2005 NMR User Training Course

National Program for Genomic Medicine High-Field NMR Core Facility,
The Genomic Research Center, Academia Sinica
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Course Handout

Concepts on protein triple resonance experiments

by

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- **R.F pulse field strength**
- **Decoupling**
- **Shaped pulse (compared to rectangular pulses)**
- **Brief description of a 3D HNCA experiment**
- **Water suppression in triple resonance experiments**
- **Constant time HSQC (15N-constant time, 13C constant time)**
- **TROSY type experiments**
- **Deuterium decoupling**

R.F pulse field strength

•For a single 90-degree pulse:

Field strength (bandwidth) γB_1 (Hz) =1/(4*PW90), PW90 is length of the 90-degree hard pulse.

For example: a field strength of 6 kHz on a ^{15}N channel

γB_1 = 6000=1/4*PW90, PW90 = 41.6 usec

•For composite pulse decoupling:

Effective bandwidth of decoupling = Figure of merit * 1/(4*PW90)

Figure of merit (Ξ):

The ratio of effective bandwidth to the radiofrequency level, the higher the figure of merit, the larger the effective bandwidth (or weaker power required).

Composite pulse decoupling

- **Waltz-16 decoupling bandwidth= 1.8 * (1/4*pw90), Waltz-16 is good for decoupling 15N, 2H, 1H.**

- **GARP decoupling bandwidth= 4.8 * (1/4*pw90), for decoupling 13C and 15N**

- **Dipsi2 decoupling bandwidth= 1.2* (1/4*pw90), for decoupling 1H**

- **CHIRP95 decoupling bandwidth up to 50***

For a 70 usec 13C_P90 using GARP decoupling:

the decoupling bandwidth is 4.8* (1/4*70*10^{-6})=17143 Hz

This will cover 17143/125MHz=137 ppm on 500 MHz, 114 ppm on 600 MHz.

GARP decoupling sequence: under /lists/cpd directory

pcpd*0.339:0
pcpd*0.613:180
pcpd*2.864:0
pcpd*2.981:180
…… …….
…… …….

^{13}C Band selective excitation/inversion using rectangular pulse

Calculate ^{13}C rectangular pulse width according to the following equations:

$$PW\_90 = (15)^{0.5} / 4 * \Delta frequency$$
$$PW\_180 = (3)^{0.5} / 2 * \Delta frequency$$

For example: On 600 MHz ^{1}H (150 MHz ^{13}C) to excite Cα (center at 56 ppm) and a null (zero excitation) at CO center at 176 ppm.

$PW\_90 = (15)^{0.5} / [4 * (176-56)*150*10^{6}] = 53.8$ usec
$PW\_180 = (3)^{0.5} / [2 * (176-56)*150*10^{6}] = 48$ usec

Caution: The standard Bruker pulse sequences use shaped 13C pulse for band selective excitation instead of rectangular pulse.

Why using Shaped ^{13}C Pulses

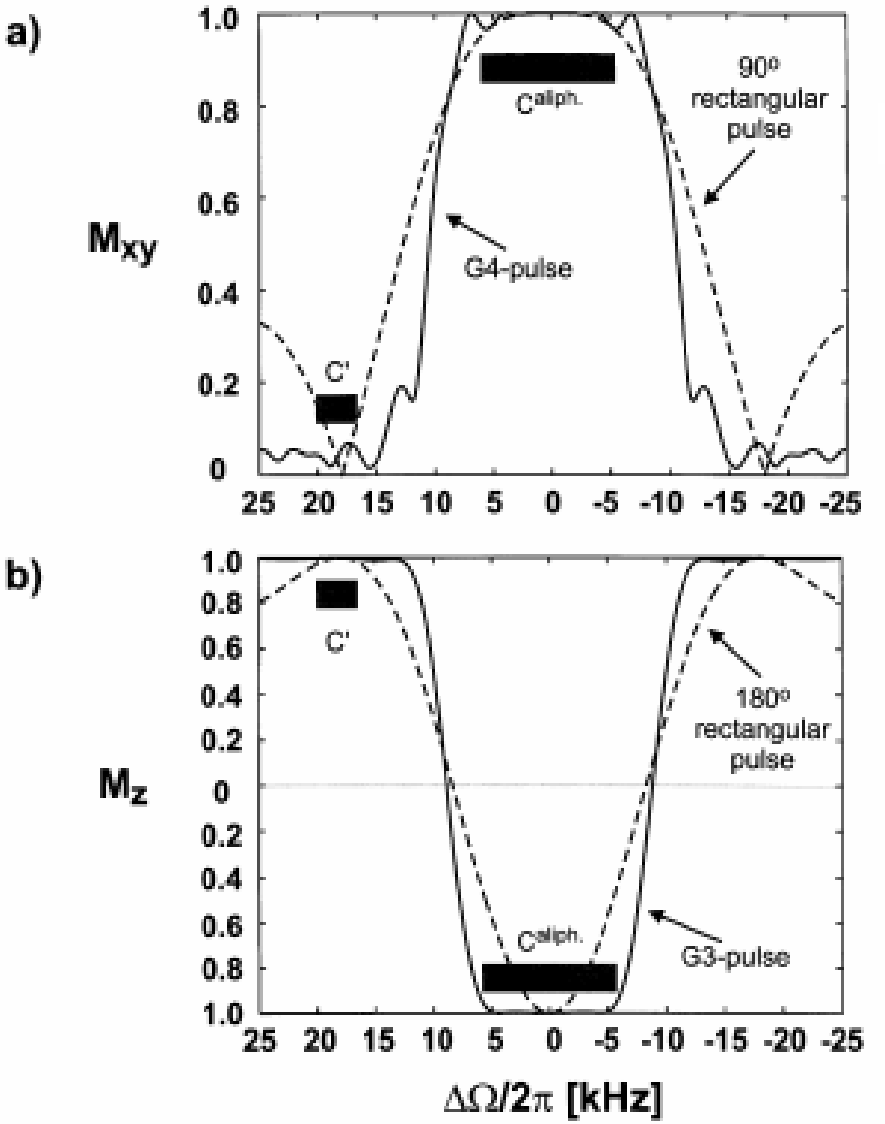


Figure from
M. Sattler et. al. „Progr.NMR Spectr“ v34 (1999), p93-158

90-degree excitation profile by :

- **a 53.8 us rectangular pulse**
- **a 400 us G4 shaped pulse**

Inversion of Z magnetization profile by :

- **a 48 us rectangular pulse**
- **a 250 us G3 pulse**

Advantage of shaped pulses over rectangular hard pulses:
excite region of interest with more homogenous and with minimal perturbation of other regions.

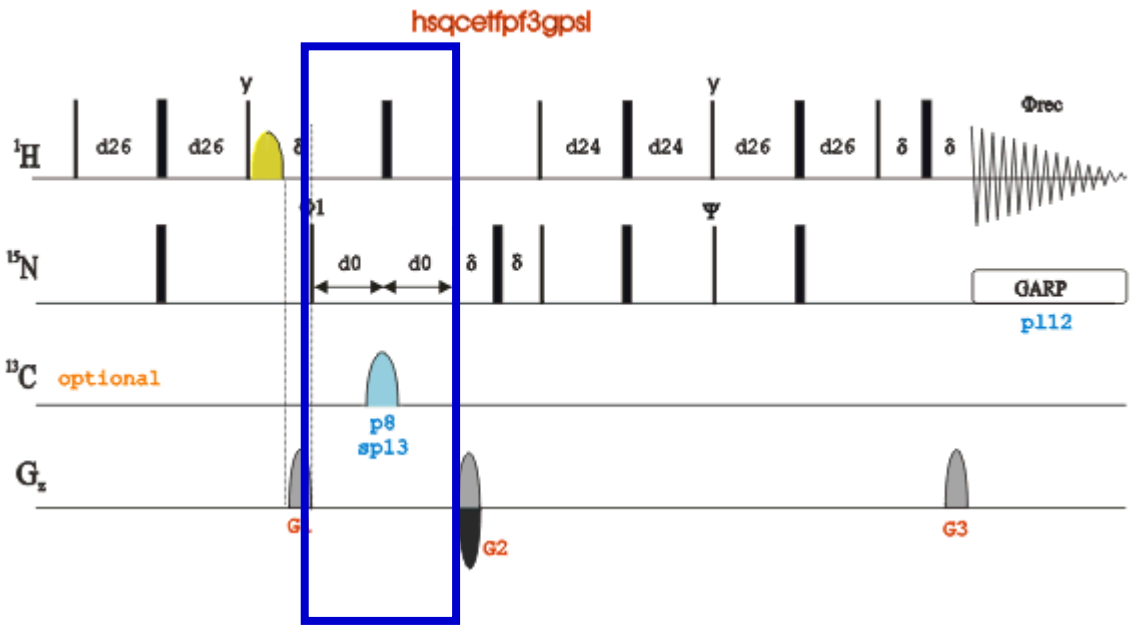
Shaped (Frequency Selective) Pulses

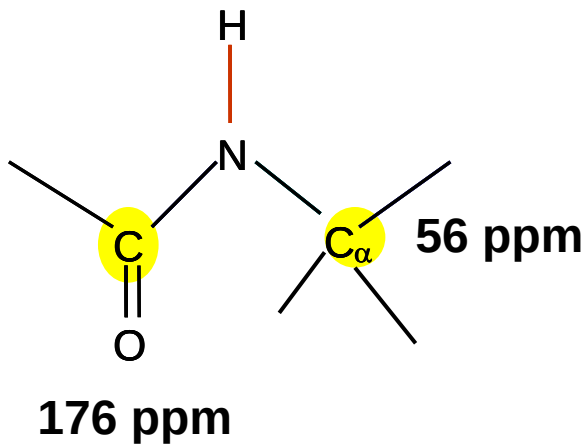
Syntax of a shaped pulse:
(p14:sp3 ph1):f2

p14: pulse length
sp3: power level of the shaped pulses
spnam3: shaped pulse name,
spoff3: ^{13}C-offset for selective decoupling

G4 or Q5.1000 for a 90-degree excitation
G3 or Q3.1000 for a 180-degree inversion

Adiabatic Inversion Pulses



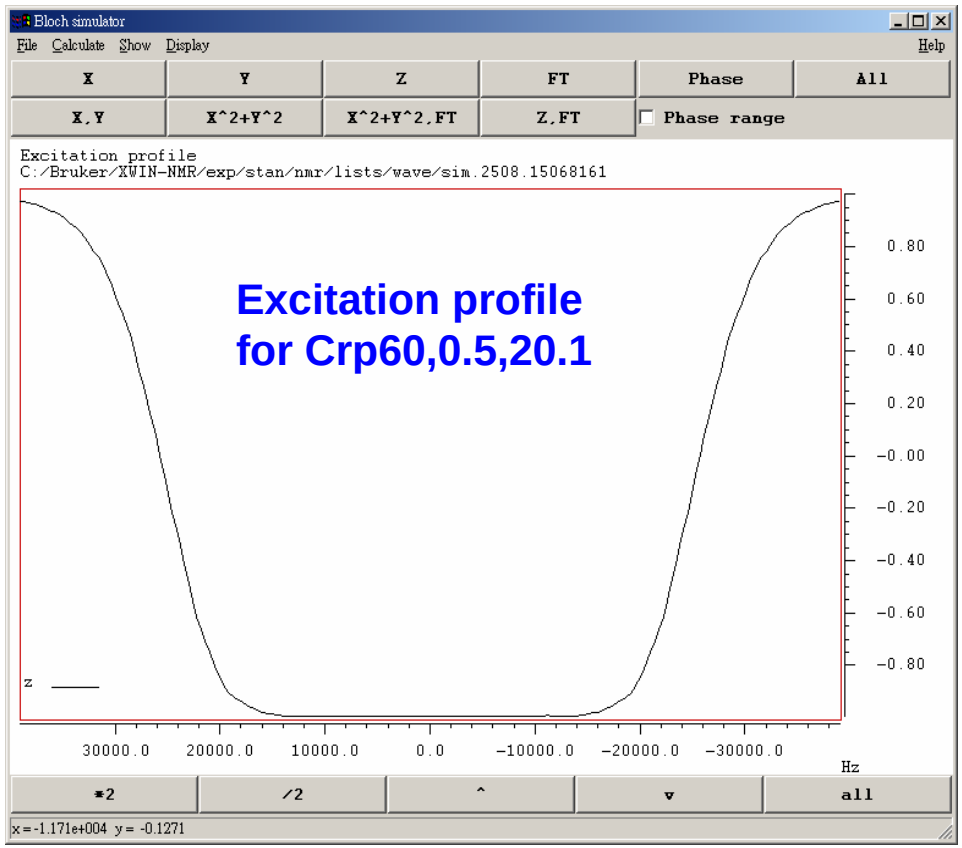


For ^{13}C, ^{15}N-sample, it is necessary to simultaneously decouple C_α (center at 56 ppm) and CO (center at 176 ppm) during ^{15}N chemical shift evolution. An adiabatic pulse is required to cover this large ^{13}C chemical shift range.

