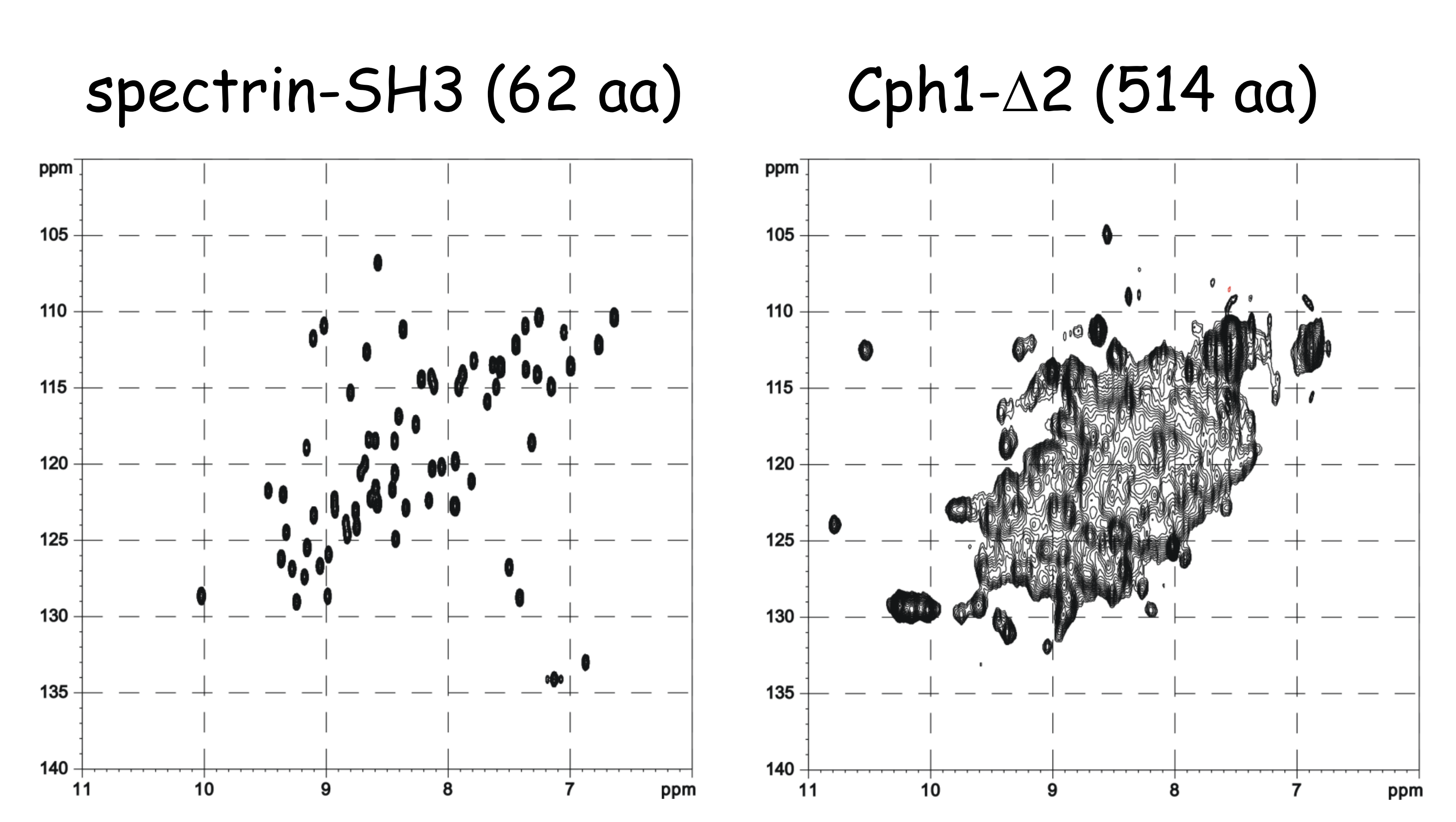
If the size of proteins under investigation by solution state NMR increases, several problems arise:

The number of resonances increases without an increase in spectral dispersion

The relaxation of the nuclei involved becomes increasingly efficient, the lines get broader and the transfer of magnetisation necessary during the NMR experiments becomes less efficient (since the J couplings do not change)

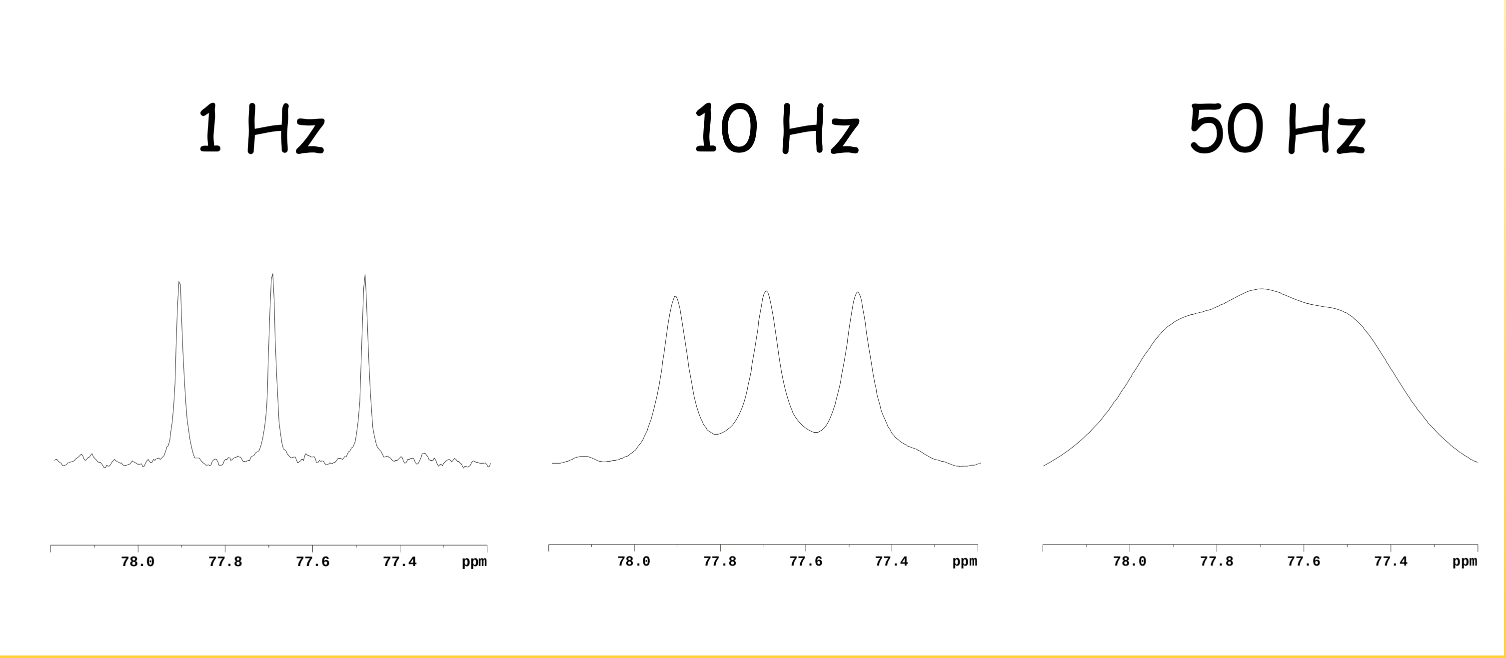
NMR on large proteins

The number of signals increases



NMR on large proteins

The lines get broader, making magnetization transfer inefficient



NMR on large proteins

Recipes against the increasing number of signals are higher dimensionality and selective labeling, those against increasingly faster relaxation are

deuteration

TROSY

Which can be used independently or jointly

Deuteration and TROSY

The main source of relaxation is different for the different nuclei involved

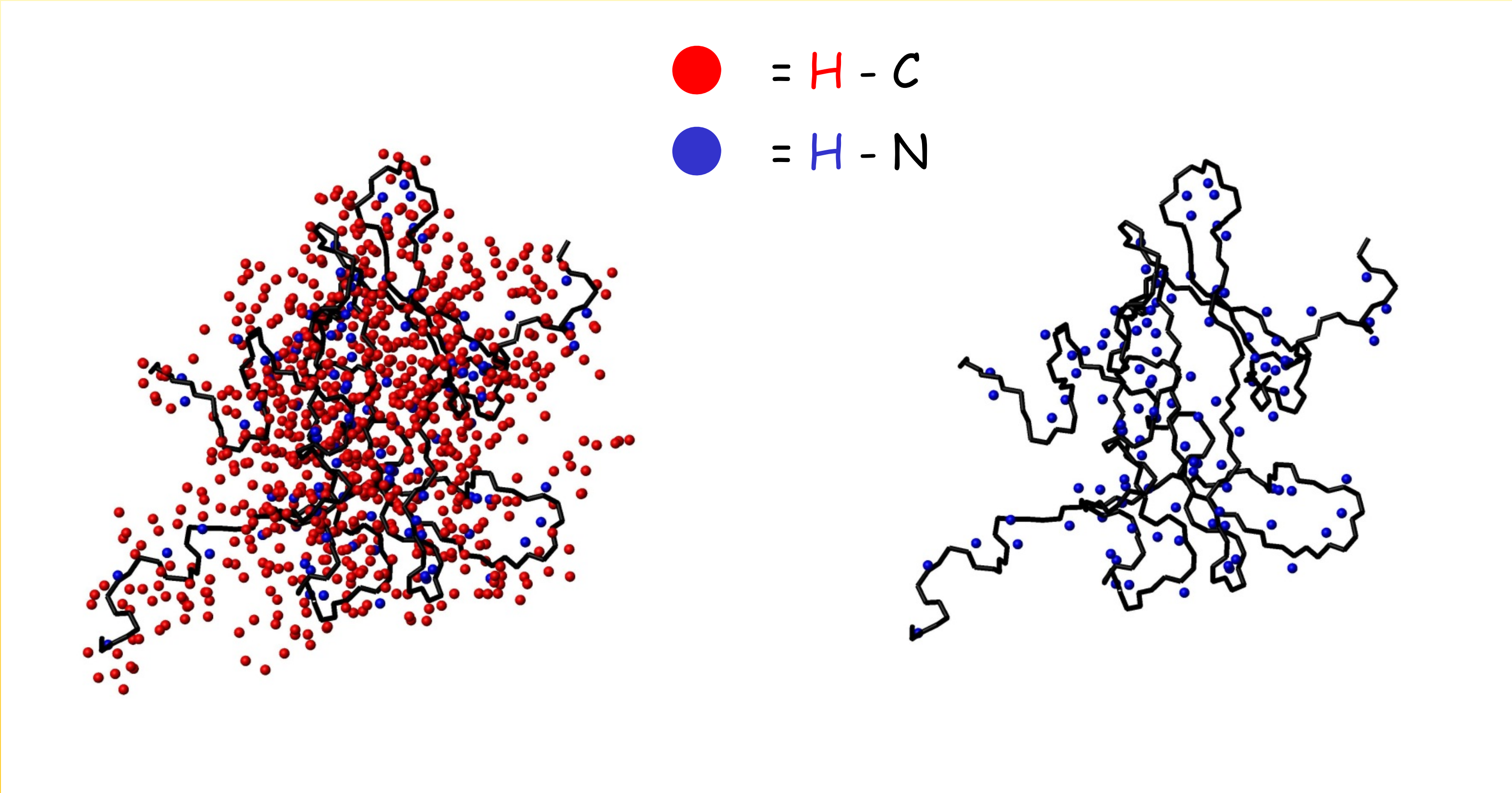
protons (^{1}H)	other protons
carbon (^{13}C)	directly bound proton
nitrogen (^{15}N)	directly bound proton

An important difference between the protons bound to carbon and to nitrogen is the exchange with water

Deuteration and TROSY

Deuteration

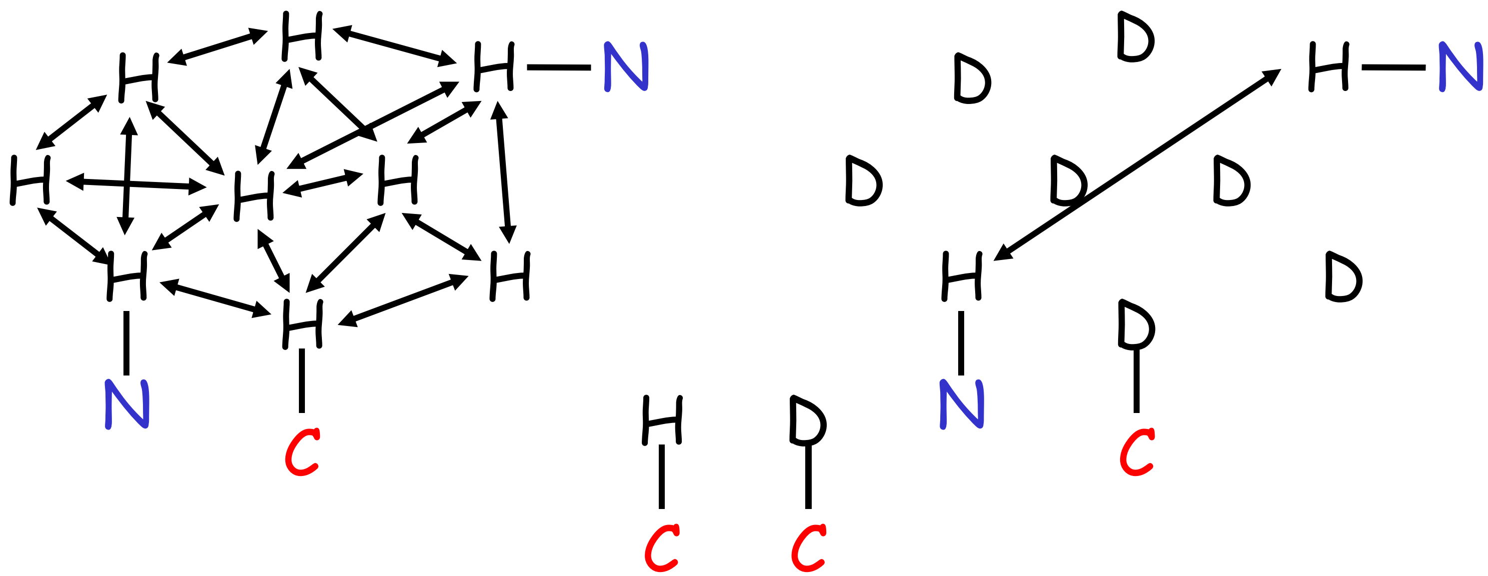
"normal" protein deuterated protein



Deuteration and TROSY

Deuteration

Proton-spins are diluted, the carbons loose their main relaxation source



$\gamma_D/\gamma_H \sim 1/6.5$

D=1 D=0,02

Deuteration and TROSY

But deuteration does not help with the nitrogen nuclei, they still have there main source of relaxation. With