Figure from Bruker NMR Guide

Adiabatic Inversion Pulses

- **An adiabatic pulse covers a very large bandwidth (chemical shift range)**
- **Adiabatic pulse is good for inversion purpose**



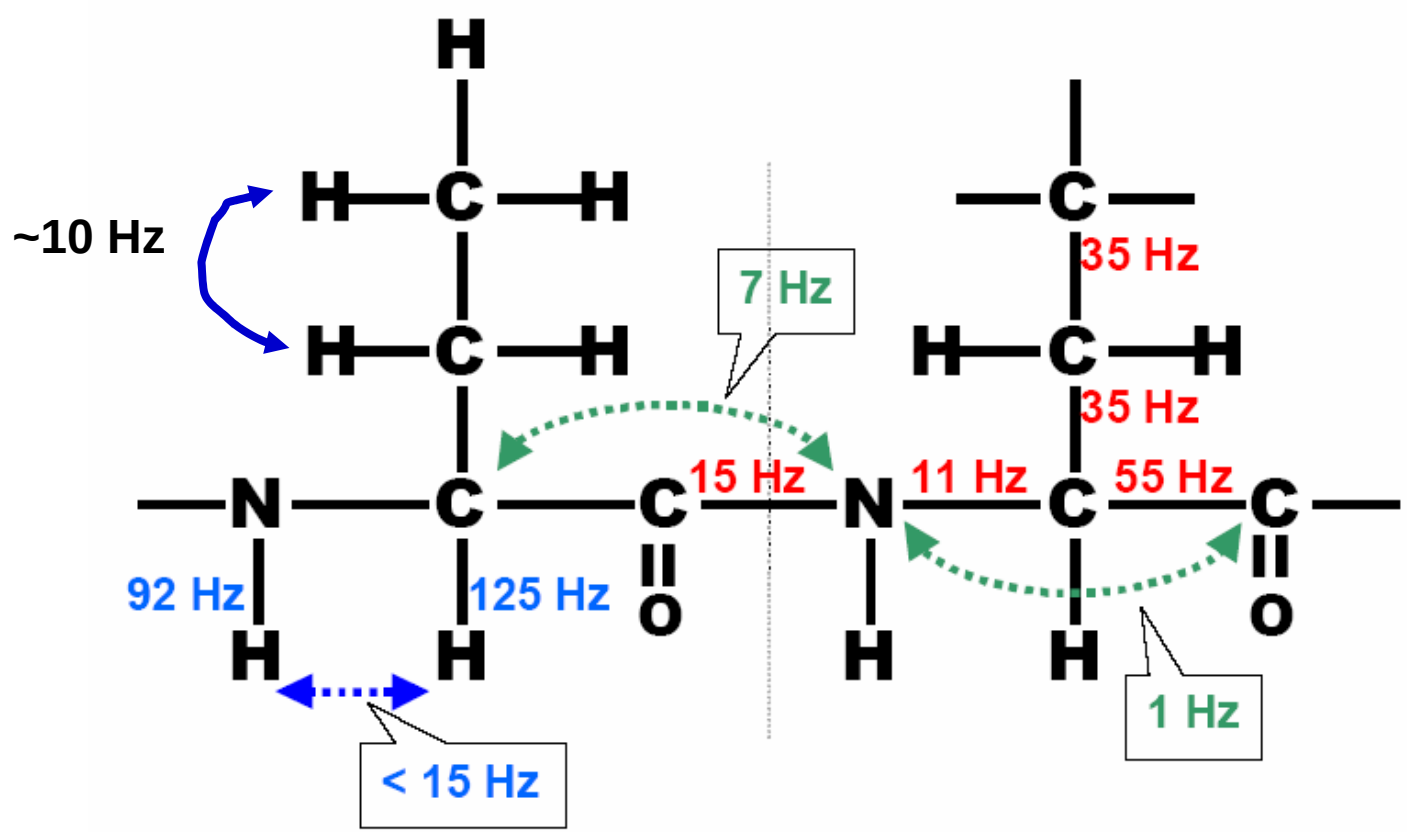
Z

-Z

Crp60,0.5,20.1

size of shape	*1000*
total sweep-width	*60000*
length of pulse	*500 (us)*
% to be smoothed	*20*
low to high field	*-1*

Coupling Constants in Polypeptides



Protein triple resonance: ^{1}H, ^{15}N, ^{13}C

Magnetization transfer is via J-coupling interaction, so the larger the J-coupling, the more efficient the transfer. The sensitivity also depends on the T2 relaxation time of the nuclei.

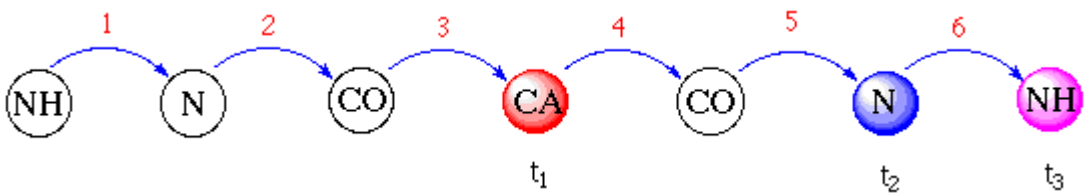
Figure from Bruker Avance 3D triple resonance

Nomenclatures of Triple Resonance Experiments

- HN, N, HA, CA, CO, HB, CB
- $^{1}H^{N}$, ^{15}N, $^{1}H^{\alpha}$, $^{13}C^{\alpha}$, ^{13}CO, $^{1}H^{\beta}$, $^{13}C^{\beta}$

- For “out and back” type triple resonance experiments experiment:

For example: **HN(CO)CA**
The magnetization transfer pathway:



(HN)N(CO)CA(CO)(N)NH

Name for the return path is dropped to shorten the name.

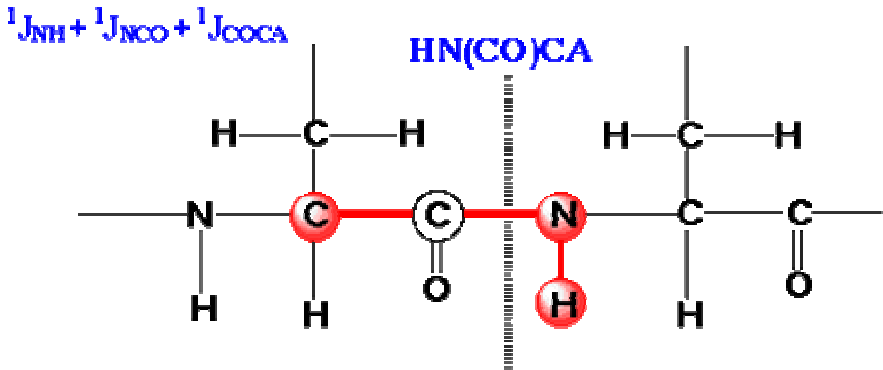
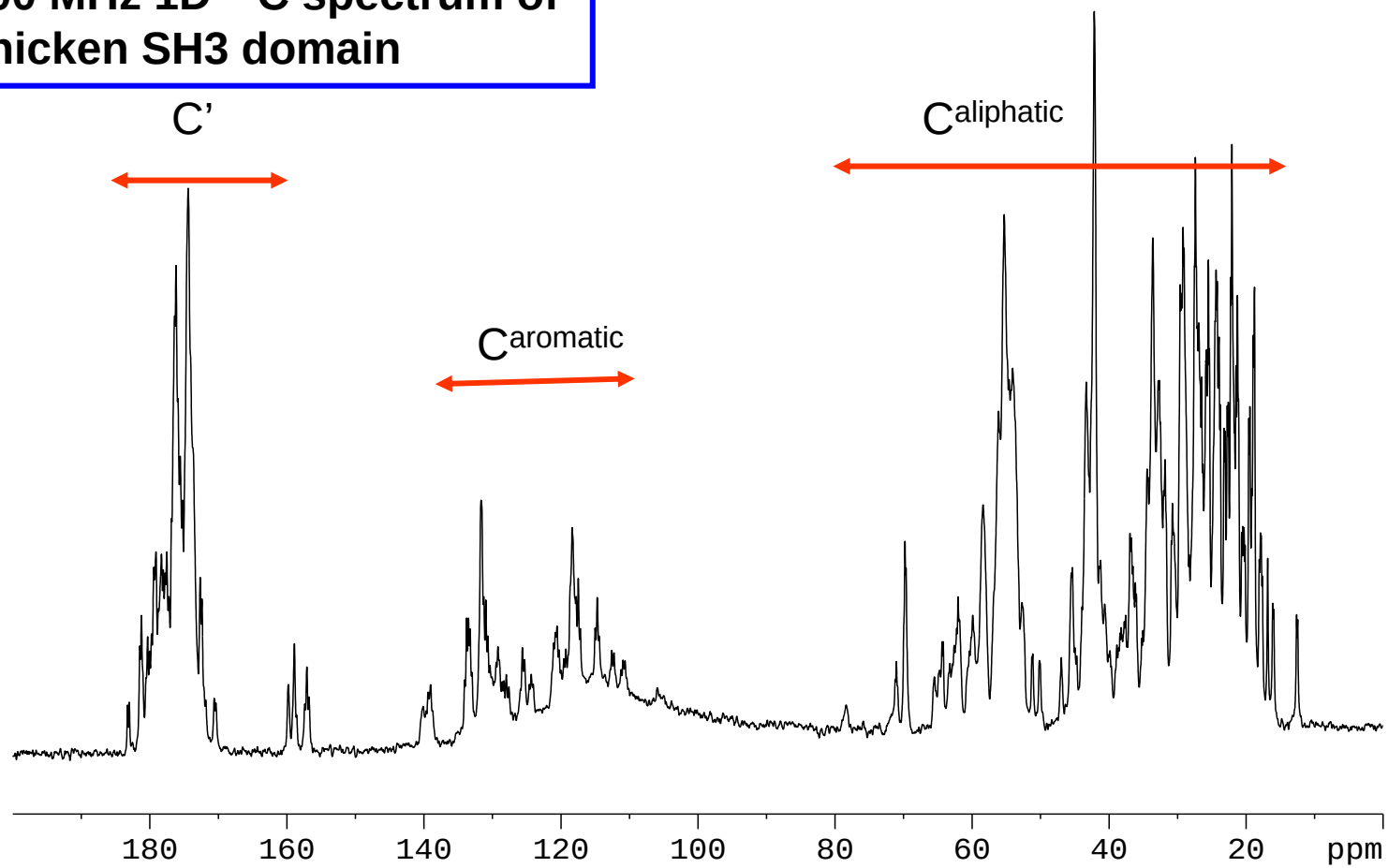


Figure from Bruker NMR Guide

200 MHz 1D ^{13}C spectrum of chicken SH3 domain



Carbonyl (C') centers at ~176 ppm (sw ~15 ppm wide)
The aliphatic ^{13}C resonance ~ 10-75 ppm
C_α centers at ~ 56 ppm (sw ~30 ppm)
C_α-C_β center at ~ 39 ppm (sw~70-75 ppm)

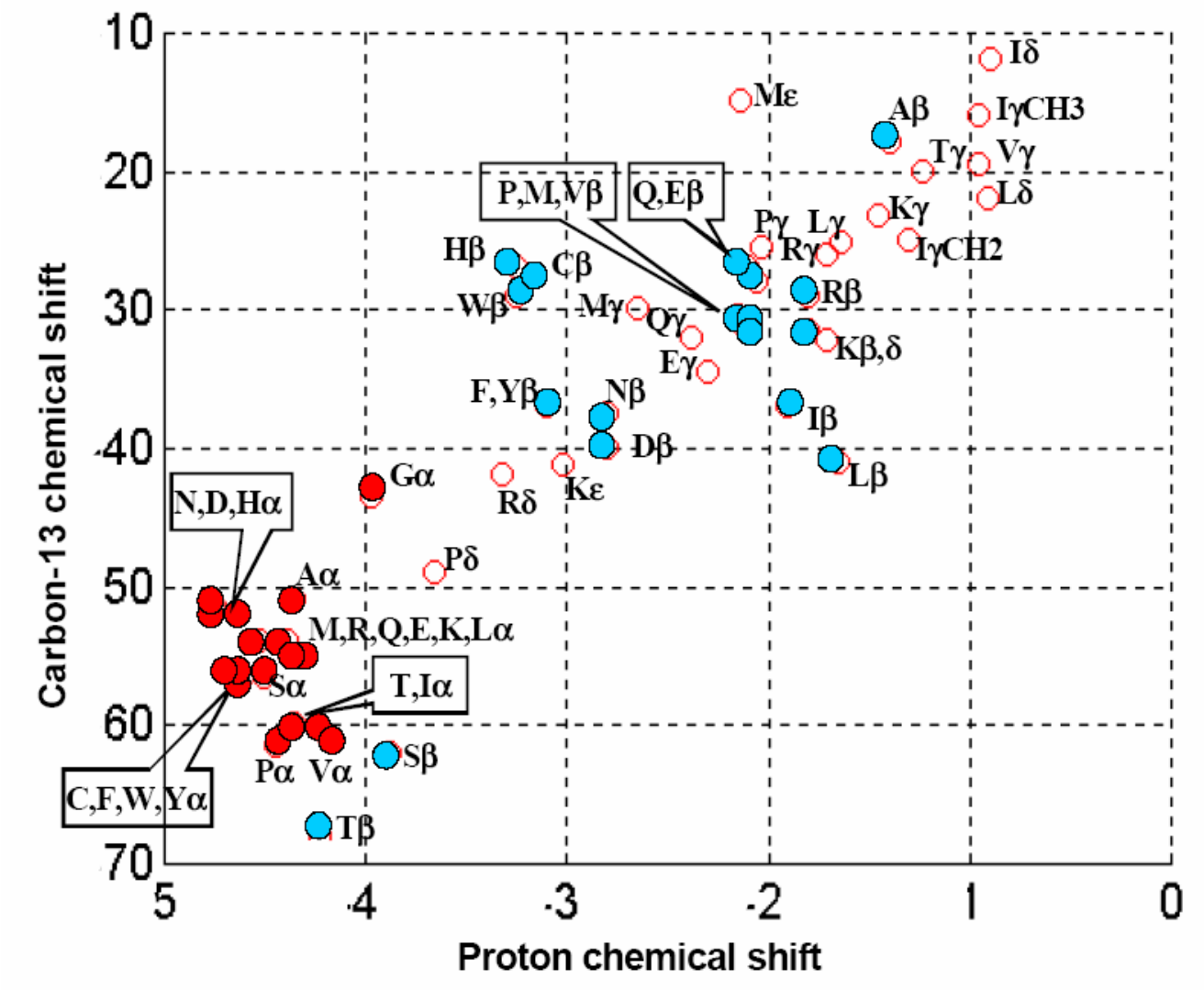
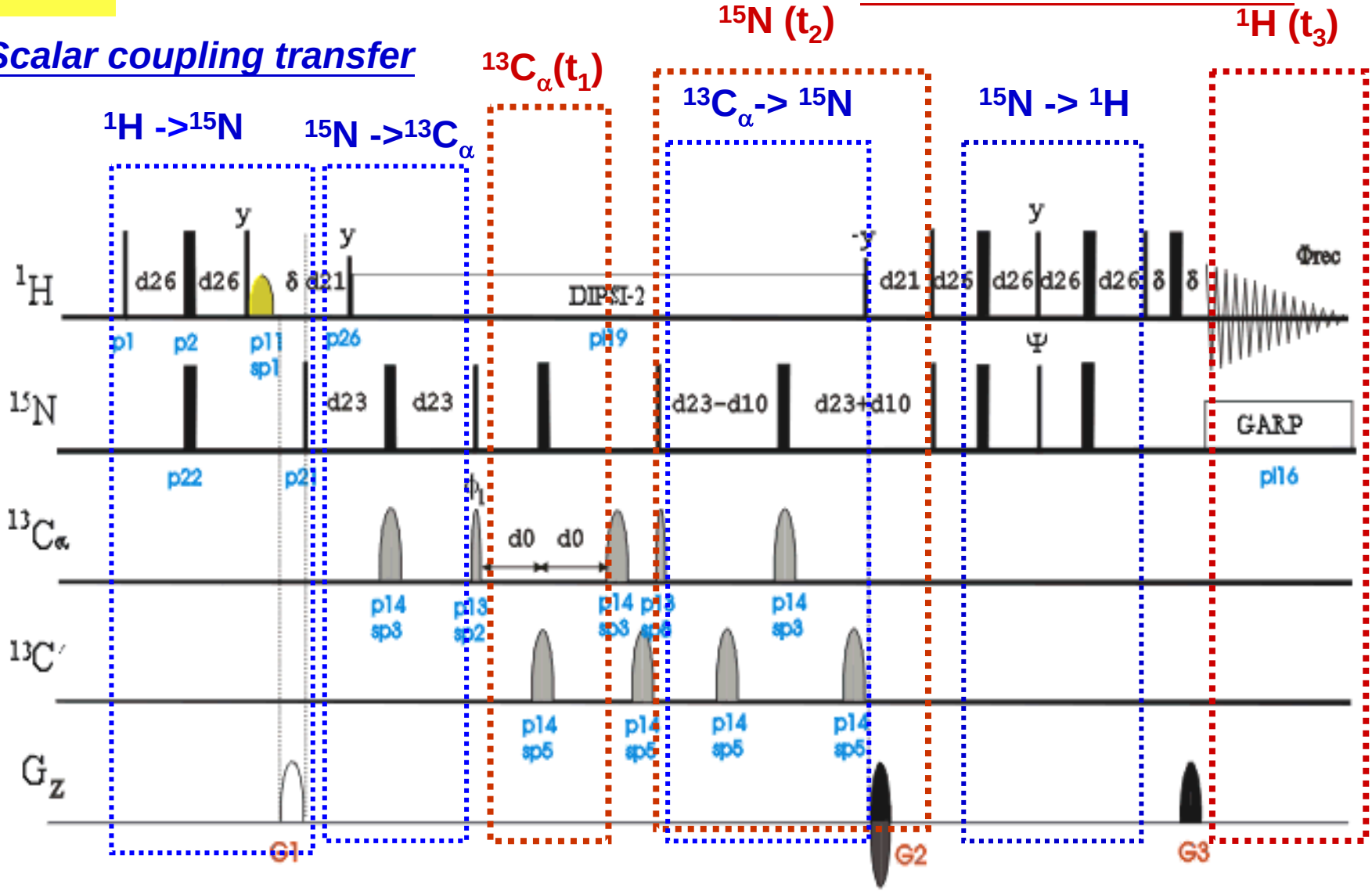


Figure from Bruker Avance 3D triple resonance

Concepts on triple resonance experiments

HNCA

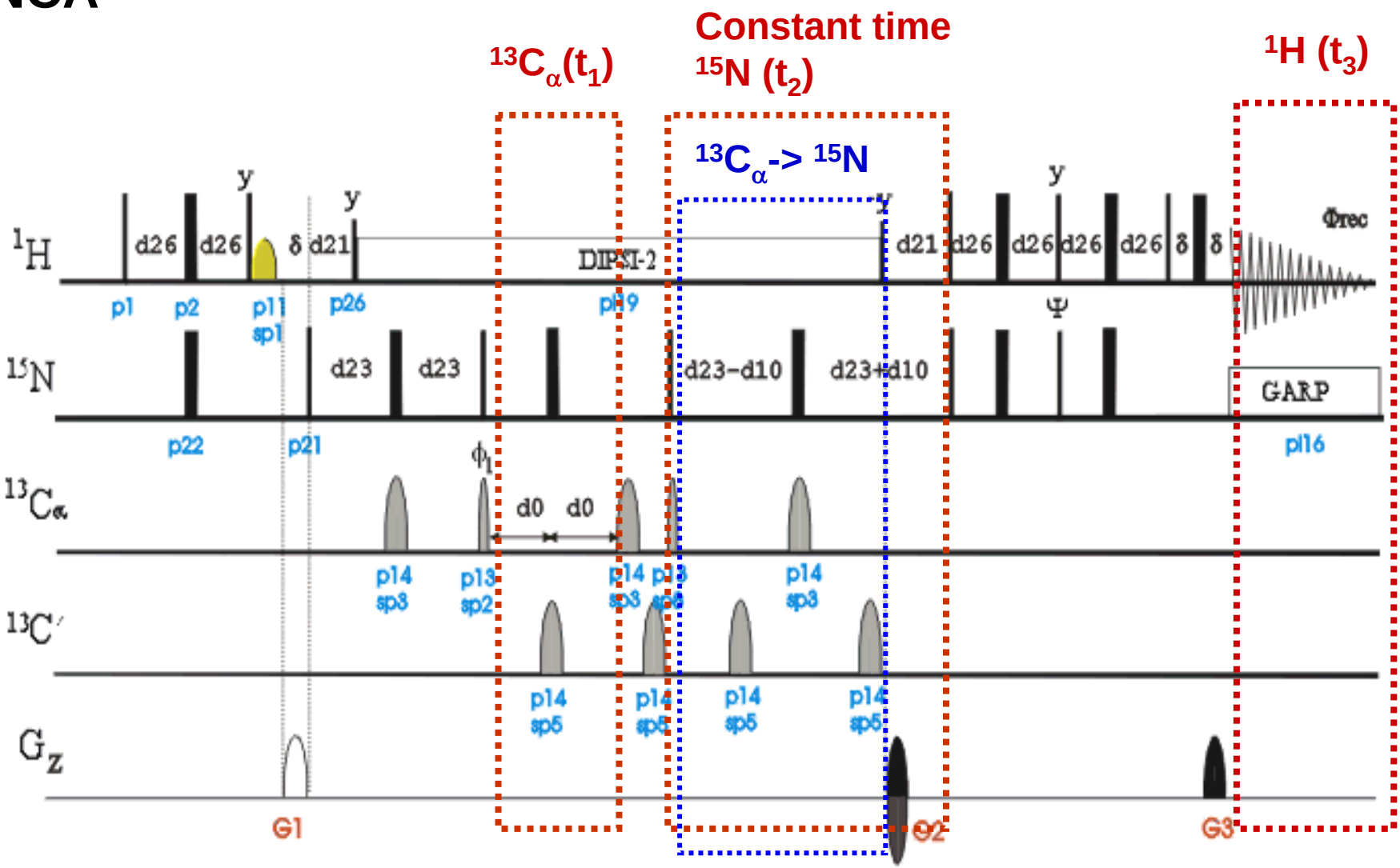
Scalar coupling transfer ***Chemical shift evolution***



d26: 1/(4J$_{NH}$)= 2.3 msec **d21:** 1/(2J$_{NH}$) =5.5 msec **d23:** 1/(4J$_{NC\alpha}$)=12 msec

Figure from Bruker NMR Guide

HNCA



^{15}N constant time evolution: simultaneous chemical shift evolution of ^{15}N and ^{15}N-^{13}C scalar coupling to transfer magnetization from ^{13}C to ^{15}N.

Advantage: shorter pulse sequence, reduction in transverse relaxation loss.

Figure from Bruker NMR Guide

Transformations of magnetization

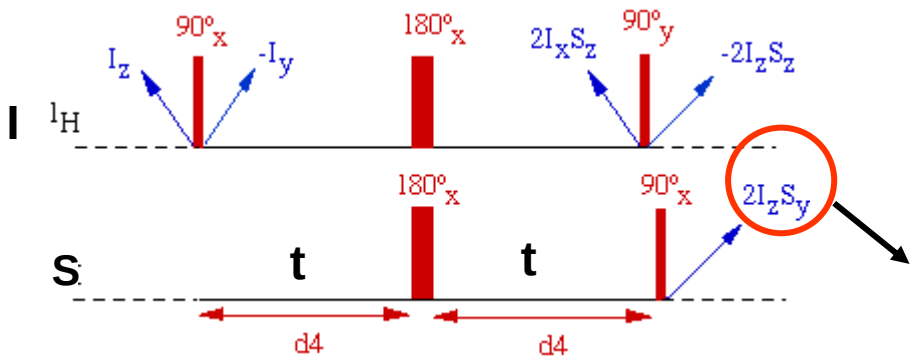
Pulses: change the orientation of the magnetization

Chemical shift: label the magnetization with characteristic frequency during evolution time t

Scalar coupling (J-coupling): transfer magnetization between J-coupled spins.

Gradients: dephase/rephase magnetization

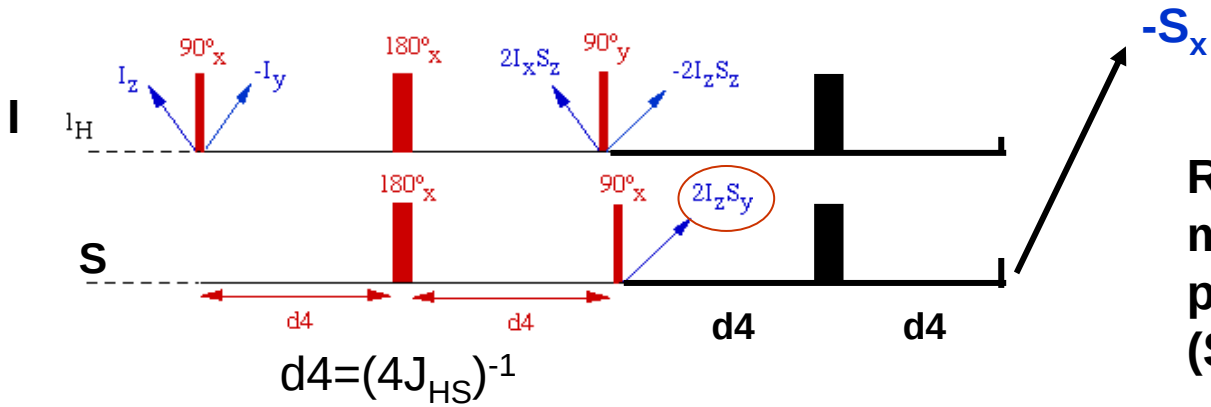
INEPT Sequence: I_z -> $2I_zS_y$



d4= $(4J_{HS})^{-1}$

when t=d4= $(4J_{HS})^{-1}$
$2I_zS_y\sin(2\pi J_{HS}t)=2I_zS_y$
maximal transfer of magnetization from ^1H to S,

Refocused INEPT Sequence: I_z -> $-S_x$

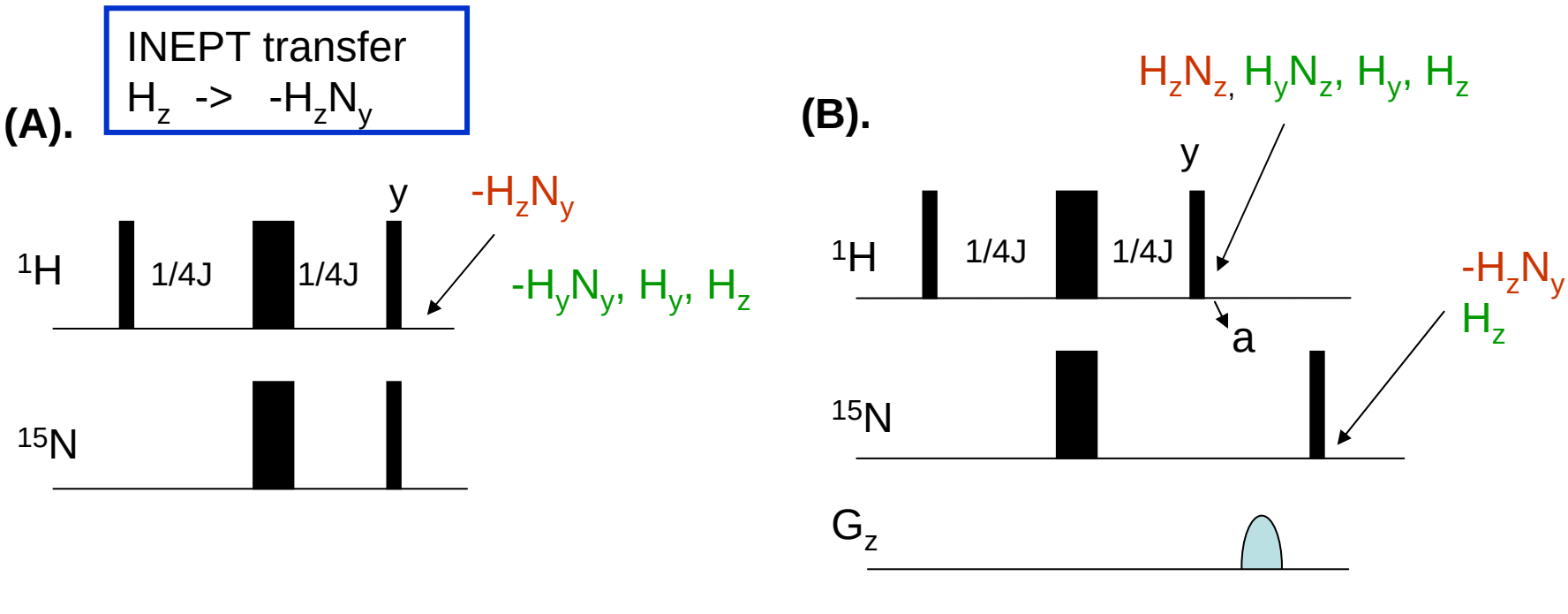


d4=$(4J_{HS})^{-1}$

Refocus anti-phase S magnetization (I_zS_y) to in-phase S magnetization (S_x).