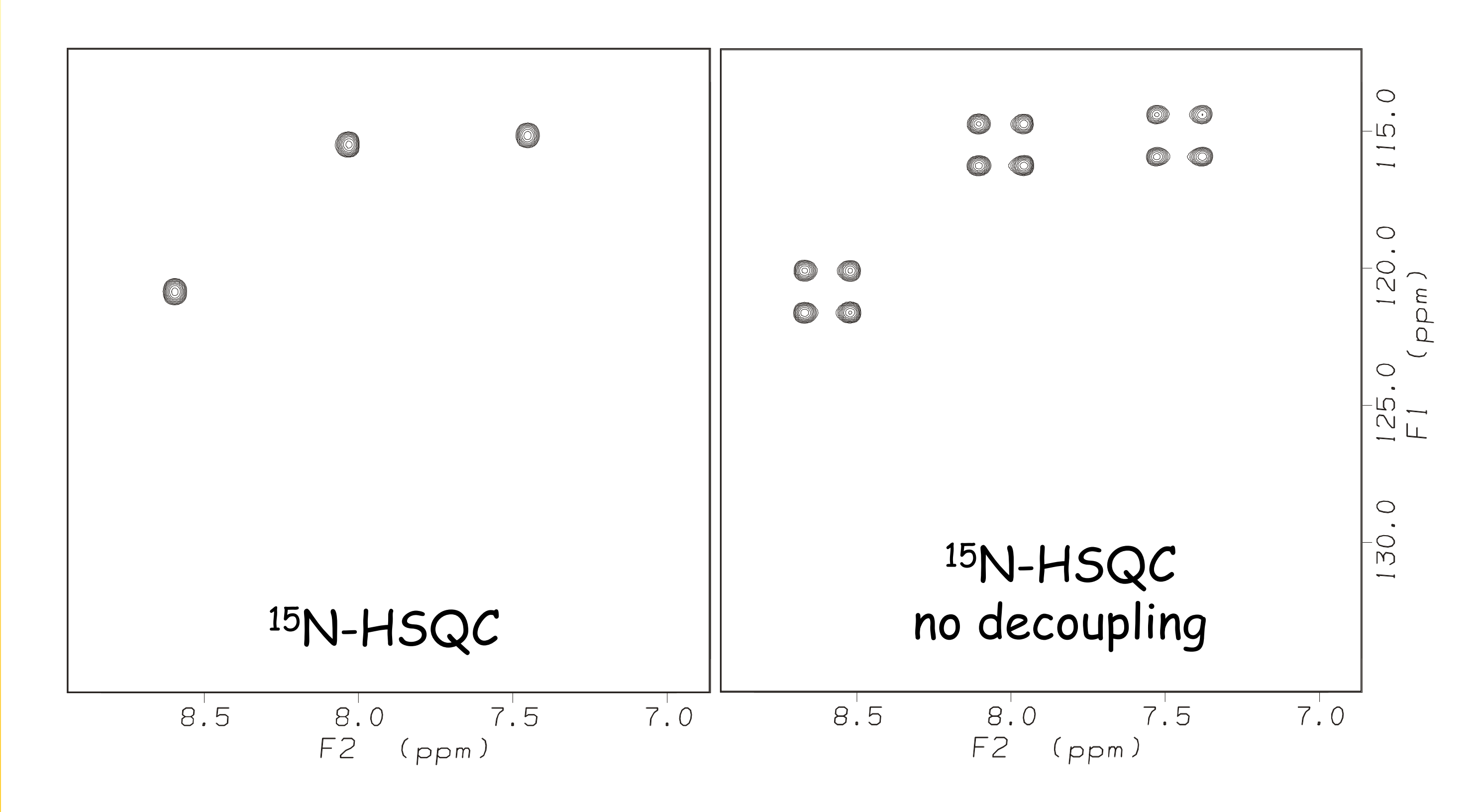
TROSY

(Transverse Relaxation Optimized SpectroscopY)

we use *relaxation interference* to obtain narrower lines for nitrogen and nitrogen-bound protons

Deuteration and TROSY

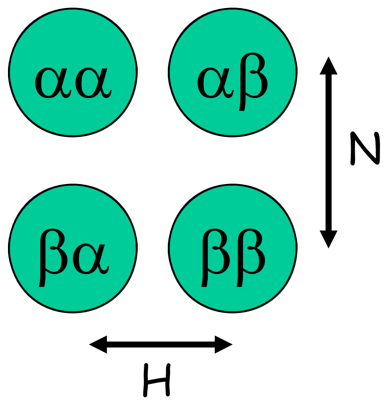
The interference between CSA and dipolar relaxation is difficult to see with small molecules



Deuteration and TROSY

But we know the four components of an HN-multiplet represent different spin states (α and β).

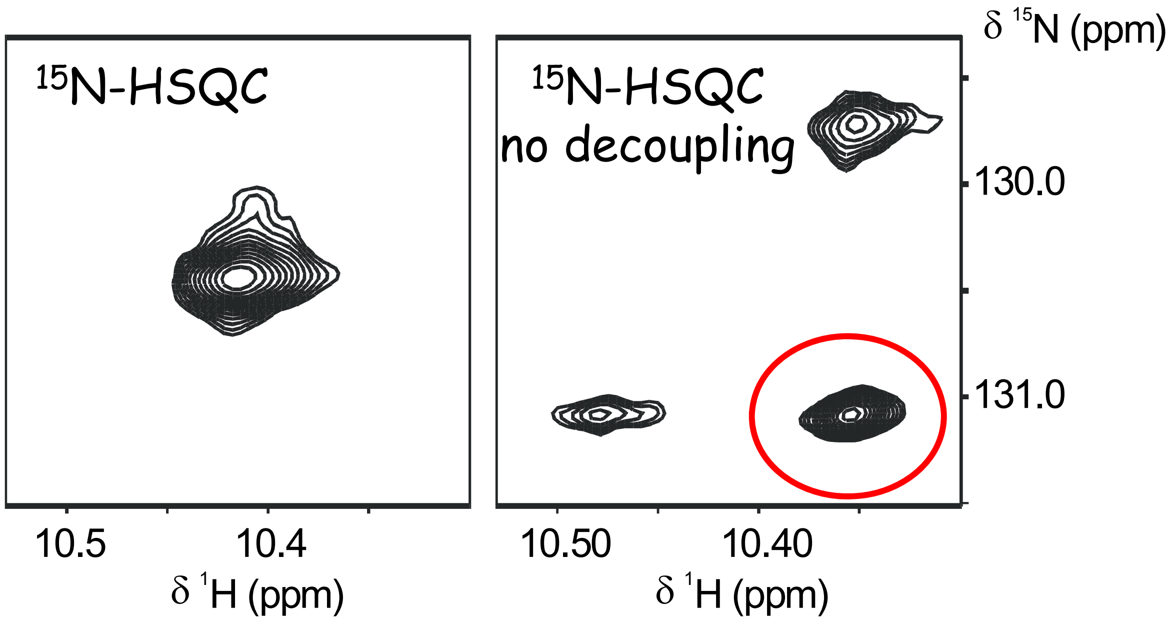
As it turns out the interaction between CSA and DD is different for each component



$$R_{\alpha H} \sim (D_{HN} + CSA_H)$$
$$R_{\beta H} \sim (D_{HN} - CSA_H)$$
$$R_{\alpha N} \sim (D_{HN} + CSA_N)$$
$$R_{\beta N} \sim (D_{HN} - CSA_N)$$

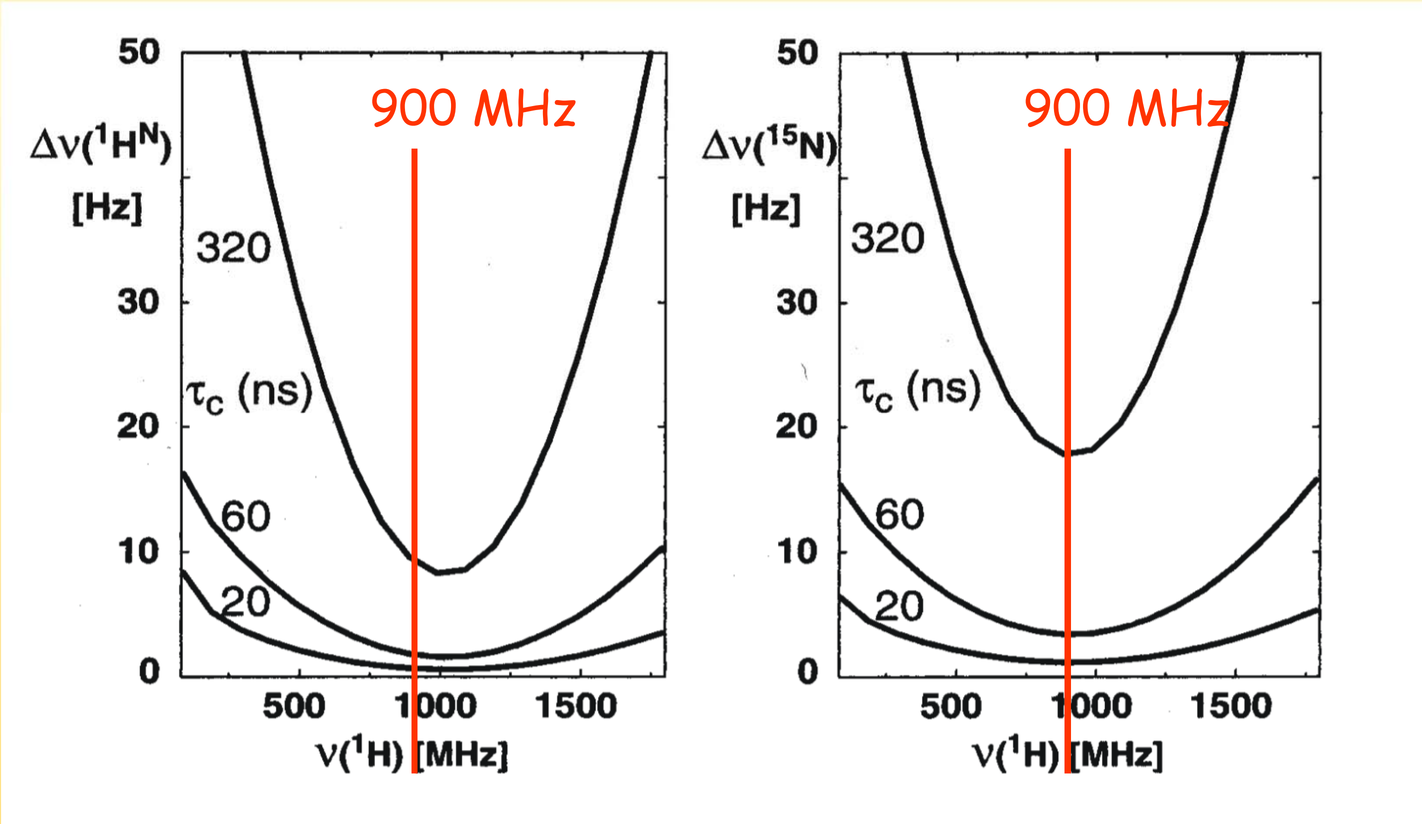
Deuteration and TROSY

And indeed: with large molecules one line each of ^{1}H and ^{15}N is narrow, so one of the four components will be particularly narrow as well