Figure from Bruker NMR Guide

Artifact suppression by a Z-gradient pulse



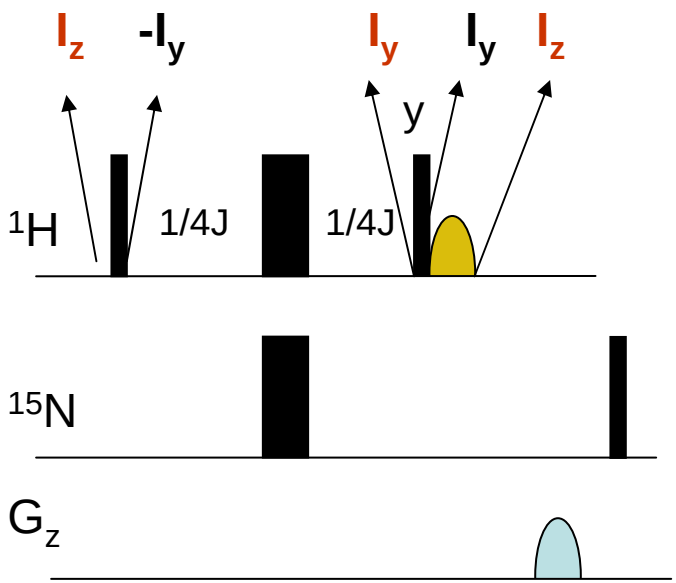
There are several magnetization created at point “a”:

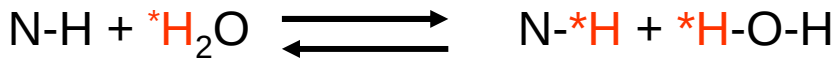
Desired magnetization: H_zN_z
Unwanted magnetization: H_yN_z, H_y, H_z

•The unwanted magnetization with transverse component can be destroyed by a short Z-gradient pulse; this also reduces phase cycling for artifact removal.

A. Bax et al. *J. Mag. Res.*, 99, 638-643 (1992)

Water Suppression



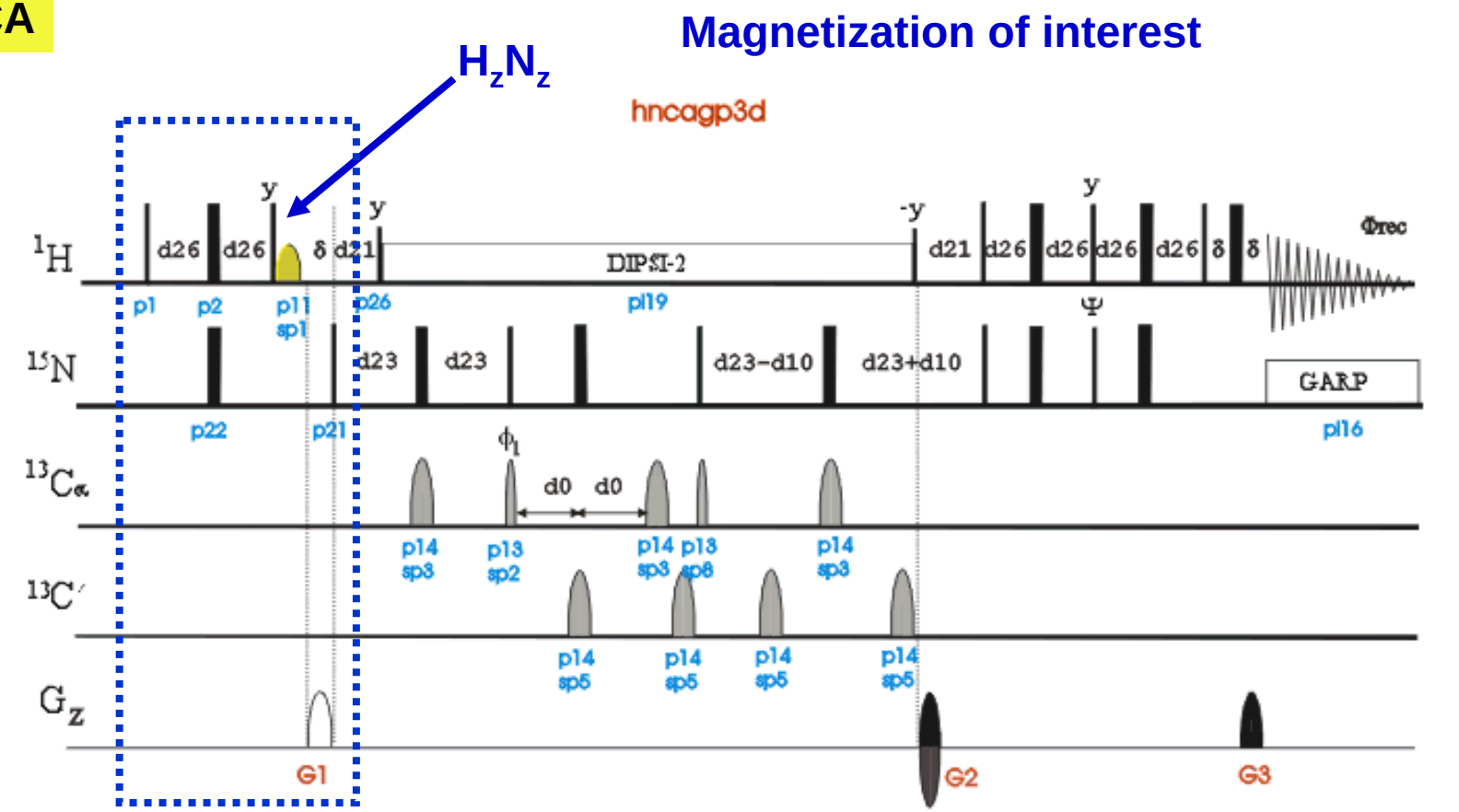


The selective pulse on water (yellow shaped) restores the water magnetization to the +Z axis before applying the de-phasing gradient pulse. This avoids destroying the transverse water magnetization, and reduces saturation transfer to labile amide protons.

L.E. Kay, G.Y. Xu & T. Yamazaki, J. Magn. Reson. A109, 129-133 (1994)

Artifact Suppression by Z-Gradient Pulse

HNCA

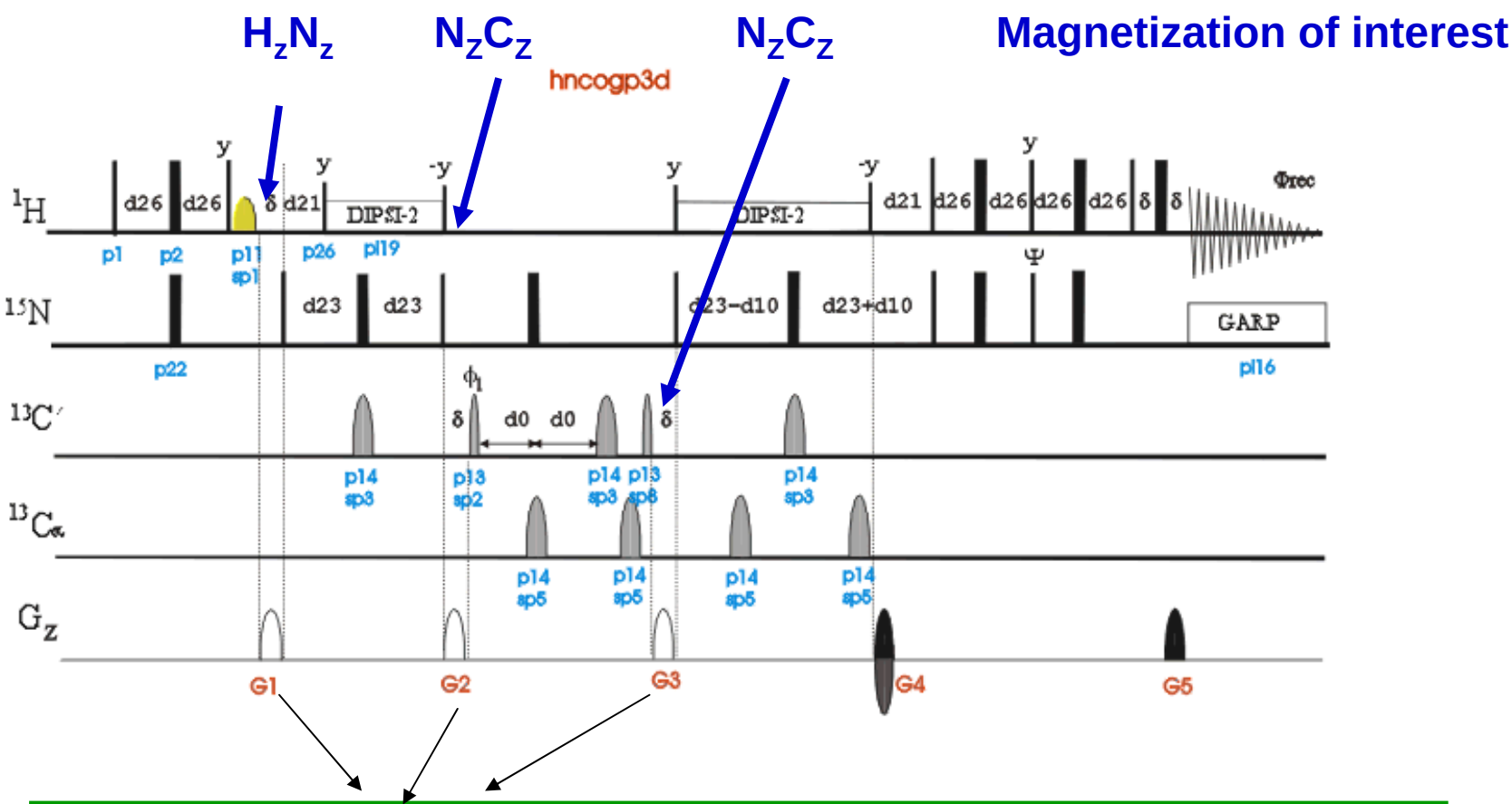


G1 gradient is used to suppress artifact on x/y plane, while the magnetization of interest is along the Z axis.

Figure from Bruker NMR Guide

Artifact Suppression by Z-Gradient Pulse: another example

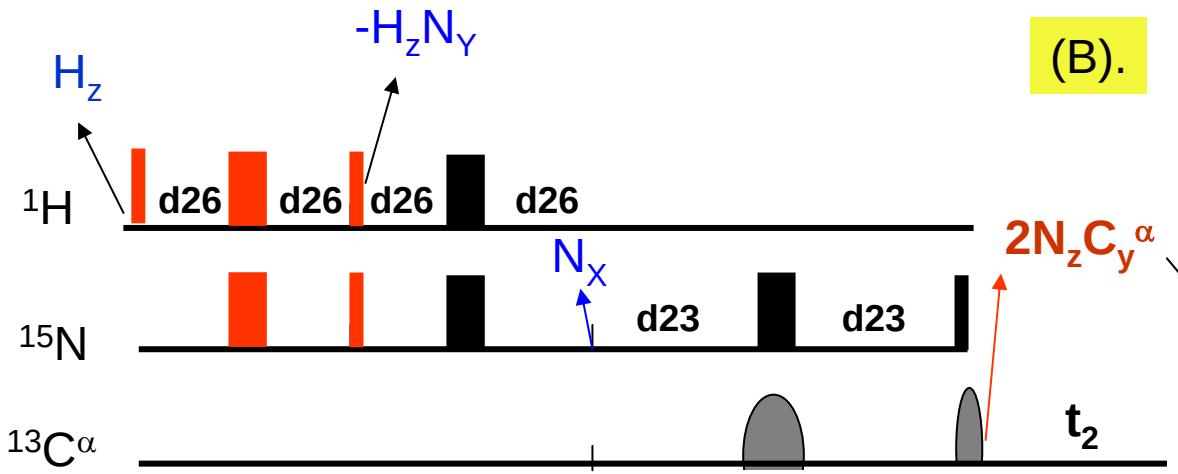
HNCO



G1, G2, G3 are used to suppress artifact on x/y plane, while the magnetization of interest is along the Z axis.

Figure from Bruker NMR Guide

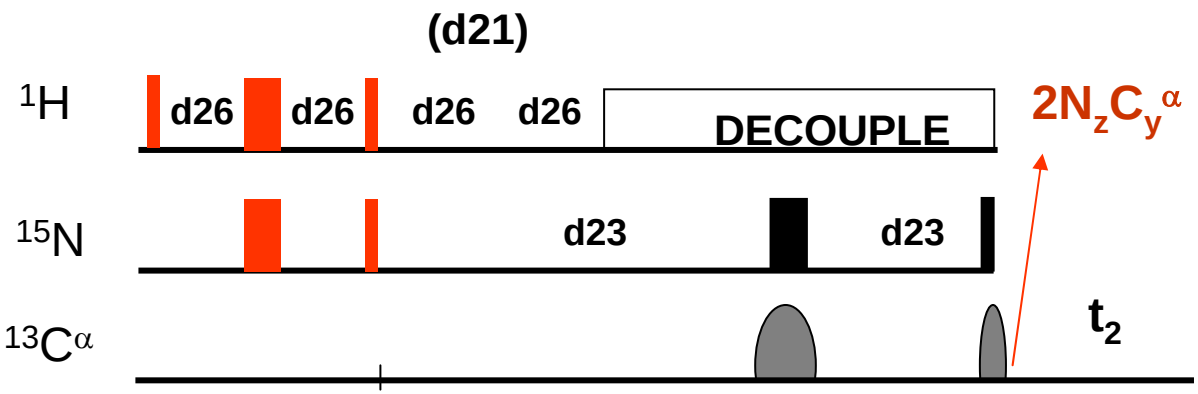
Shortening a pulse sequence



same output !

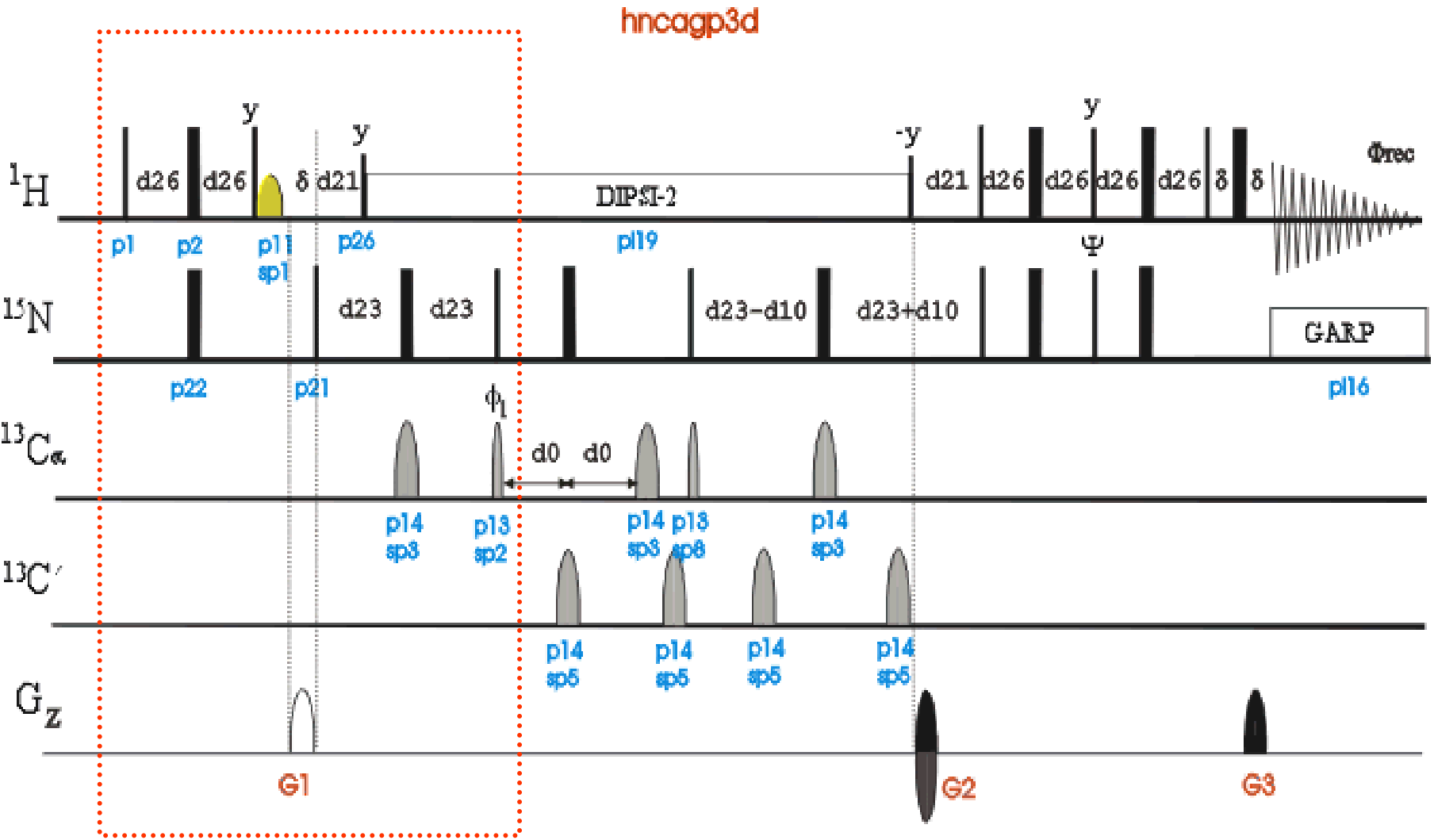
d26: $1/(4J_{NH})$= 2.4 ms **d21:** $1/(2J_{NH})$ =5.5 ms **d23:** $1/(4J_{NC\alpha})$=12 ms

(A).



Advantage of (A): shorter pulse sequence (5.5 ms shorter, and one less 180° ^{15}N pulse).

Edison et al., Method in Enzymol. V239, p3, 1994

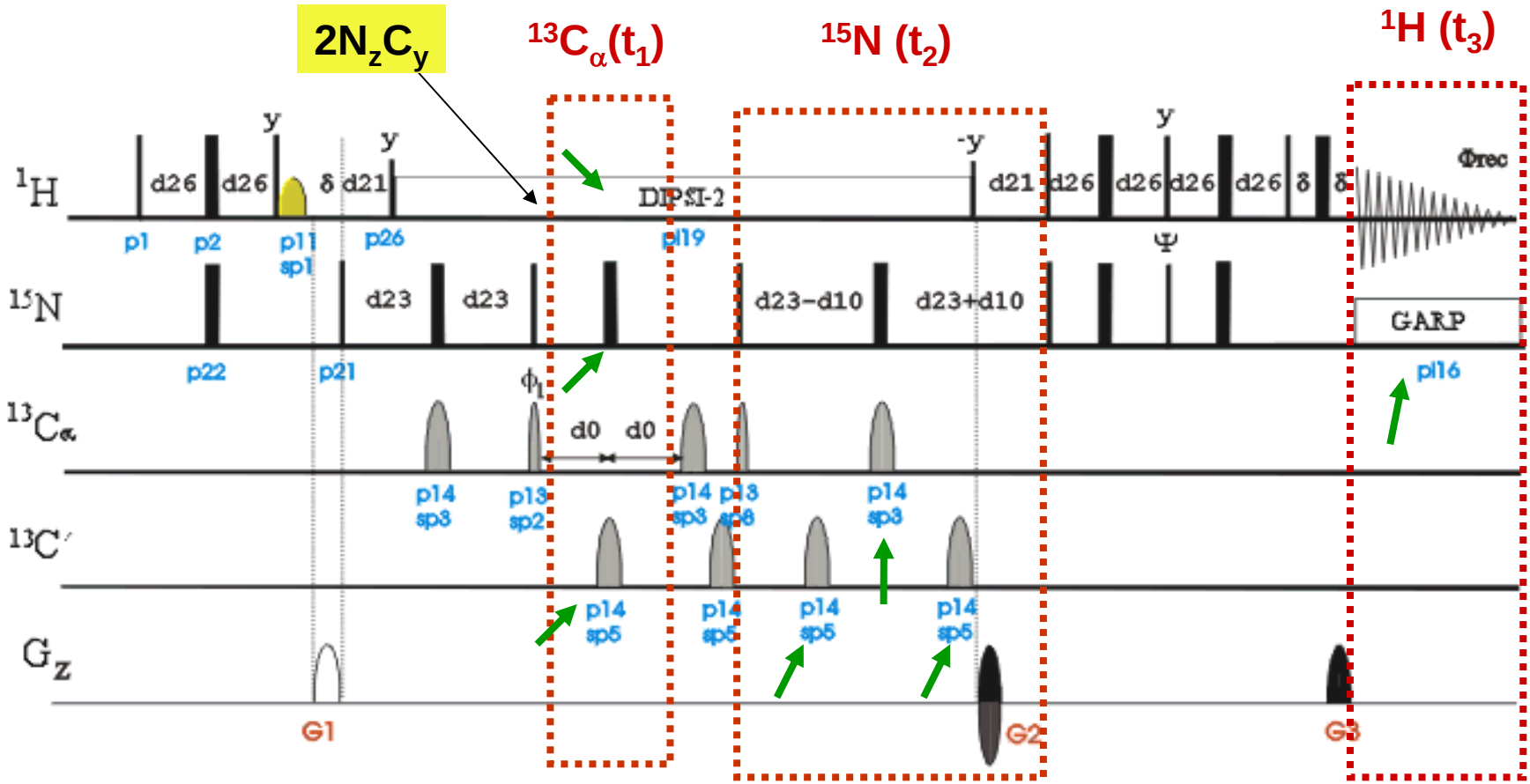


d26: $1/(4J_{NH})$= 2.4 ms **d21:** $1/(2J_{NH})$ =5.5 ms **d23:** $1/(4J_{NC\alpha})$=12 ms

Figure from Bruker NMR Guide

HNCA

Heteronuclear decoupling during chemical shift evolution



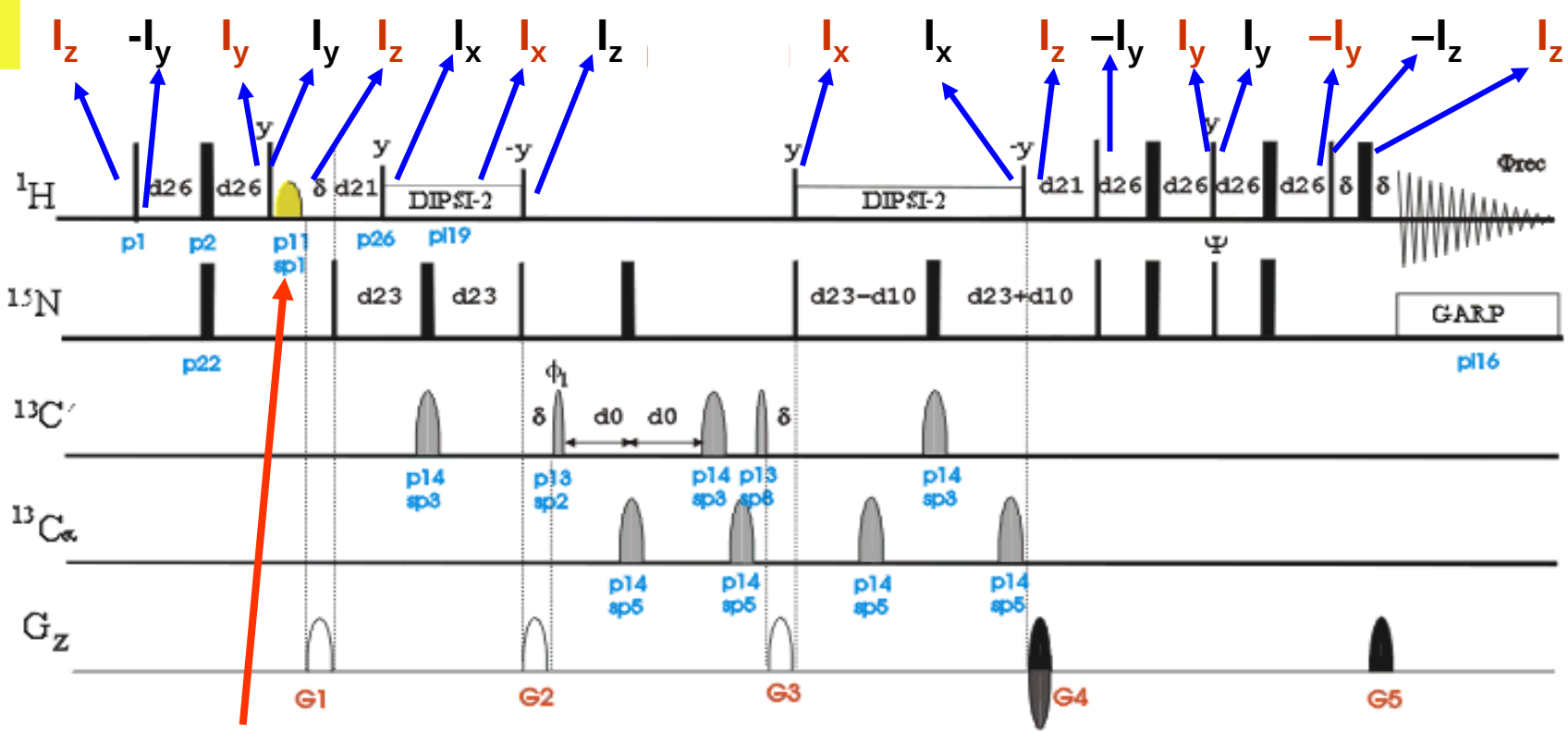
During the chemical shift evolutions:

180-degree inversion pulses (or CPD decoupling pulses) are applied to J-coupled heteronuclei in the middle of chemical shift evolution to remove heteronuclear J-coupling.

Water suppression in triple resonance experiment

Example: hncogp3d

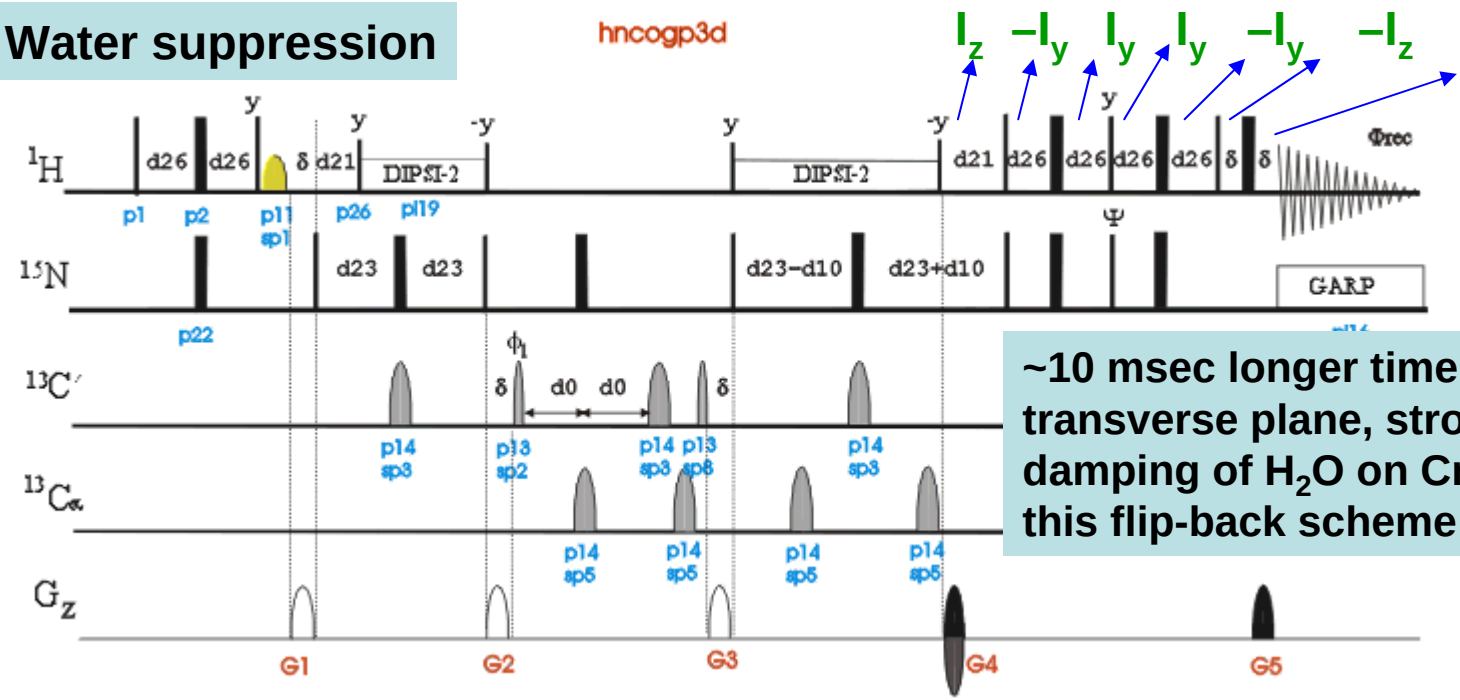
H_2O:



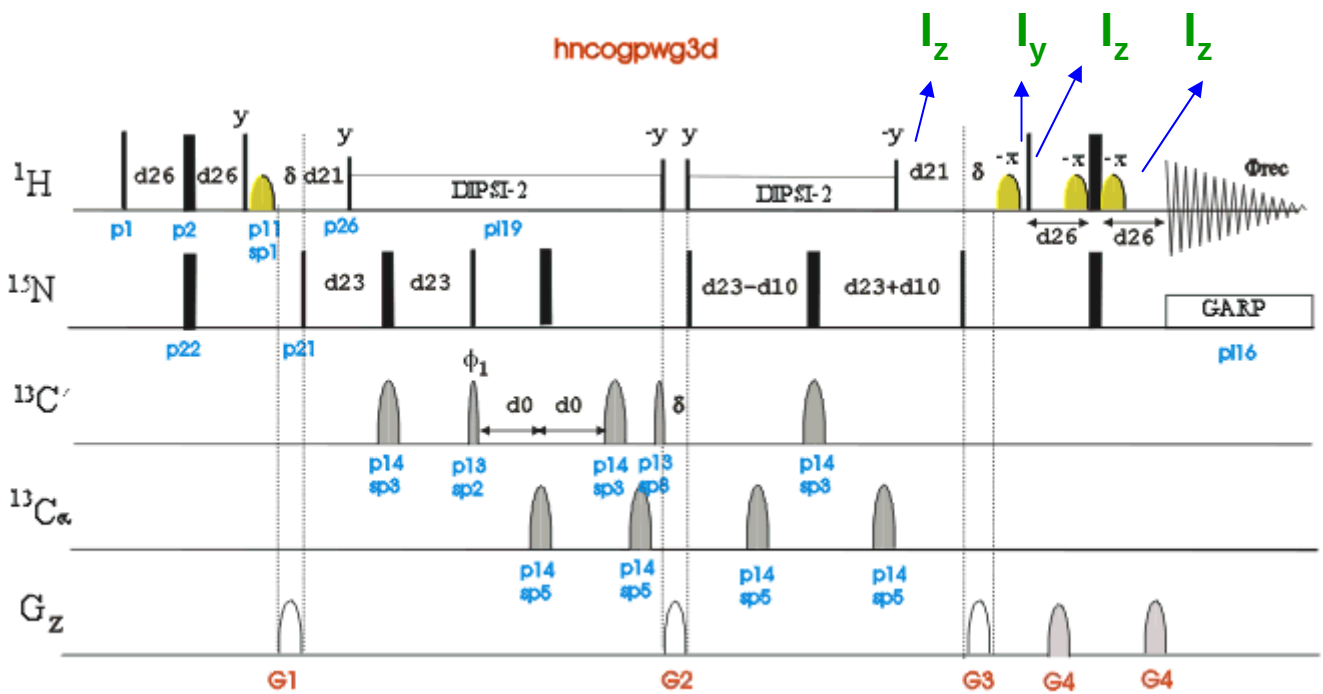
•The selective pulse on water (yellow shaped) restores the water magnetization to the +Z axis before applying the G1 de-phasing gradient pulse. This avoids destroying the transverse water magnetization, and saturation transfer to labile protons (NH's).

L.E. Kay, G.Y. Xu & T. Yamazaki, J. Magn. Reson. A109, 129-133 (1994)

Water suppression



~10 msec longer time on the transverse plane, strong radiation damping of H_2O on CryoProbe makes this flip-back scheme less effective.



WATERGATE provides higher degree of water suppression on CryoProbe. (Note: “hncogp3d” is sensitivity enhanced).

Figure from Bruker NMR Guide

Water Suppression on CryoProbe

- **Radiation damping: The presence of transverse magnetization in a sample induces oscillating current in the coil of the NMR probe. In turn, this current generates a transverse magnetic field which, it turns out, has a tendency to rotate the original magnetization towards the z-axis. This effect, known as radiation damping, is generally only significant for very intense resonances, such as that from solvent H_2O.**

- **Radiation damping is significant for high-Q (sensitivity) probe.**

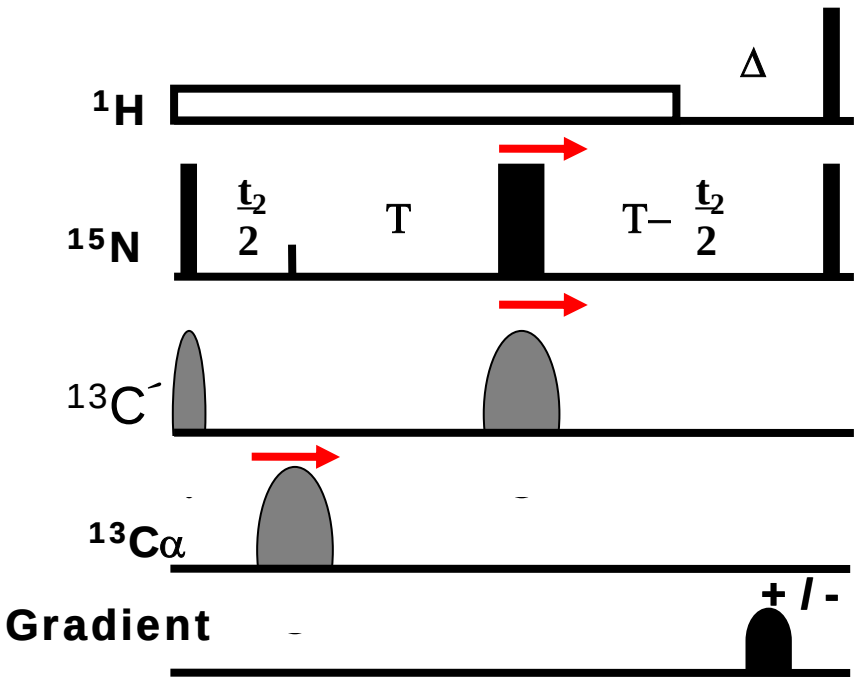
- **On CryoProbe, many "WATERGATE" type experiments (i.e. "hncogpwg3d") give higher degree of water suppression than the non- WATERGATE type experiments (i.e. "hncogp3d"). On the other hand, the "hncogp3d" is "sensitivity enhanced", therefore the relative sensitivities should be compared.**

- **Use stronger gradient for water suppression gradient. Use a more squared shaped "sqsm.100" (compared to "sine.100").**

^{15}N Constant time evolution

^{15}N constant time evolution: simultaneous chemical shift evolution of ^{15}N and ^{15}N-^{13}C scalar coupling to transfer magnetization from ^{13}C to ^{15}N.

Advantage: Shorter pulse sequence, reduction in transverse relaxation loss.



For ^{15}N ($T_{2)}$ =100 ms, ^{1}J(C‘,N)=15Hz, 2T=33 ms in a HNCO experiment:

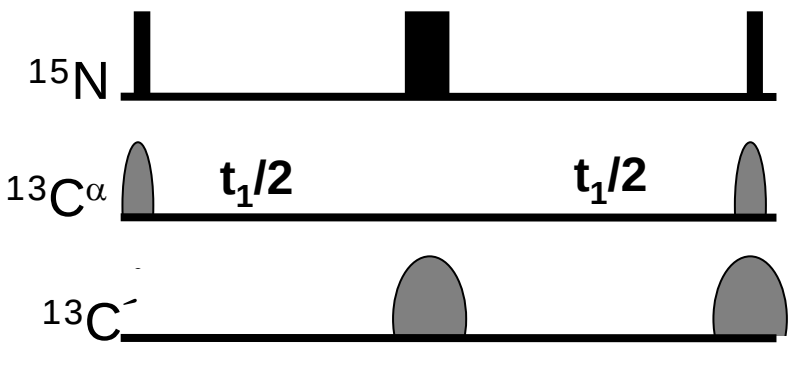
Signal intensity S_{CT}/S_{RT} = 1.2

M. Sattler et. al. „Progr.NMR Spectr“ v34 (1999), p93-158

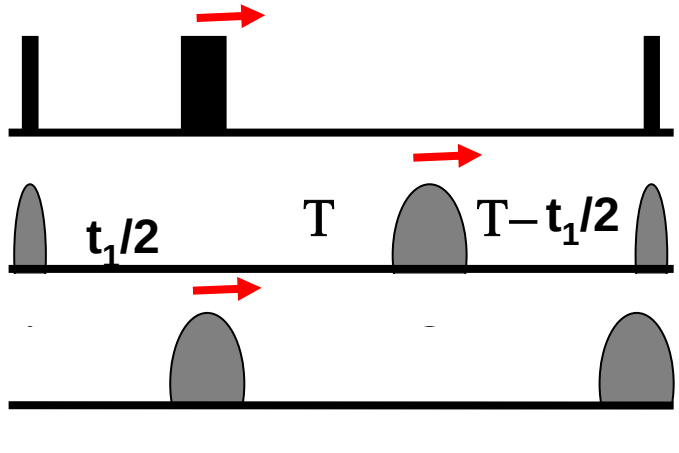
^{13}C Chemical shift evolution

HNCA

Real time



Constant time



higher sensitivity

higher resolution

Relaxation $\exp[-t_1/T_2(C^\alpha)]$ $\exp[-2T/T_2(C^\alpha)]$

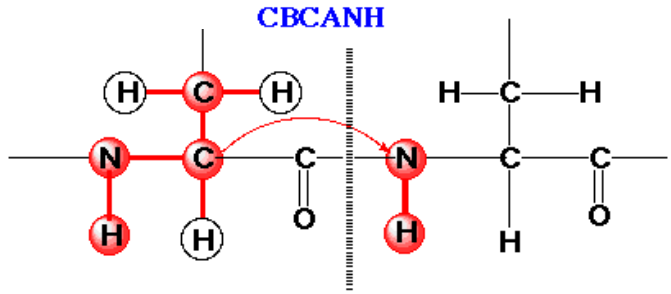
$t1_{max}$ <1/3*$Jc_\alpha c_\beta$= **10 ms**
Otherwise line-broadening or coupled signals

$t1_{max}$ < 2T=1/$Jc_\alpha c_\beta$ ~ **26 ms**

$J_{C\alpha,C\beta}$ =35Hz, Cα (T2)=20ms,
$t1_{max}$=2T=1/ $J_{C\alpha,C\beta}$ =26ms (constant time), $t1_{max}$=1/3 $J_{C\alpha,C\beta}$ =10 ms (real time)

In a HNCA experiment: *signal intensity* S_{RT}/S_{CT} *= 2.8*
However, constant time ^{13}C *provides higher resolution (without 35 Hz C-C coupling), Used with small proteins when enhanced resolution is needed or* ^{2}H*-proteins.*

HNCACB V.S. CBCANH

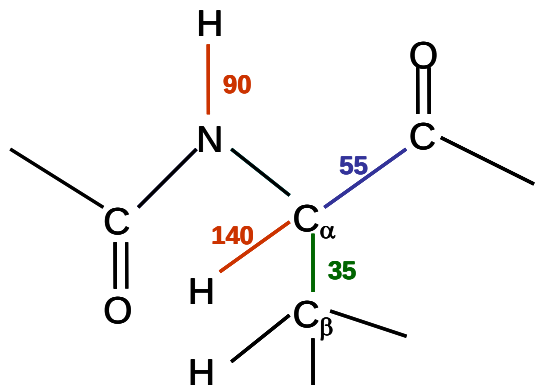


HNCACB: pulprog=hncacbgp3d

"real time" ^{13}C chemical shift evolution.

- ^{13}C magnetization is transverse for ~ **15 ms**.
- More sensitive, but ^{13}C resolution is compromised by the C, C coupling.

(for bigger proteins).



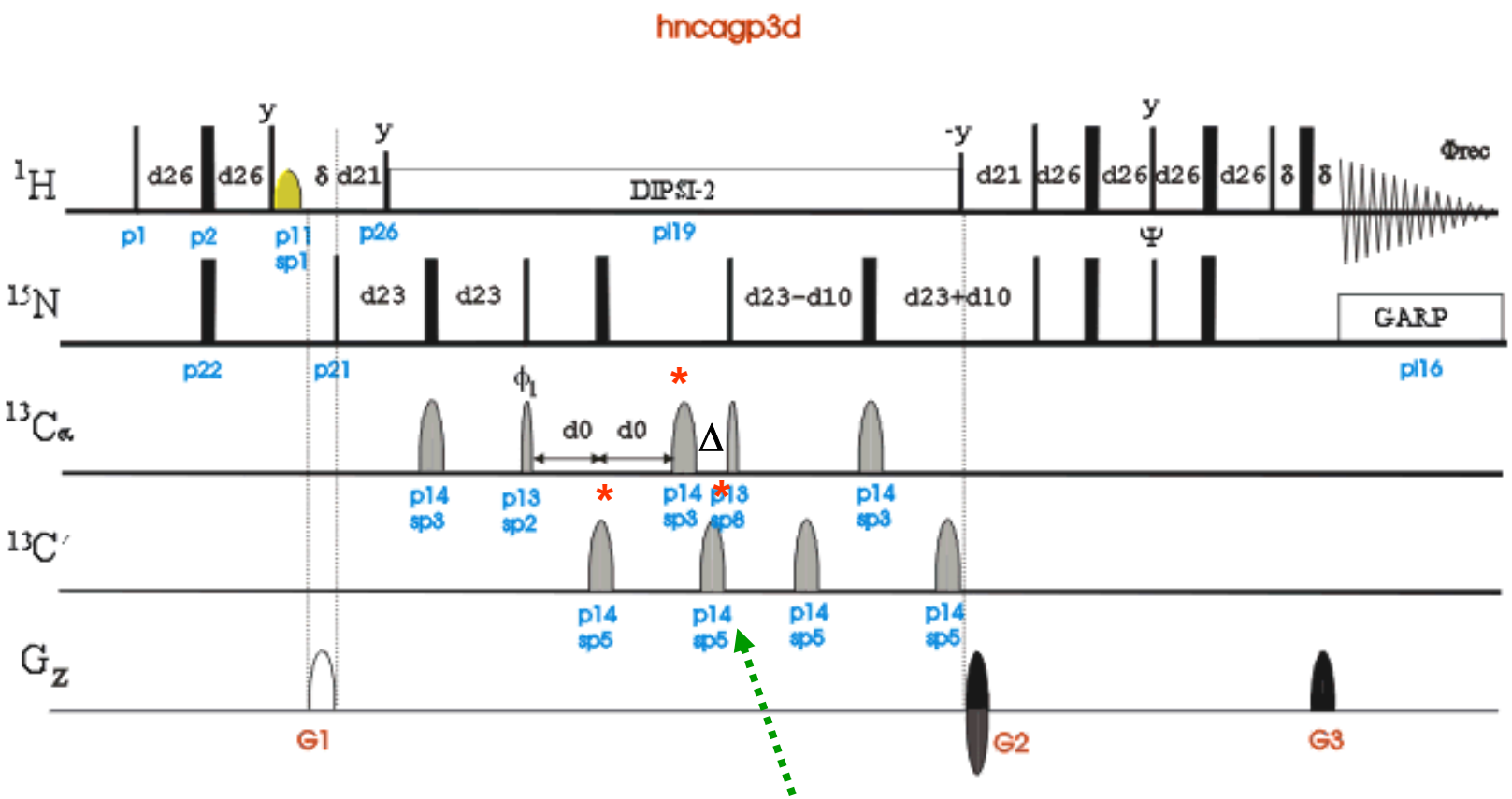
CBCANH: pulprog=cbcanhgp3d

"constant time" ^{13}C chemical shift evolution

- ^{13}C magnetization is transverse for ~ **30 ms**
- Less sensitive, but higher resolution in ^{13}C due to the absence of C-C coupling.