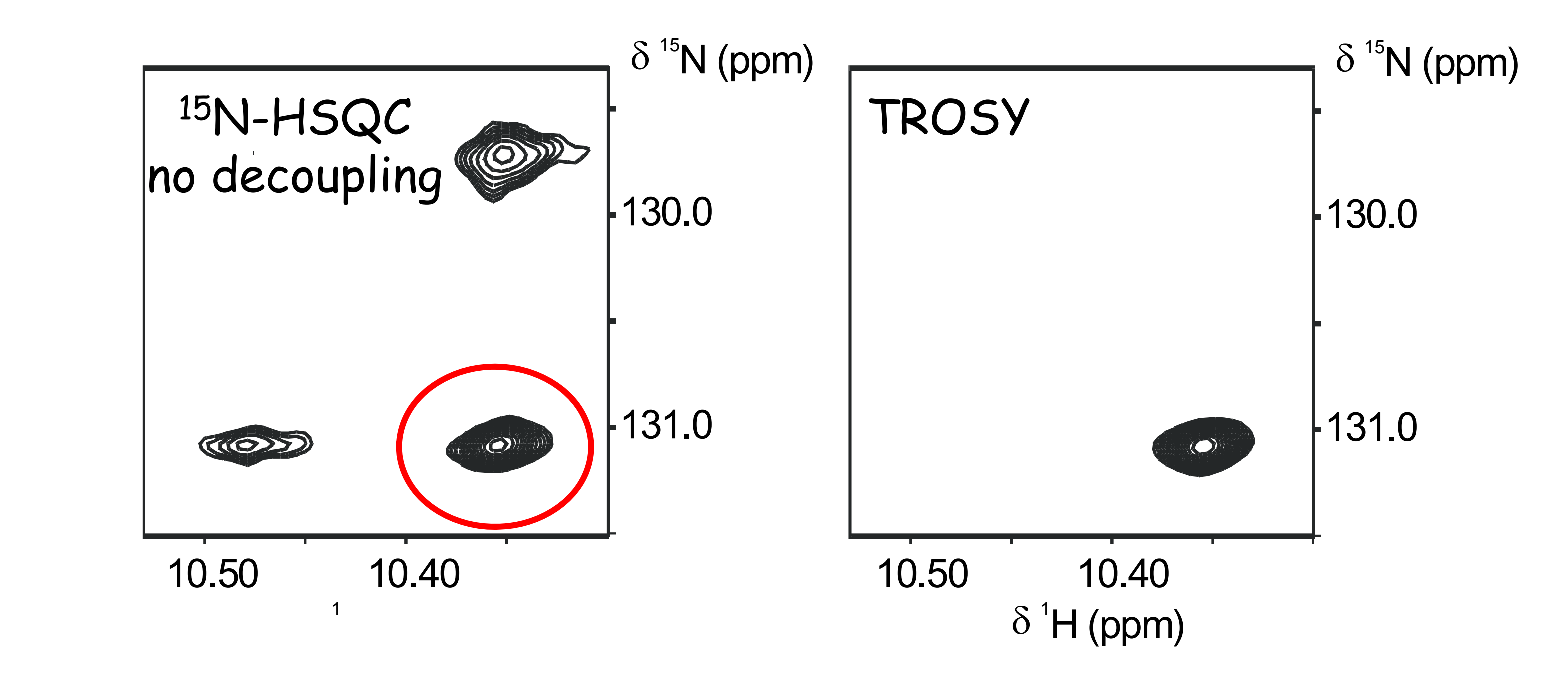
Deuteration and TROSY

The rates are field-dependent and thus the maximum effect is achieved at 950 MHz



Deuteration and TROSY

To obtain a TROSY- spectrum we have to eliminate three lines which can be done by appropriate phase cycling



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AG NMR

Methyl protonation: precursors

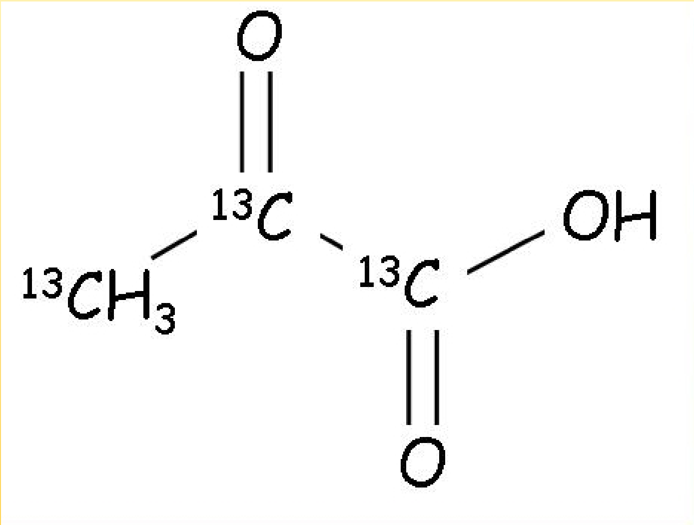
But having thus eliminated all the carbon bound protons there is also the problem of a lack of restraints in structure calculation

An initial solution was a deuteration of 75%, but that caused problems with isotopomers and sensitivity

A better solution was methyl protonation

Methyl protonation: precursors

The original idea was to use pyruvate



Labeling of

Val

Leu

Ile (γ-CH_3)

Ala

at different

amount

+ scrambeling

Rosen et al. *J.Mol.Biol.* **263**, 627-636 (1996)

Methyl protonation: precursors

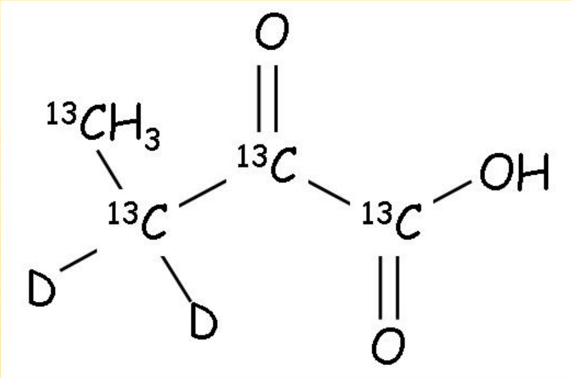
To avoid scrambeling other keto acids were used

Labeling of

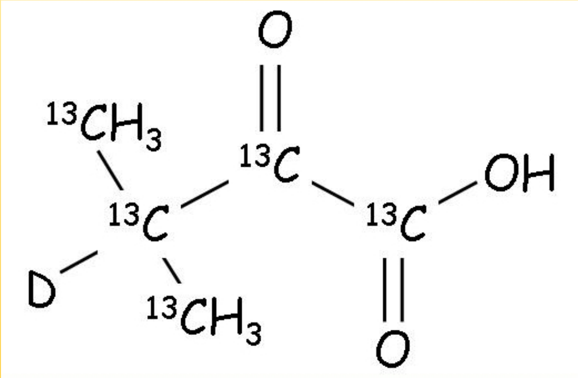
Val

Leu

Ile (δ-CH_3)



α-keto butyric acid



α-keto valeric acid

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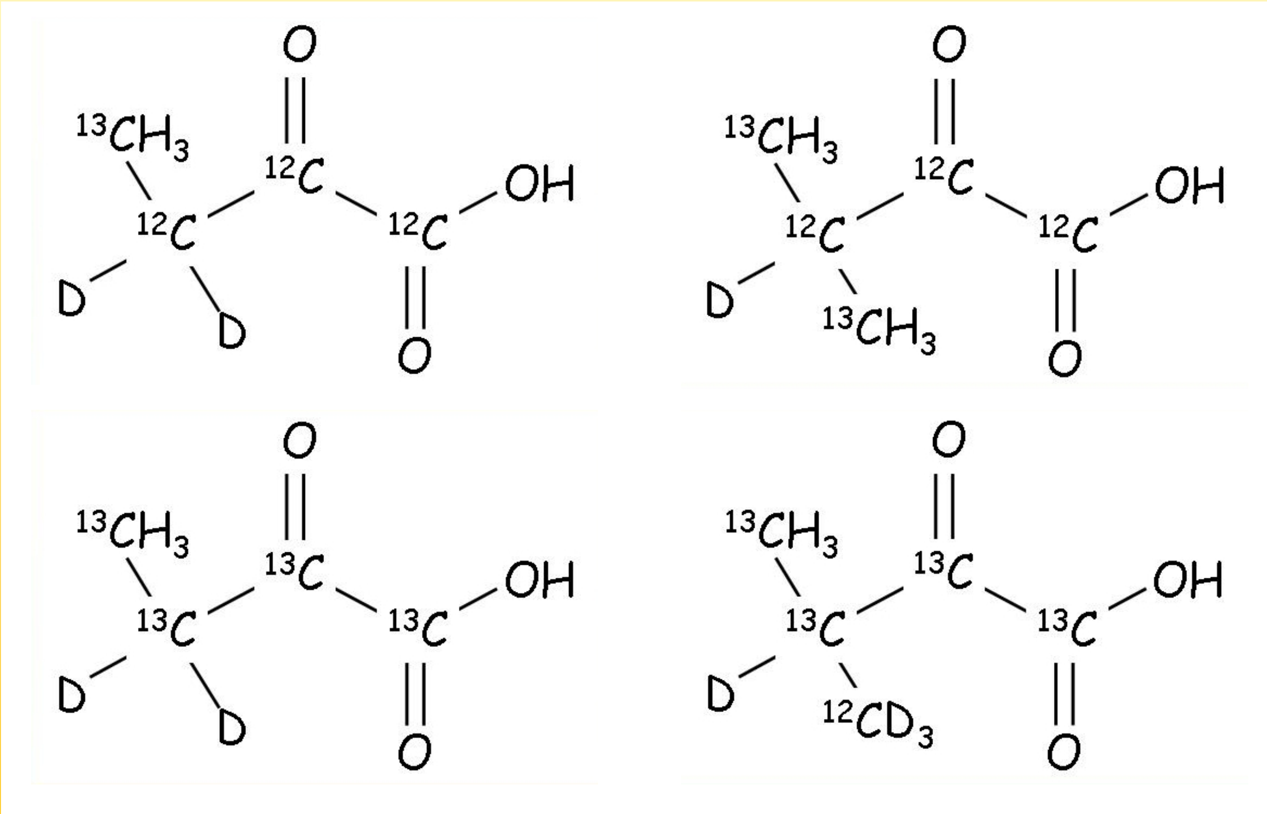
Methyl protonation: precursors

Other pattern

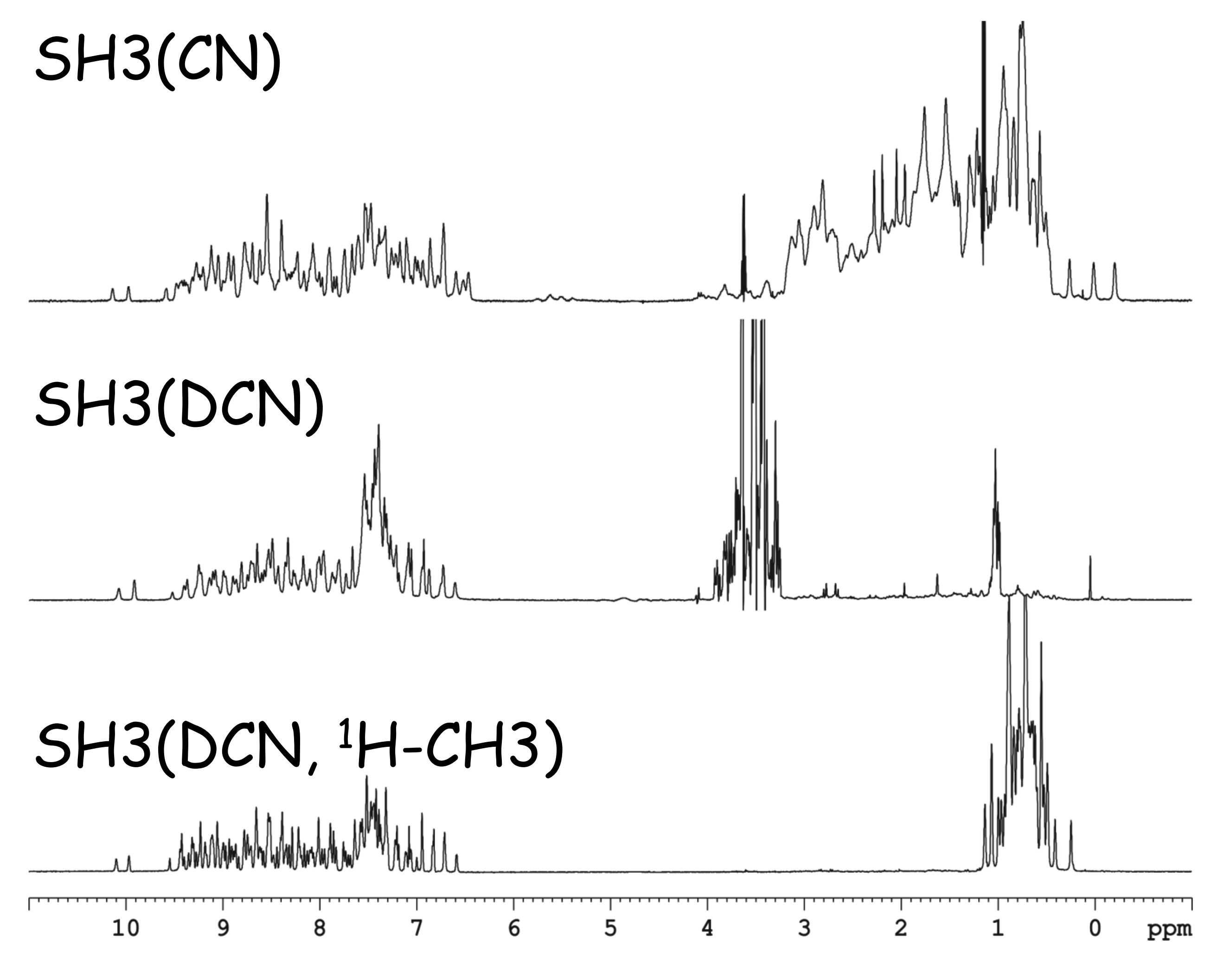
α-keto butyric acid   α-keto valeric acid



relaxation studies

linear chain

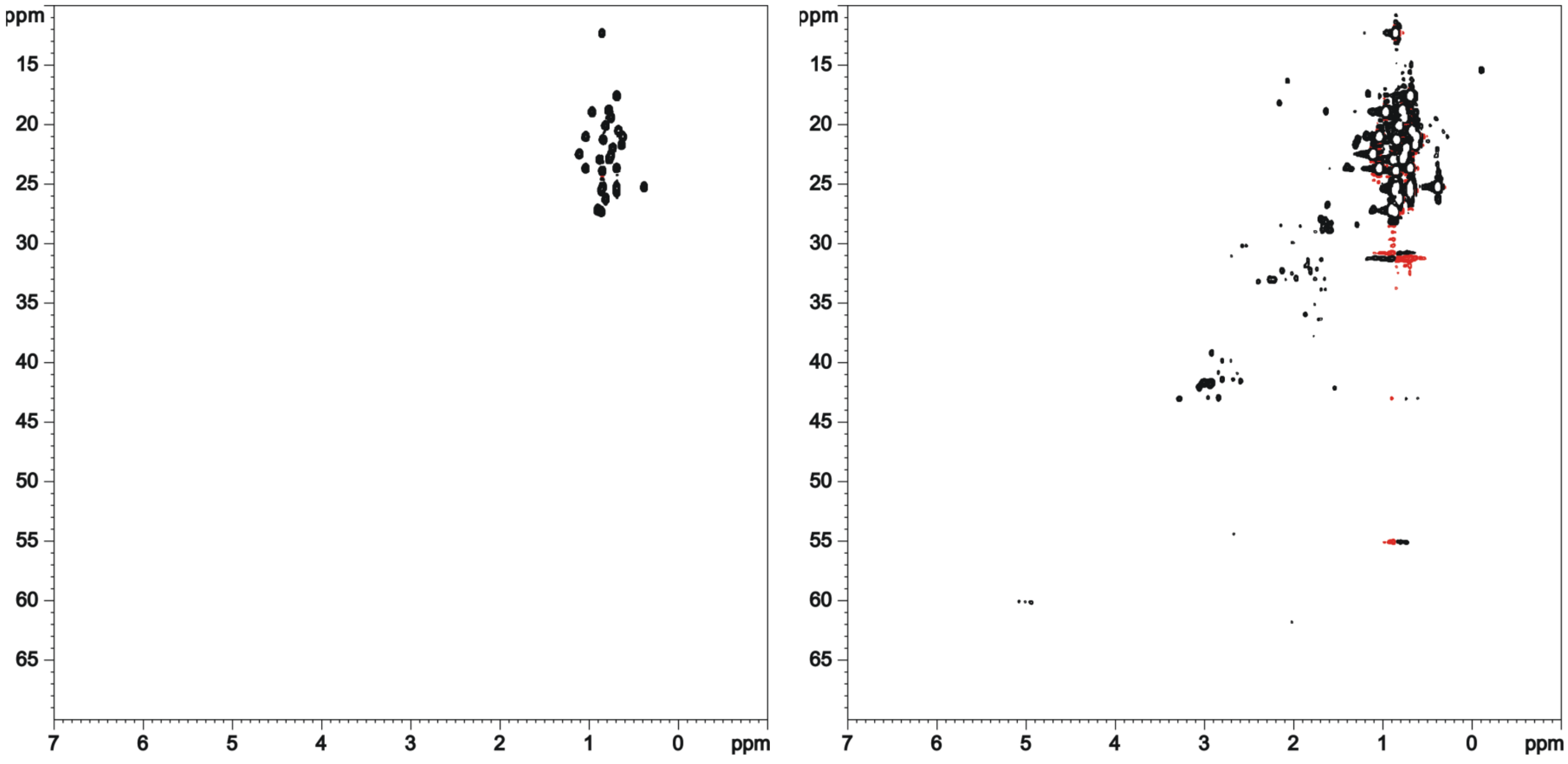
Methyl protonation: SH3



Methyl protonation: SH3

SH3(DCN, ^{1}H-CH3)

The degree of deuteration



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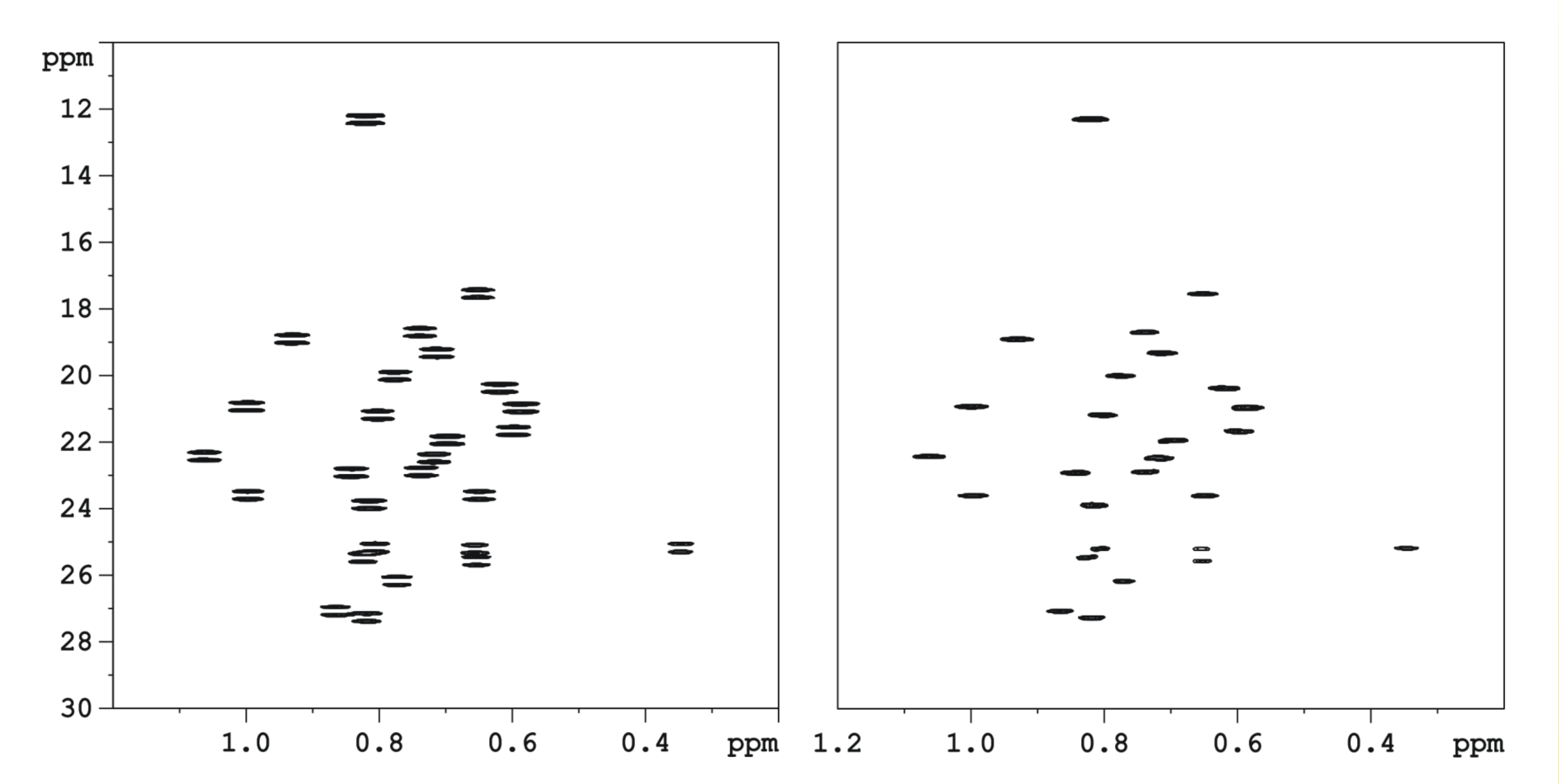
Methyl protonation: SH3

SH3(DCN, ^{1}H-CH3)

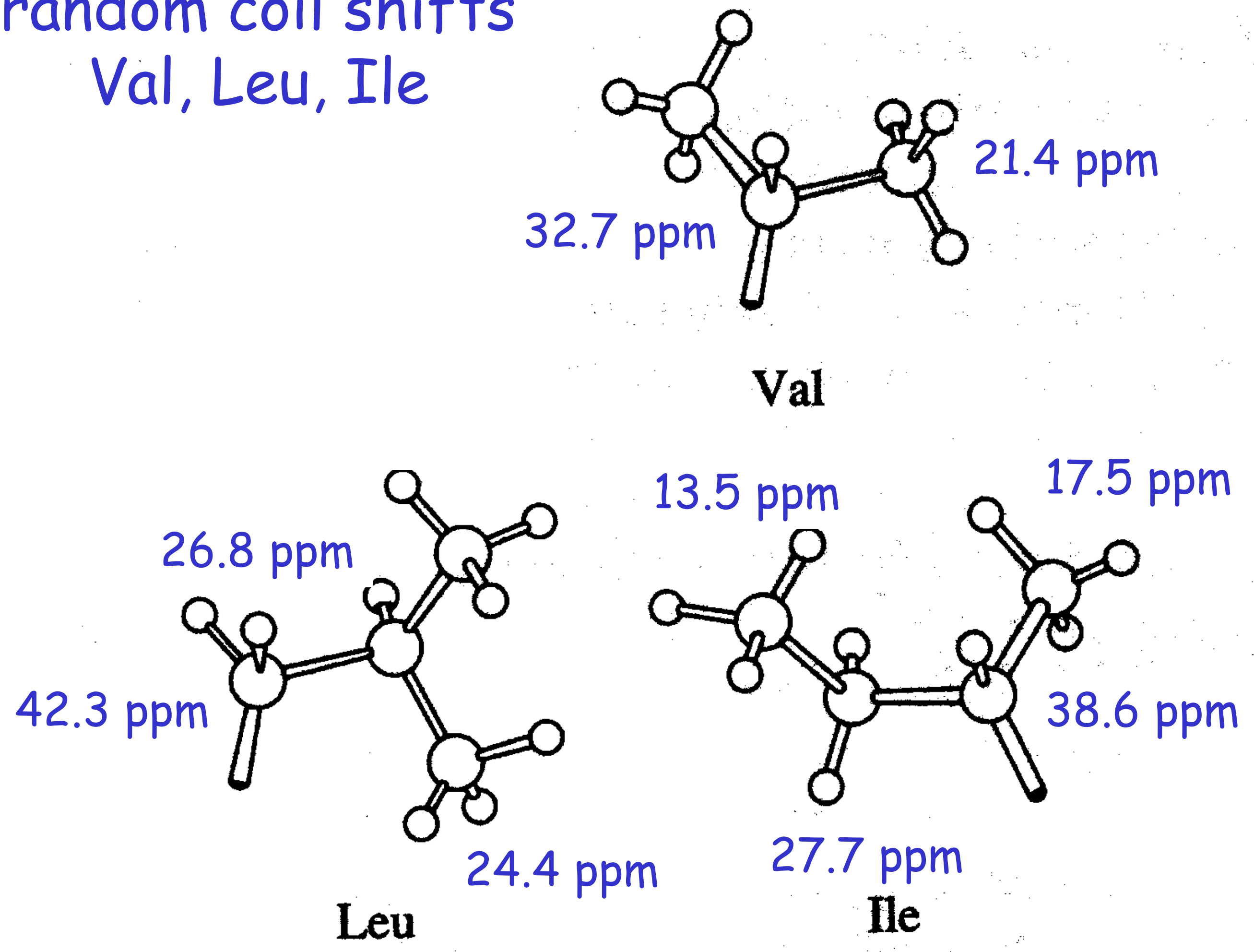
High-resolution HSQC

ct-HSQC



Methyl protonation: SH3

random coil shifts
Val, Leu, Ile



Methyl protonation: SH3

Experimental schemes for assignment

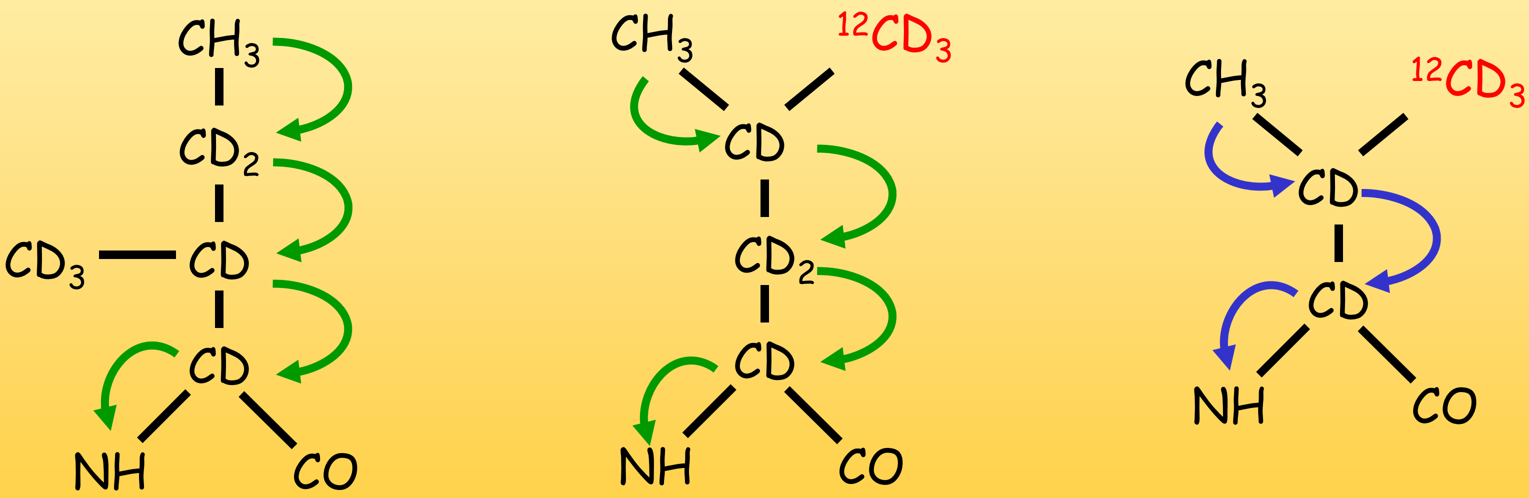
Tugarinov and Kay *J. Am. Chem. Soc.* **125**, 13868-13878 (2003)