(for smaller proteins).

Correction for Bloch-Siegert Phase Shift



to correct for Bloch-Siegert phase shift

Bloch-Siegert phase shift:

Interference of the selective CO_180 pulse with the evolution of off-resonance transverse magnetization (Cα), even if Cα is not excited by the selective pulse.

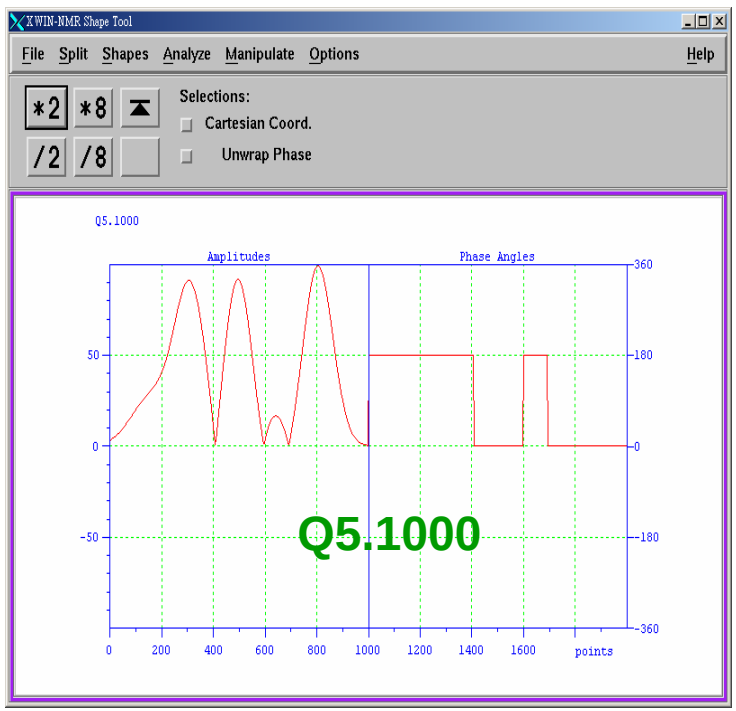
Figure from Bruker NMR Guide

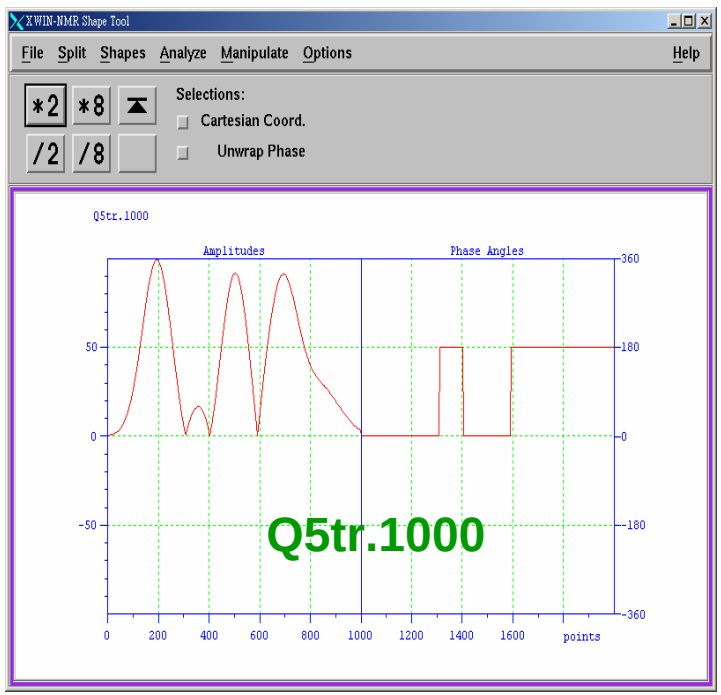
Time reversed shaped pulse

- A time-reversed pulse is “time reversed” in order to accomplish a pure 90-degree rotation in the reverse direction made by the previous ^{13}C 90°-pulse

$$NC^{\alpha}_{z} \xrightarrow[p13:sp2]{Q5.1000} NC^{\alpha}_{y} \xrightarrow[p13:sp8]{Q5tr.1000} NC^{\alpha}_{z}$$

- Same power level and duration for Q5.1000 and Q5tr.1000, just “reversed shaped”.

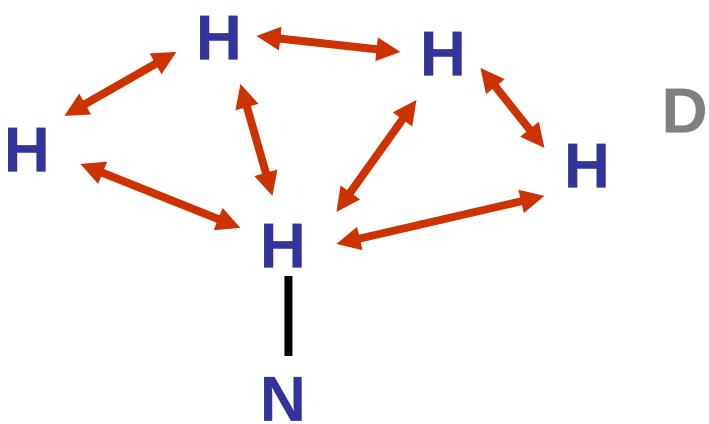


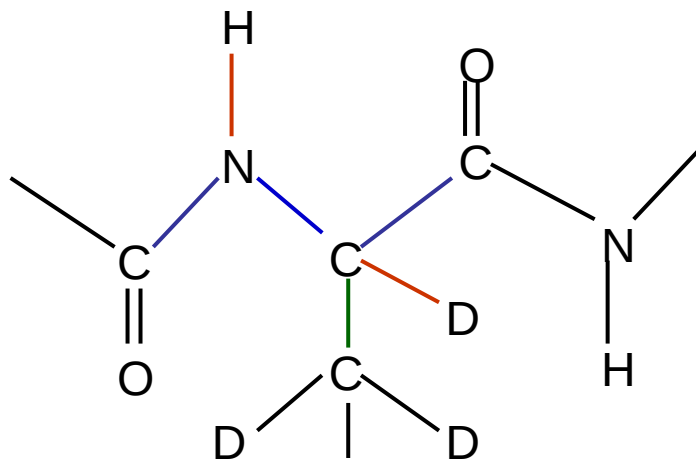


Deuteration

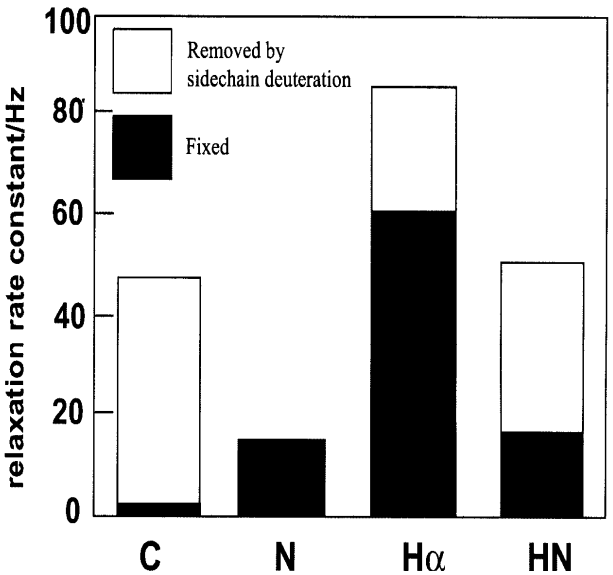
- **Reduce relaxation ($\gamma_{D/}\ \gamma_H$ = 1/6.5). (a maximal 16 fold reduction).**
- **Reduce number of signals.**
- **Suppress spin-spin diffusion**

Dipole/dipole relaxation

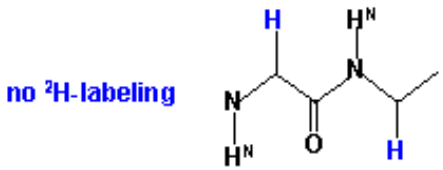


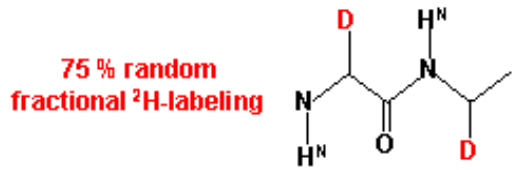


Impact of Deuteration on Relaxation and Sensitivity



(~ 12 ns correlation time)





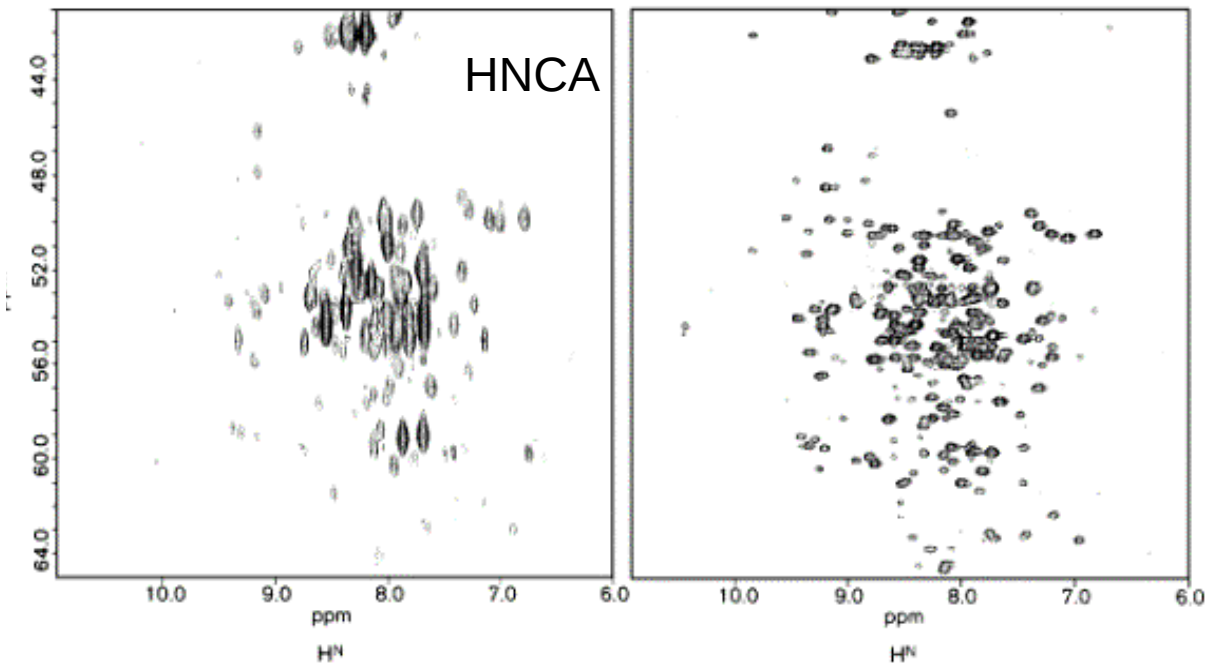
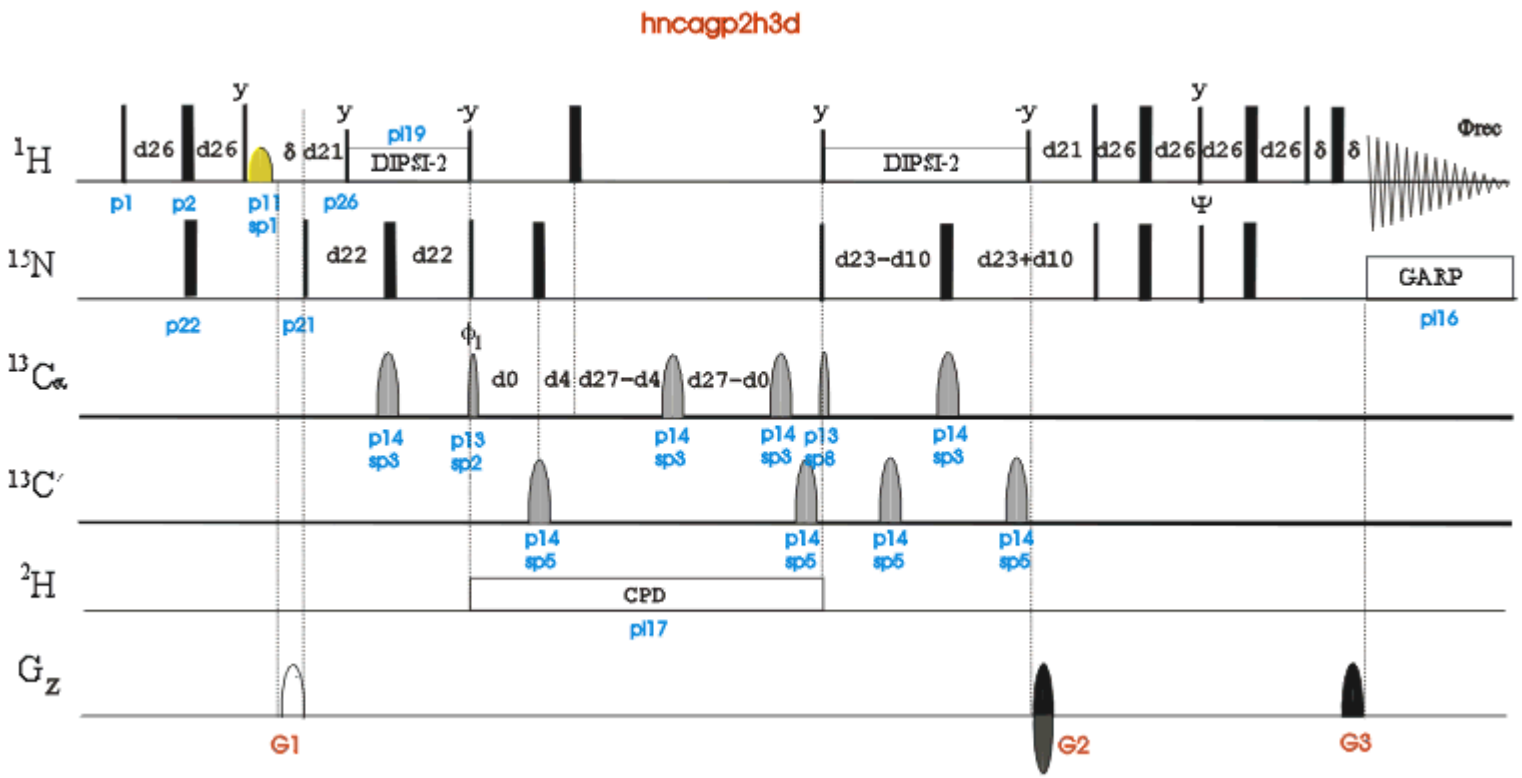


Figure from D. Nietlispach in "EMBO NMR course, 2003".