straight-through

Leu, Ile-(HM)CM(CGCBCA)NNH

Val-(HM)CM(CBCA)NNH

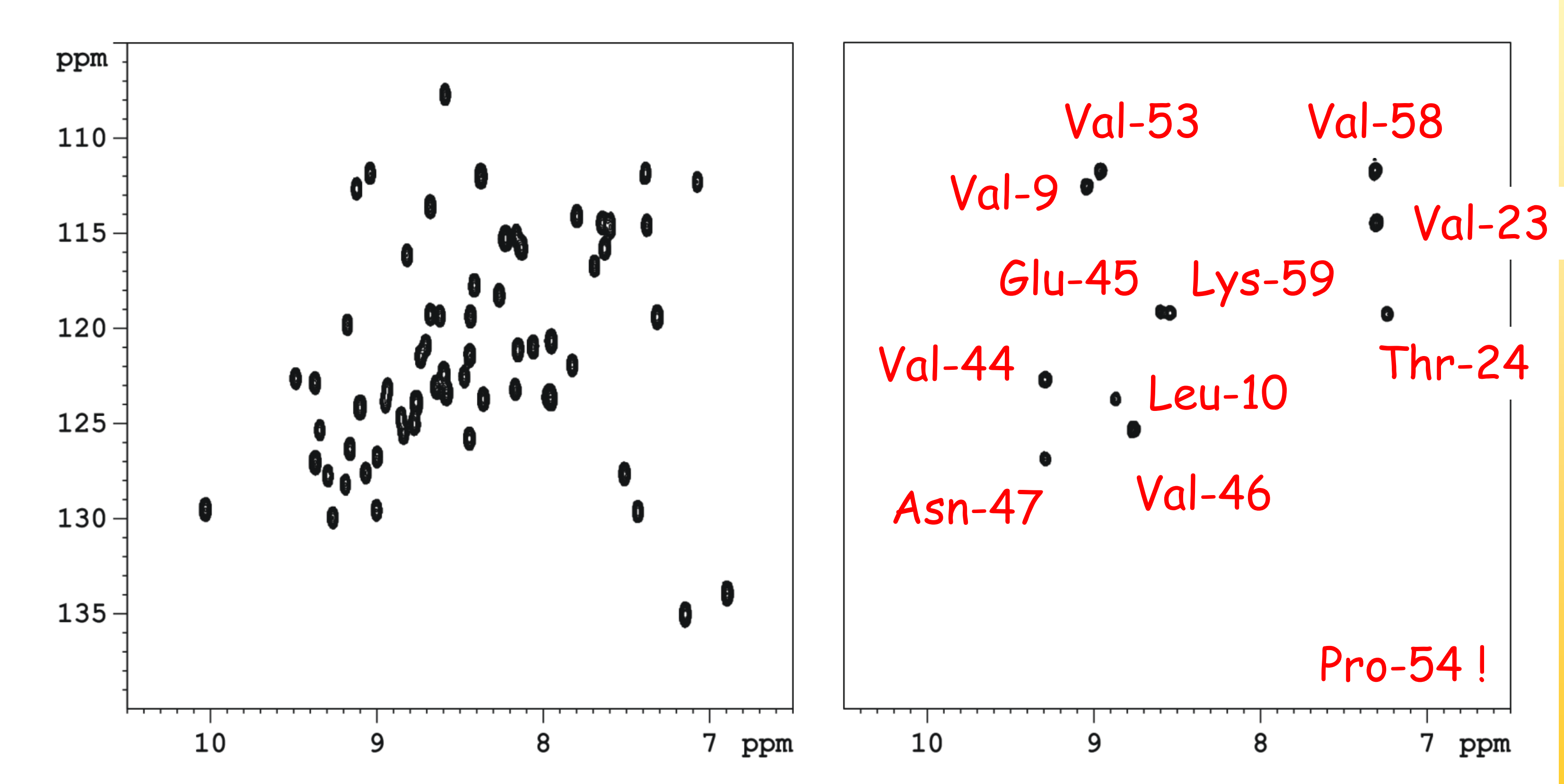
here the linear chain becomes important



Methyl protonation: SH3

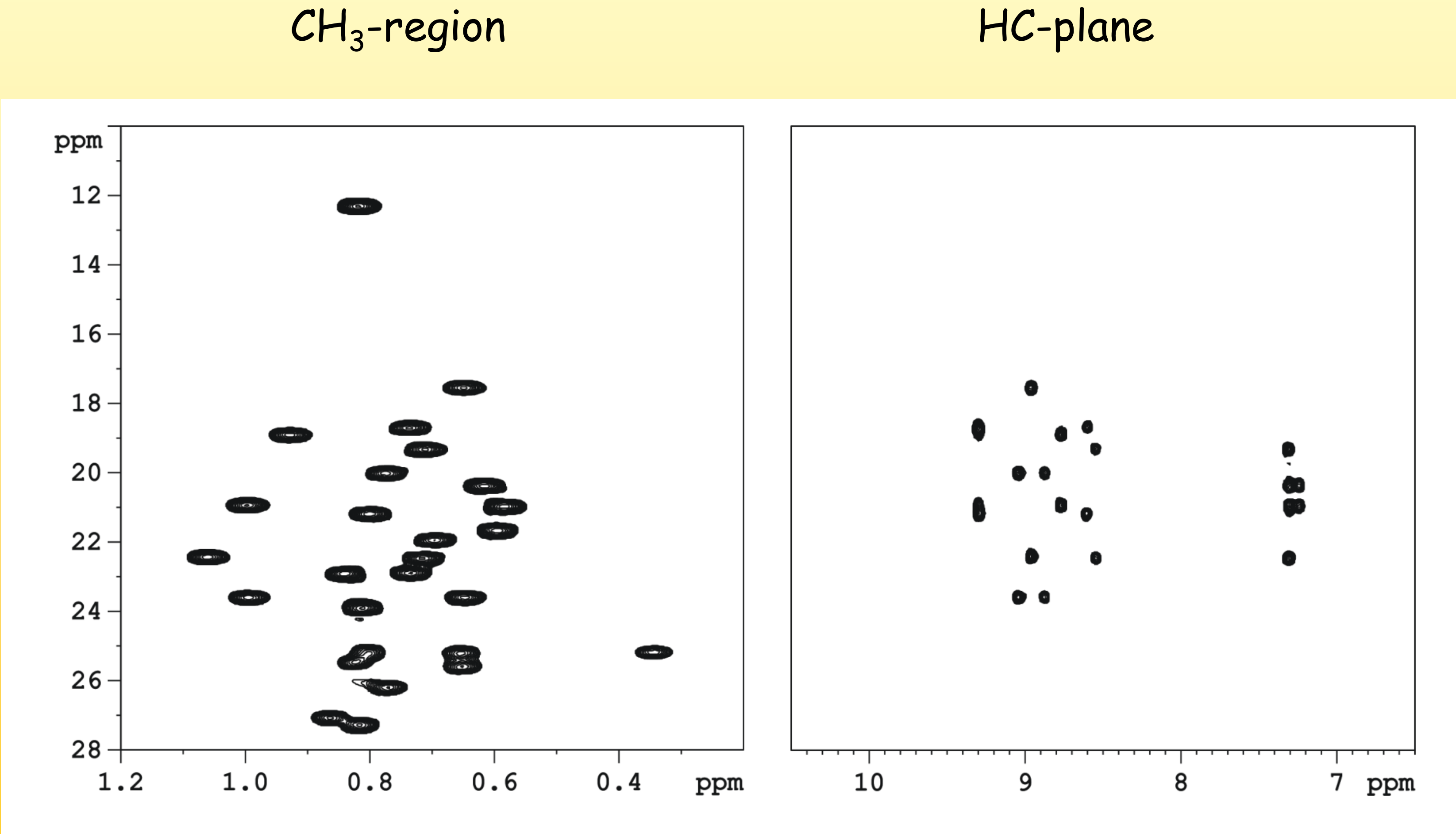
Val-(HM)CM(CBCA)NNH

TROSY HN-plane



Methyl protonation: SH3

Val-(HM)CM(CBCA)NNH



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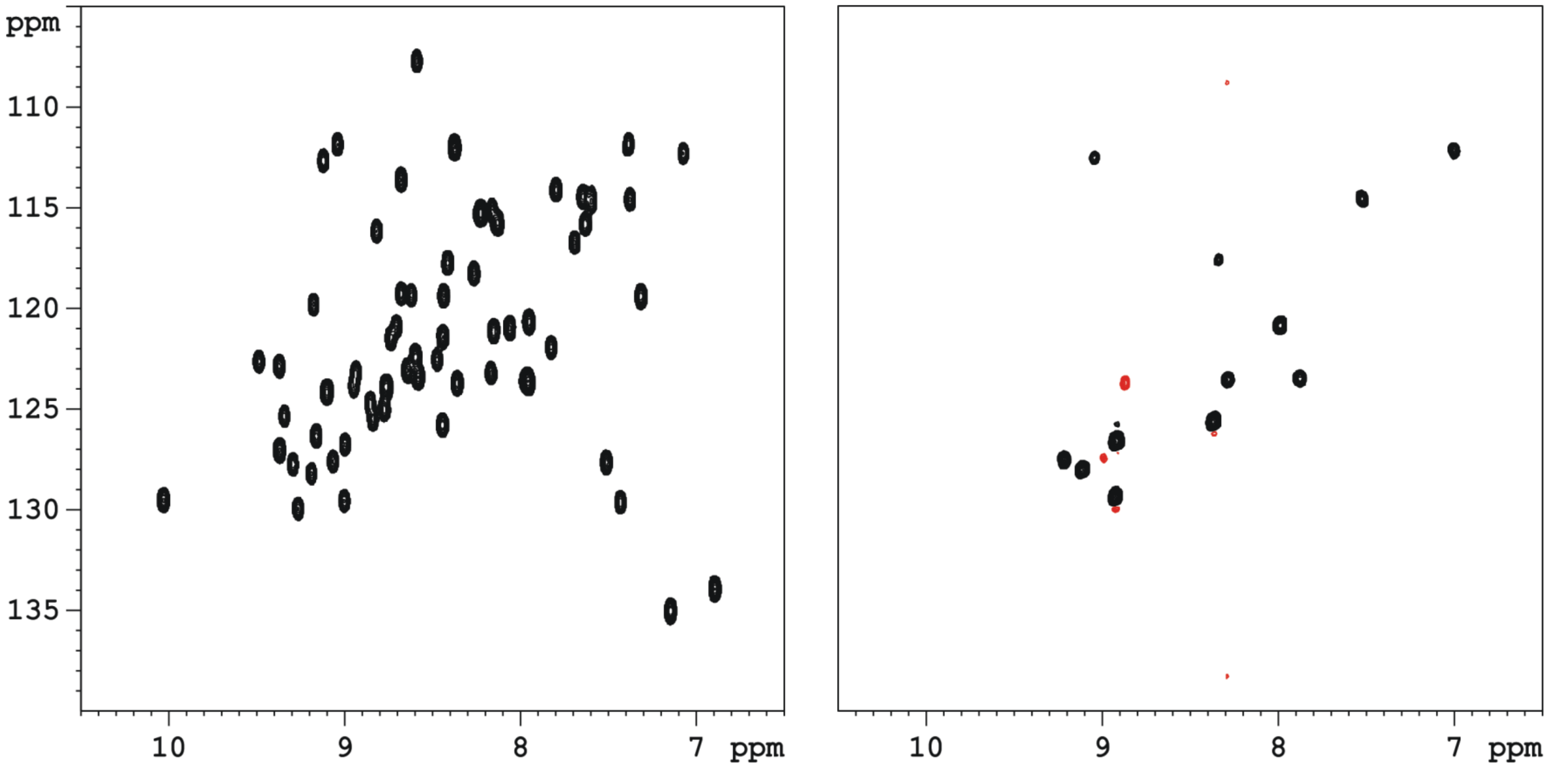
Methyl protonation: SH3

Leu,Ile-(HM)CM(CGCBCA)NNH

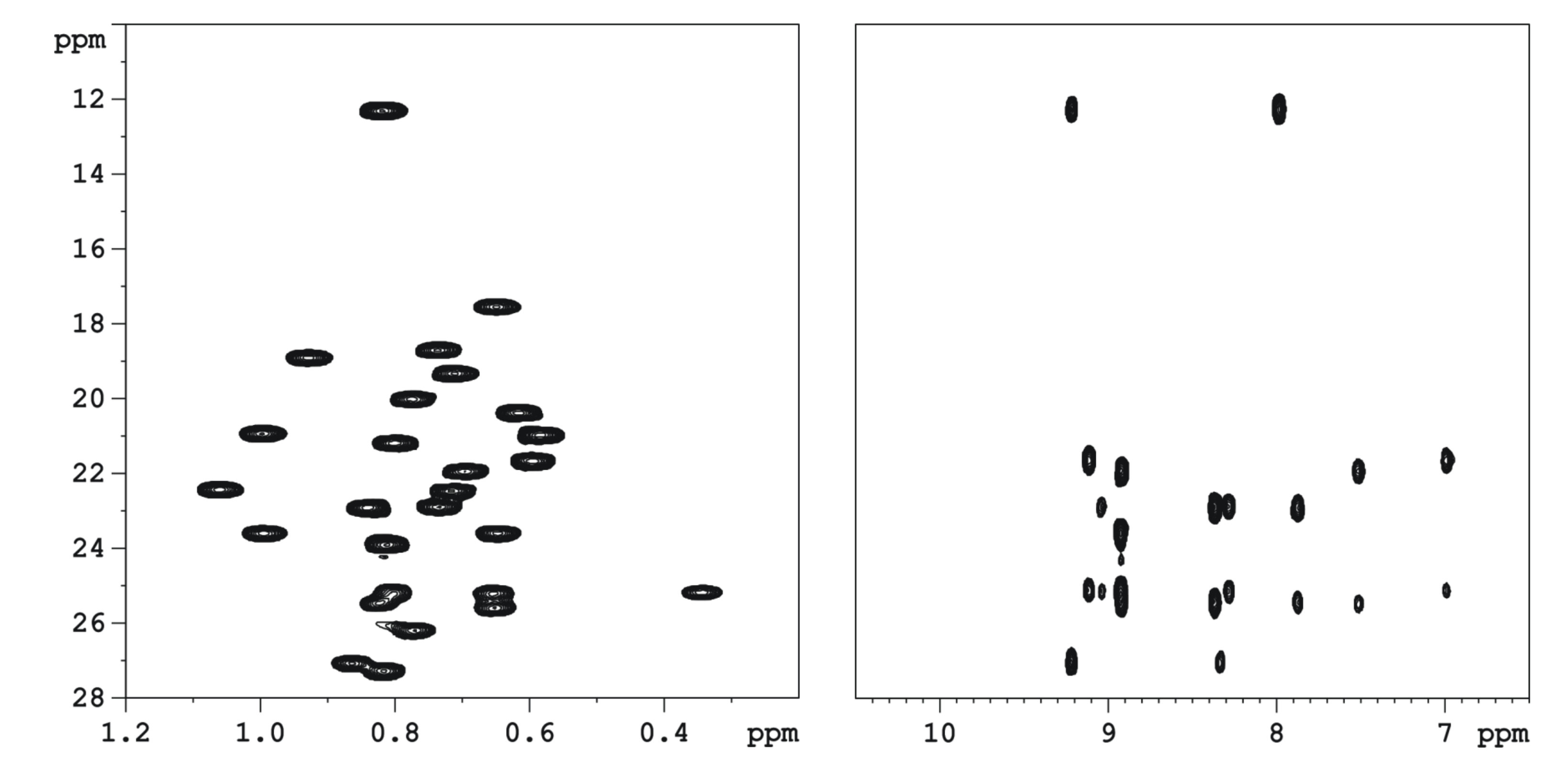
TROSY HN-plane



Methyl protonation: SH3

Leu,Ile-(HM)CM(CGCBCA)NNH

CH_3-region HC-plane



Methyl protonation: SH3

Experimental schemes for assignment

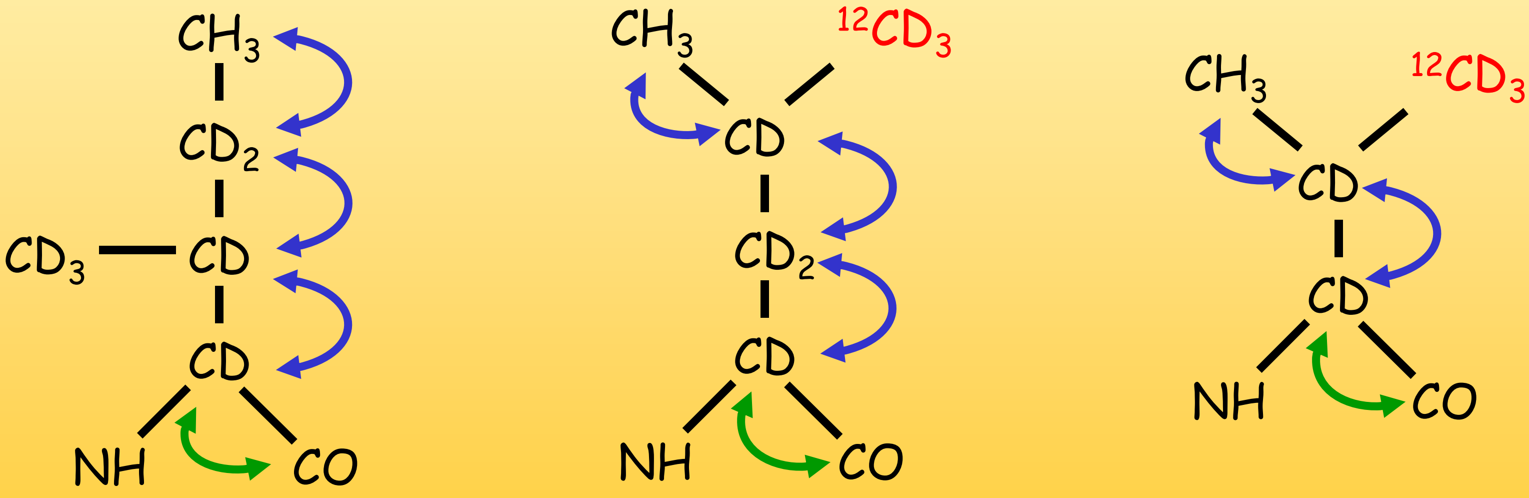
Tugarinov and Kay *J. Am. Chem. Soc.* **125**, 13868-13878 (2003)

<u>out-and-back</u>

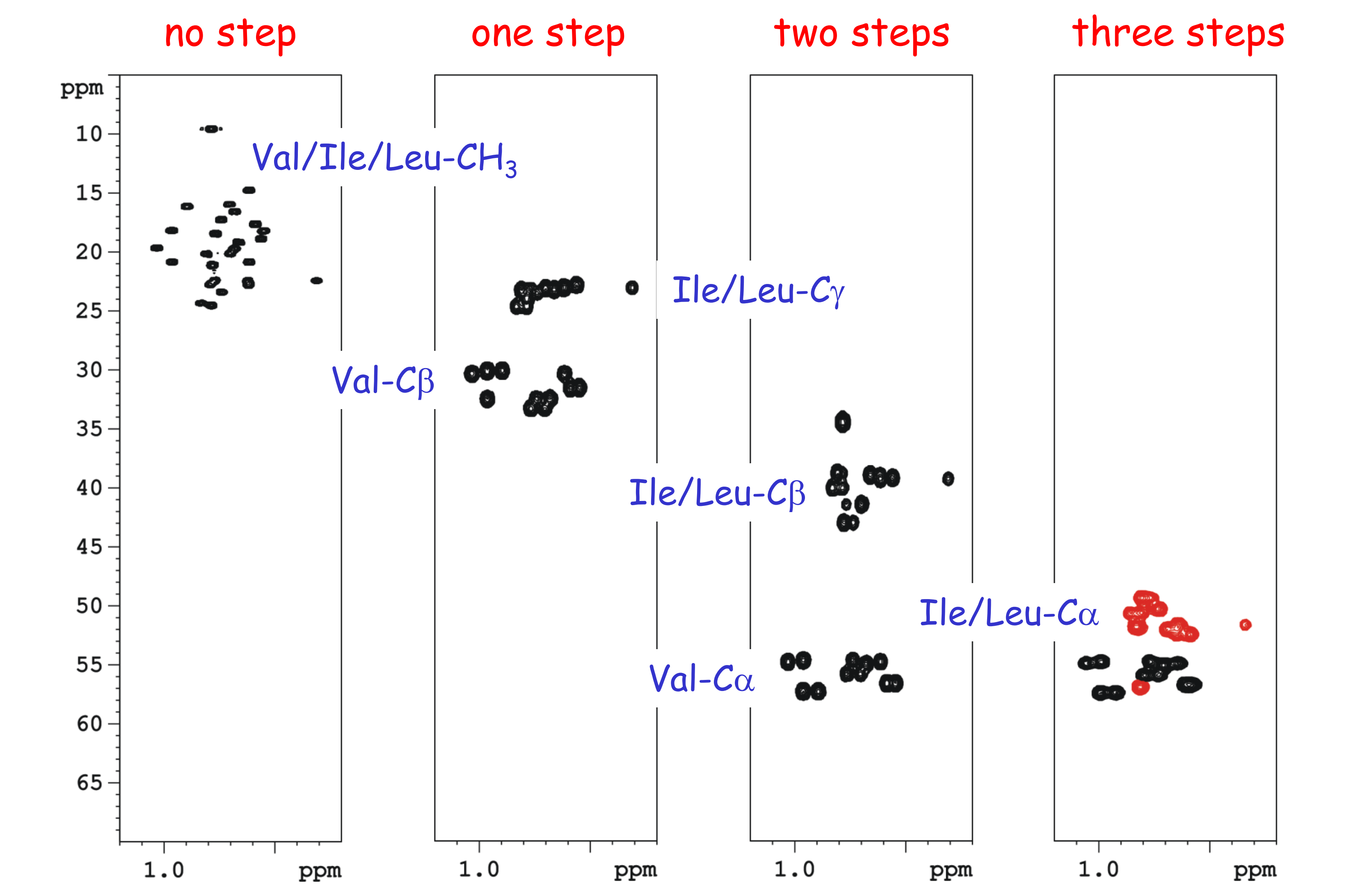
HMCM(CG)CBCA

HMCM(CGCBCA)CO

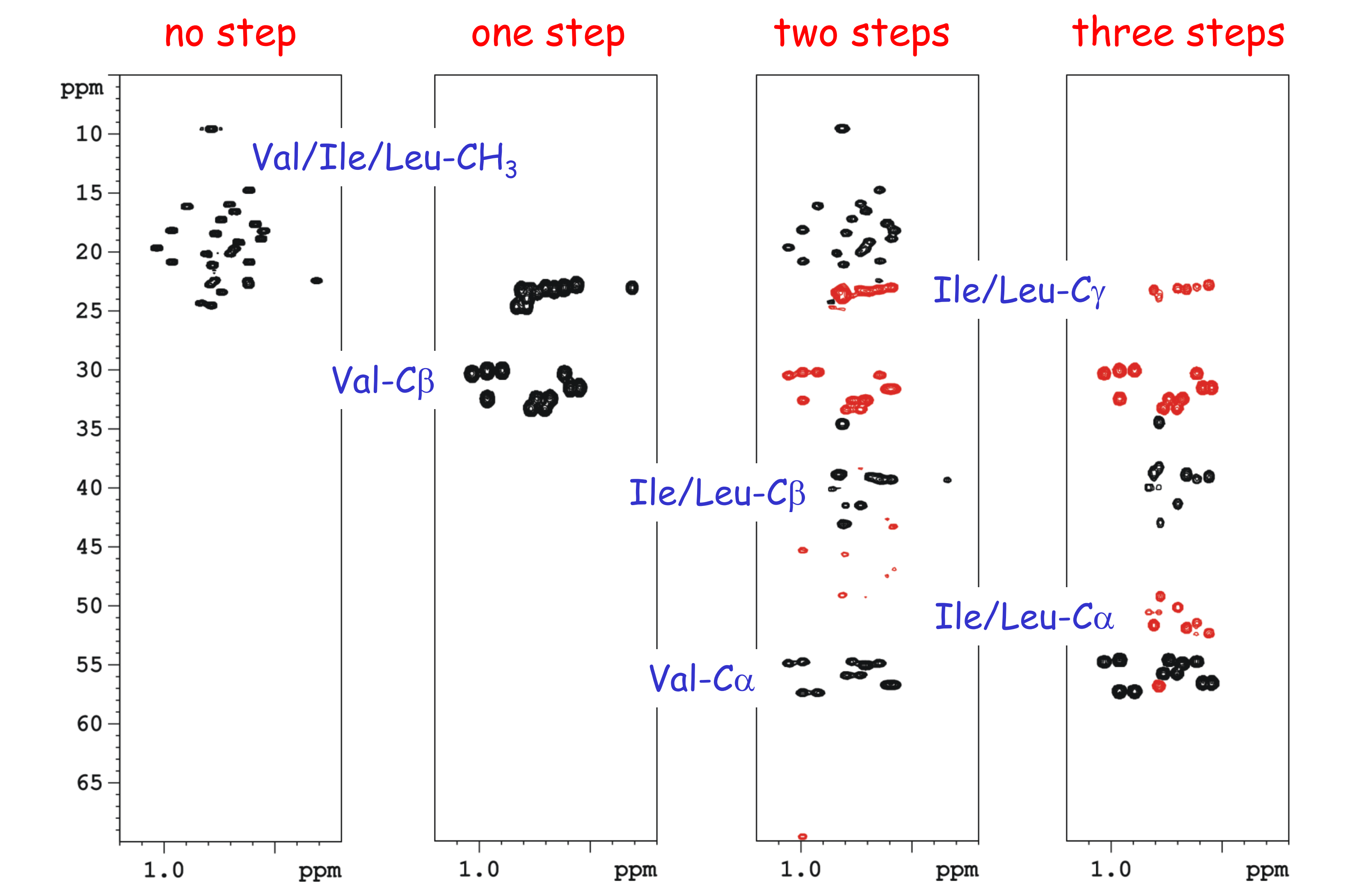
here the linear chain becomes important



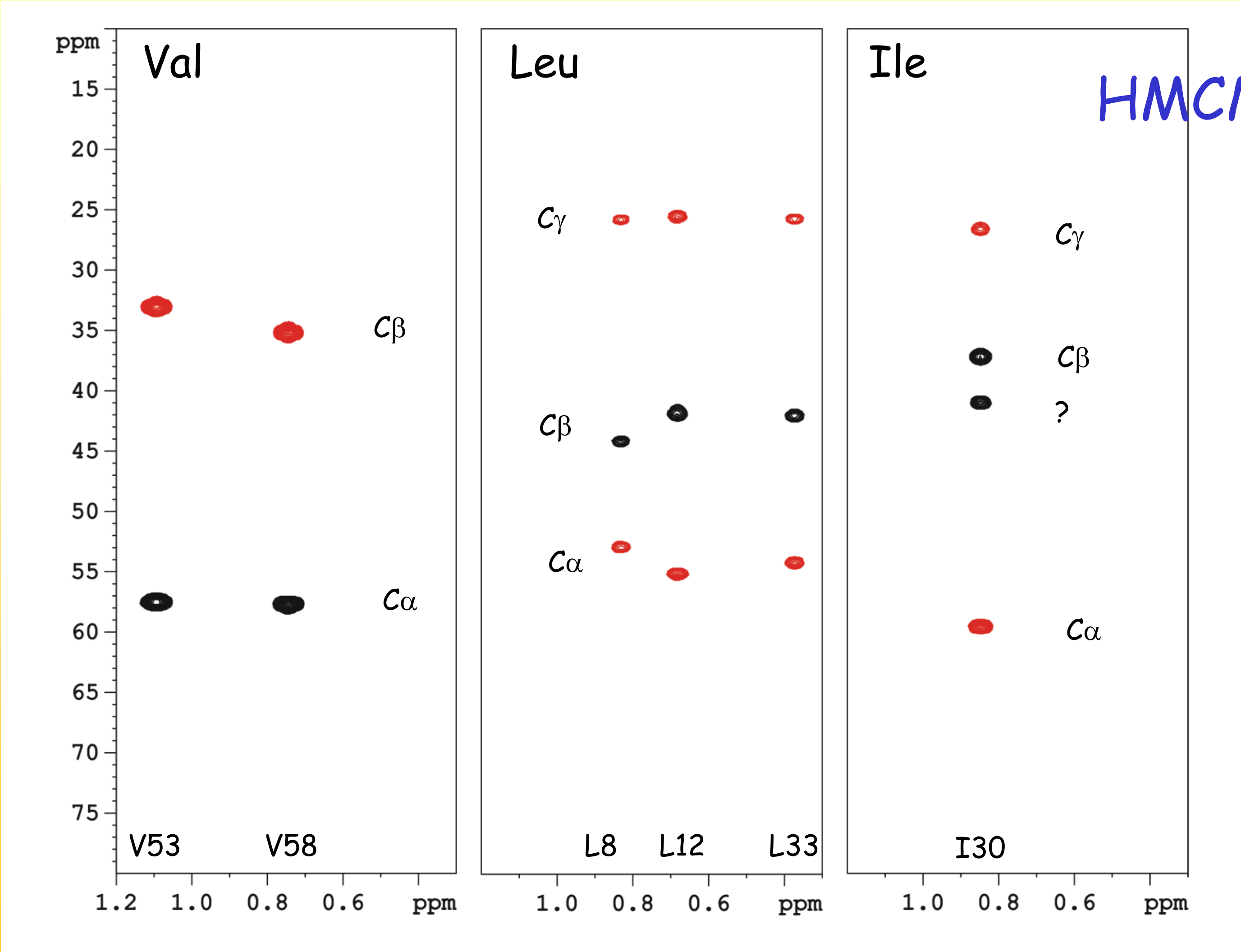
Methyl protonation: SH3



Methyl protonation: SH3



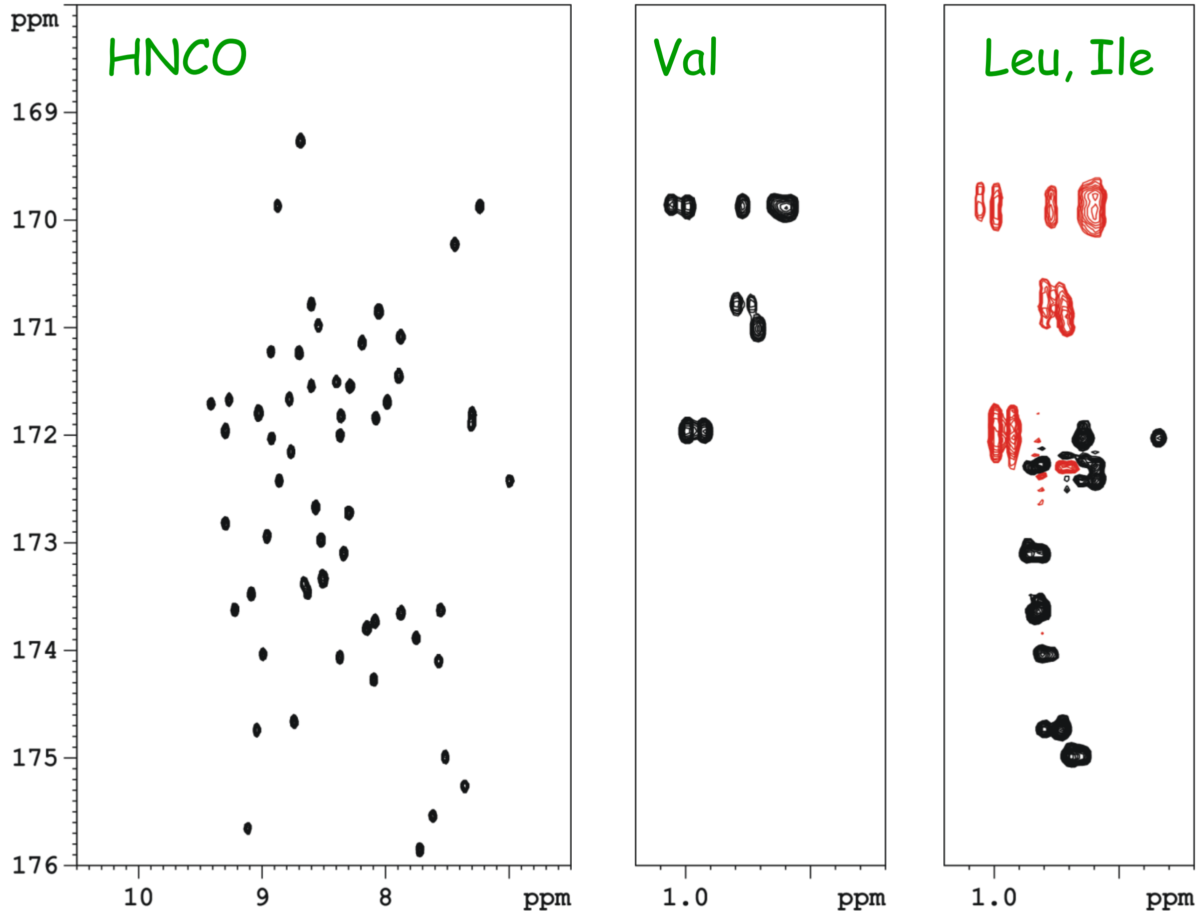
Methyl protonation: SH3



HMCM[CG]CBCA

Methyl protonation: SH3

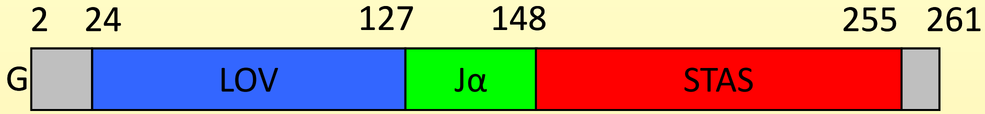
HMCM(CGCBCA)CO



Methyl protonation: „real" examples

Expression

YtvA



HCDF

DCN

using 16 g d_{12}-^{13}C-Glucose, 4 g ^{15}N-NH4Cl

36 mg protein from 1 l

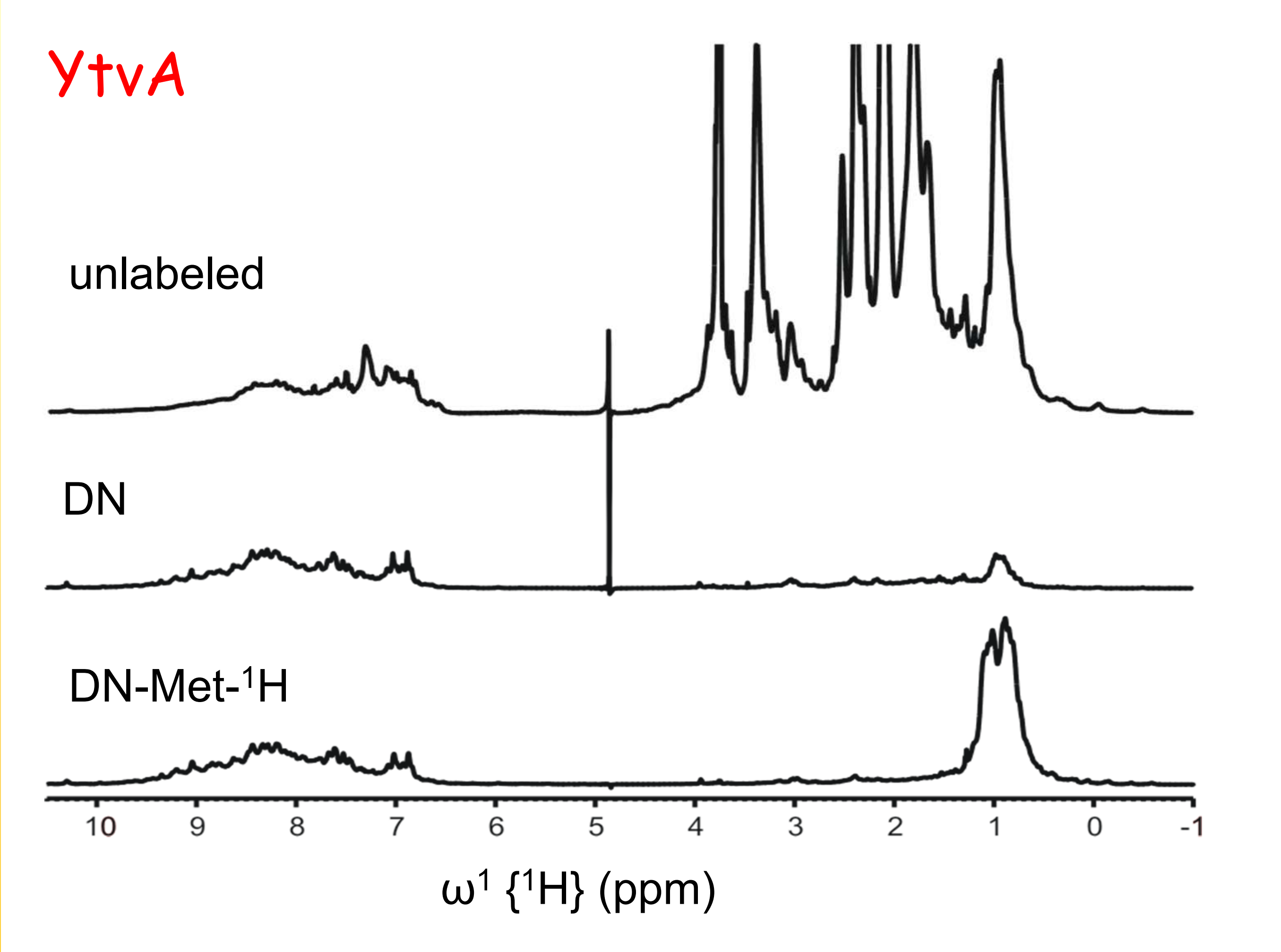
DCN-Met

mit 16 g d_{12}-^{13}C-Glucose, 4 g ^{15}N-NH4Cl

400 mg 2-ketobutyrate, 200 mg 2-ketoisovalerate

70 mg protein from 1 l

Methyl protonation: „real" examples



Methyl protonation: „real“ examples

Expression

B2709

„high yield“ shaking flask

DCN

using 5 g d_{12}-^{13}C-Glucose, 1 g ^{15}N-NH4Cl

115 mg protein from 1 l

DCN-Met

mit 5 g d_{12}-^{13}C-Glucose, 1 g ^{15}N-NH4Cl

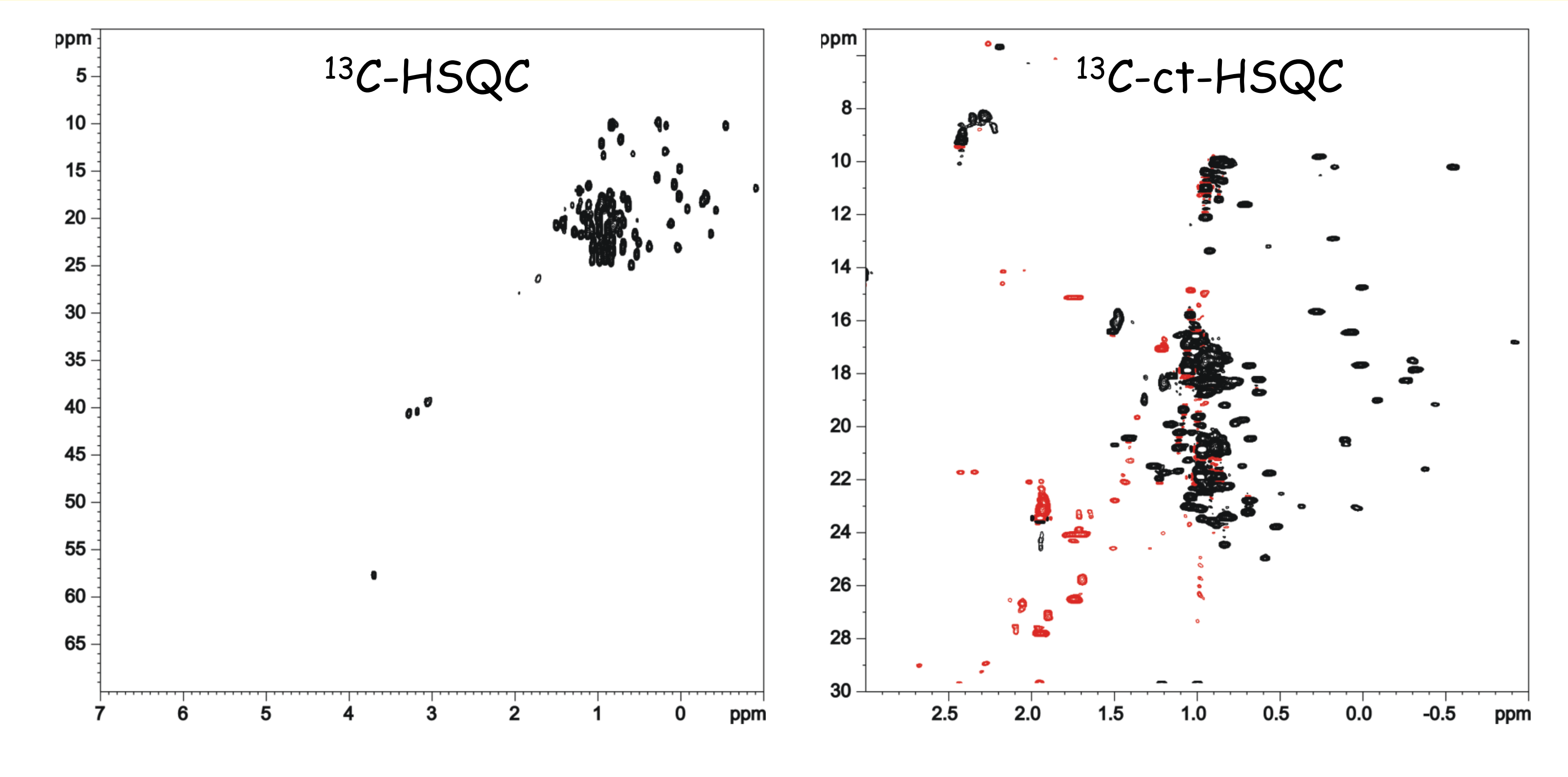
50 mg 2-ketobutyrate, 200 mg 2-ketoisovalerate

70 mg protein from 1 l

Methyl protonation: „real“ examples

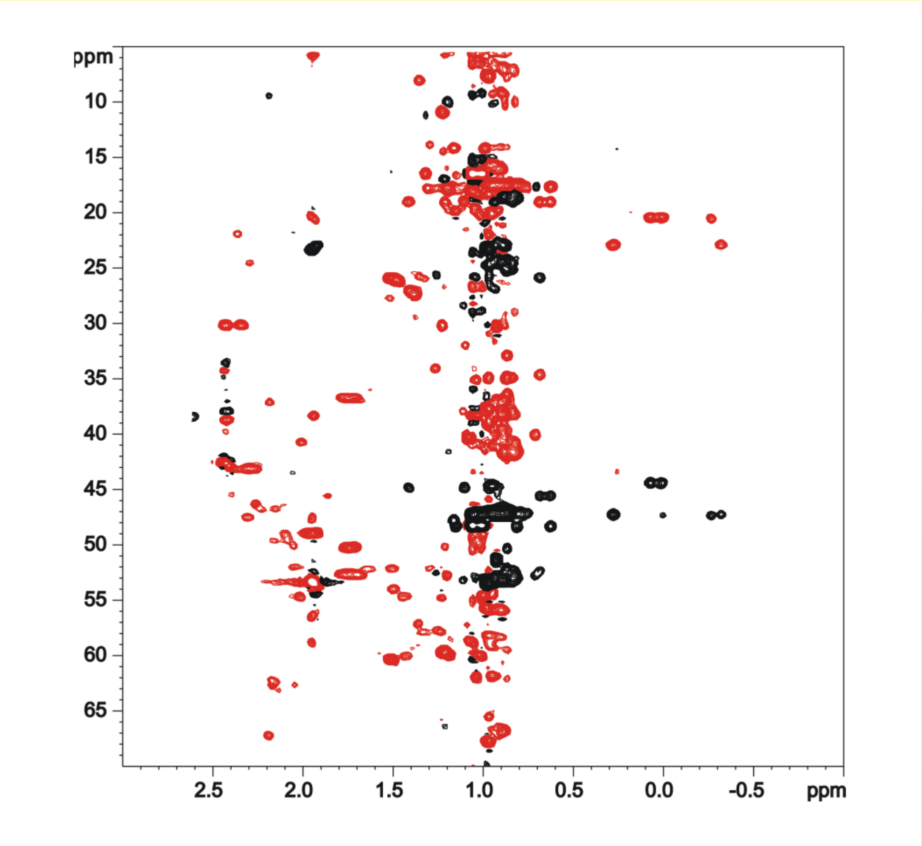
B2709

13 Val, 21 Leu, 7 Ile = 75 CH_3-groups



Methyl protonation: „real“ examples

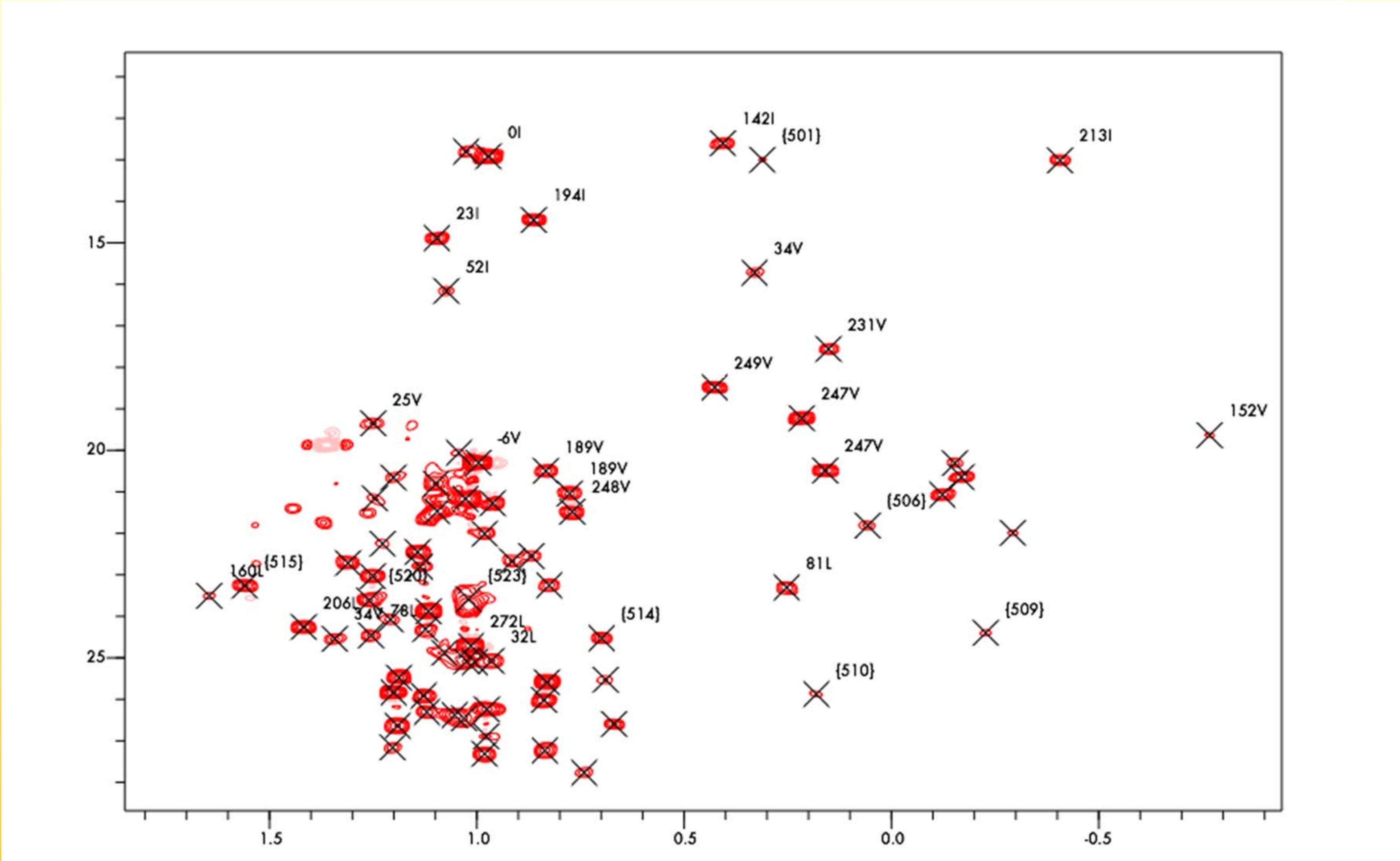
B2709



HMCM[CG]CBCA

Methyl protonation: „real“ examples

B2709



Methyl protonation: „real" examples

The ratio between butyric and isovaleric acid should be calculated from the abundance of the amino acids in the protein:

B2709: 13 Val, 21 Leu, 7 Ile = 75 CH3-groups

YtvA: 19 Val, 28 Leu, 24 Ile = 118 CH3-groups

It should be mentioned that the experiments presented above do not work for proteins of all sizes.

Several alternative approaches have been utilized, e.g. mutations, use of X-ray-structures etc.

Methyl protonation: more options

There are also other amino acids that are of interest for the study of dynamics and interaction of proteins and several further labeling options have been presented:

Alanine

Methionine

γ_2-Isoleucin

pro-R and pro-S Valine and Leucine

Some of them require more complicated precursor compositions and not all work without scambeling

Methyl protonation: more options

Most of the precursors that have been introduced over the years are commercially available by now

IN VIVO ALANINE METHYL LABELING KIT

This kit contains four separate vials of the following compounds in the amounts indicated:

DLM-584 Succinate (U-D4), 2.5g
DLM-4646 Alpha-Keto Isovalerate (U-D7), 0.2 g
DLM-141 Isoleucine (U-D10), 0.06g
CDLM-8649 Alanine (2-D, 3-13C), 0.8 g

The materials in this kit are to be used in conjunction with 1L of deuterated minimal media[1,2]. 1L of minimal media that is suitable for use with this product typically contains 2 g of glucose-D7, 1 g of ammonium salt (either 15N labeled or unlabeled, depending on preference of the user), and 11.3 g of M9 salts in D2O.

$ 950

IN VIVO ILVA METHYL LABELING KIT

This kit contains four separate vials of the following compounds in the amounts indicated:

DLM-584 Succinate (U-D4), 2.5g
CDLM-7317 Alpha-Ketoisovaleric acid, sodium salt (3-methyl-13C: 3,4,4,4-D4), 0.12g
CDLM-7318 Alpha-Ketobutyric acid, sodium salt (3-methyl-13C; 3,3-D2), 0.06g
CDLM-8649 Alanine (2-D, 3-13C), 0.8 g

The materials in this kit are to be used in conjunction with 1L of deuterated minimal media. 1L of minimal media that is suitable for use with this product typically contains 2 g of glucose-D7, 1 g of ammonium salt (either 15N labeled or unlabeled, depending on preference of the user), and 11.3 g of M9 salts in D2O

$ 995

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That's it

www.fmp-berlin.de/schmieder/teaching/educational_seminars.htm