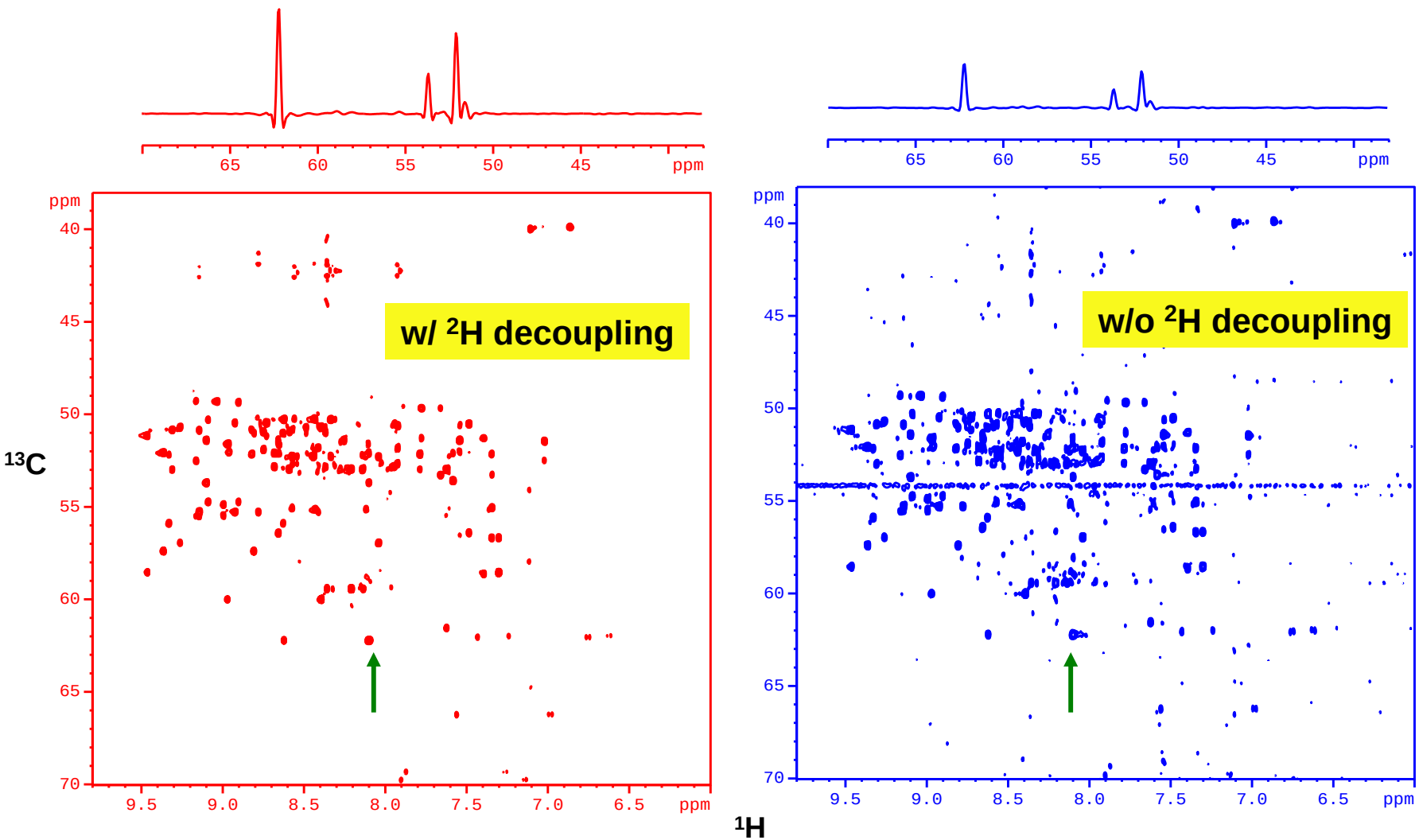
Figure from M. Sattler, http://www.embl-heidelberg.de/nmr/sattler/teaching/



^{2}H decoupling is necessary to remove 25 Hz $^{1}J_{CD}$ coupling during ^{13}C evolution for ^{2}H-proteins.

Figure from Bruker NMR Guide

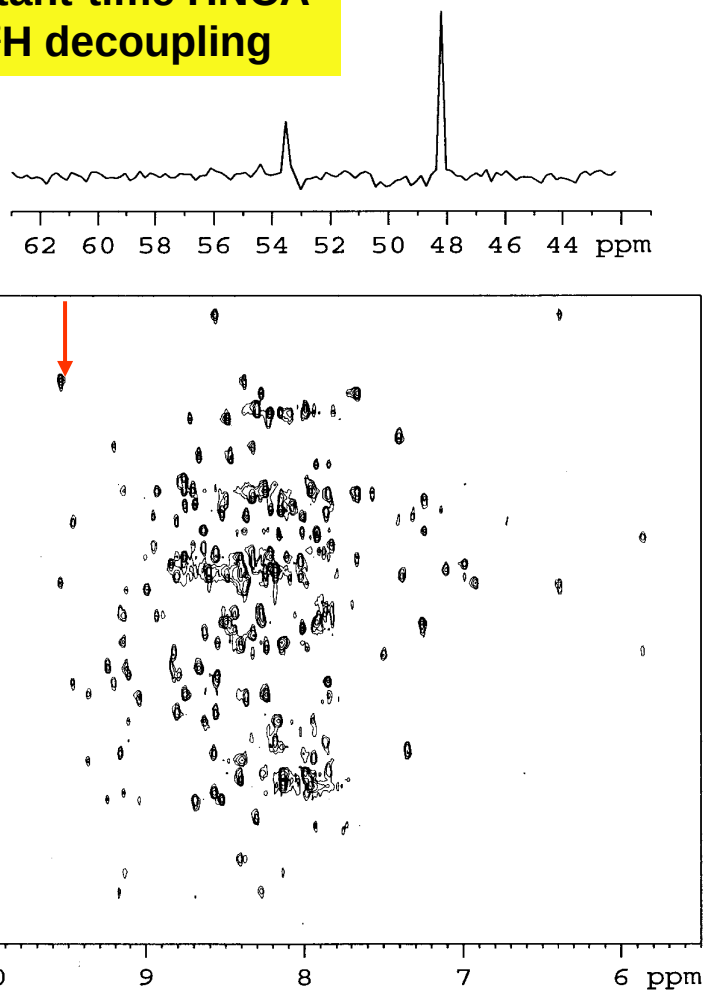
Sensitivity and Resolution Enhancement by Deuterium Decoupling for ^{2}H-Protein



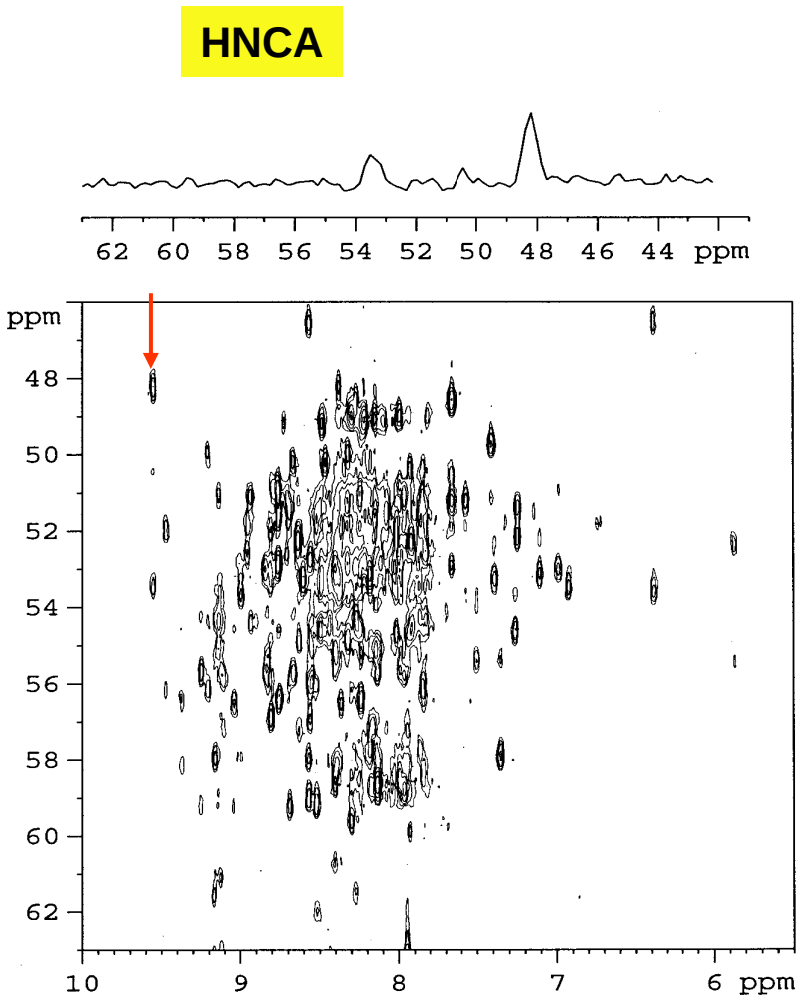
First ^{1}H-^{13}C plan of 3D constant time-HNCA of ^{2}H/^{13}C/^{15}N-SH3.
Data acquired on 800MHz CryoProbe.

Resolution enhancement by deuteration combined with ^{13}C constant time evolution

Constant-time HNCA with ^{2}H decoupling

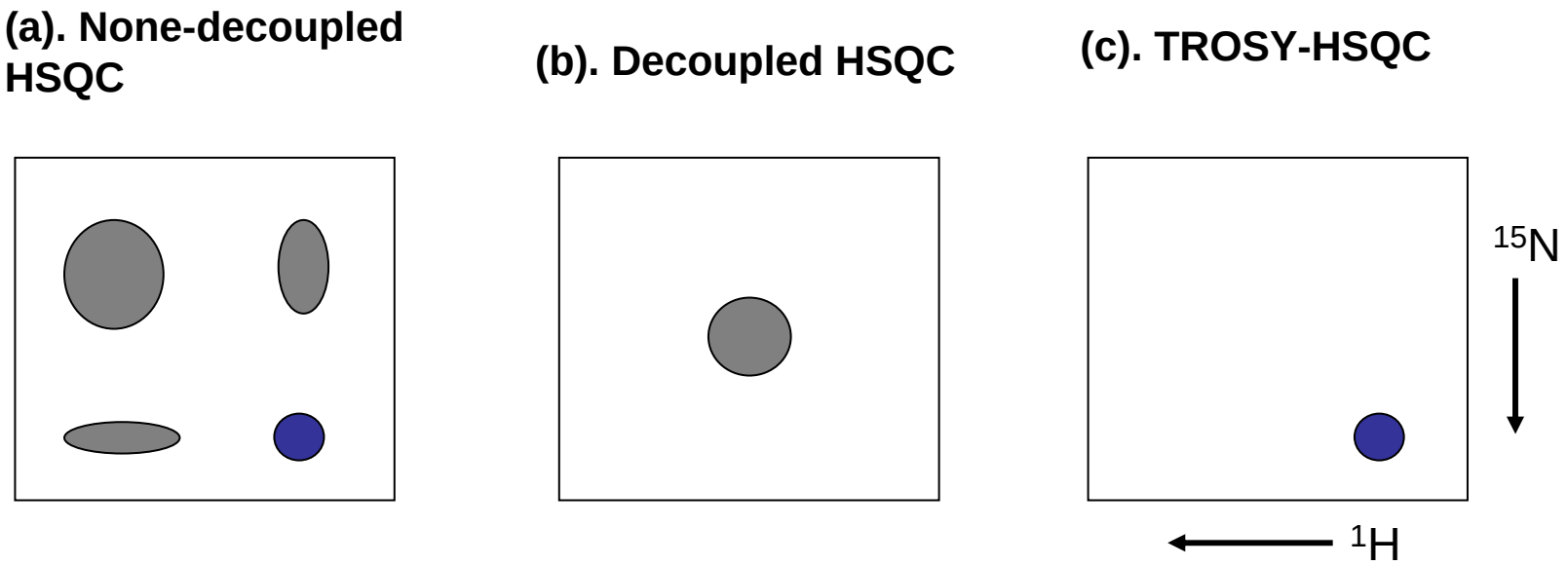


HNCA



^{2}H, ^{15}N, ^{13}C-didomain (174 a.a, from Dr. C.F. Chang)

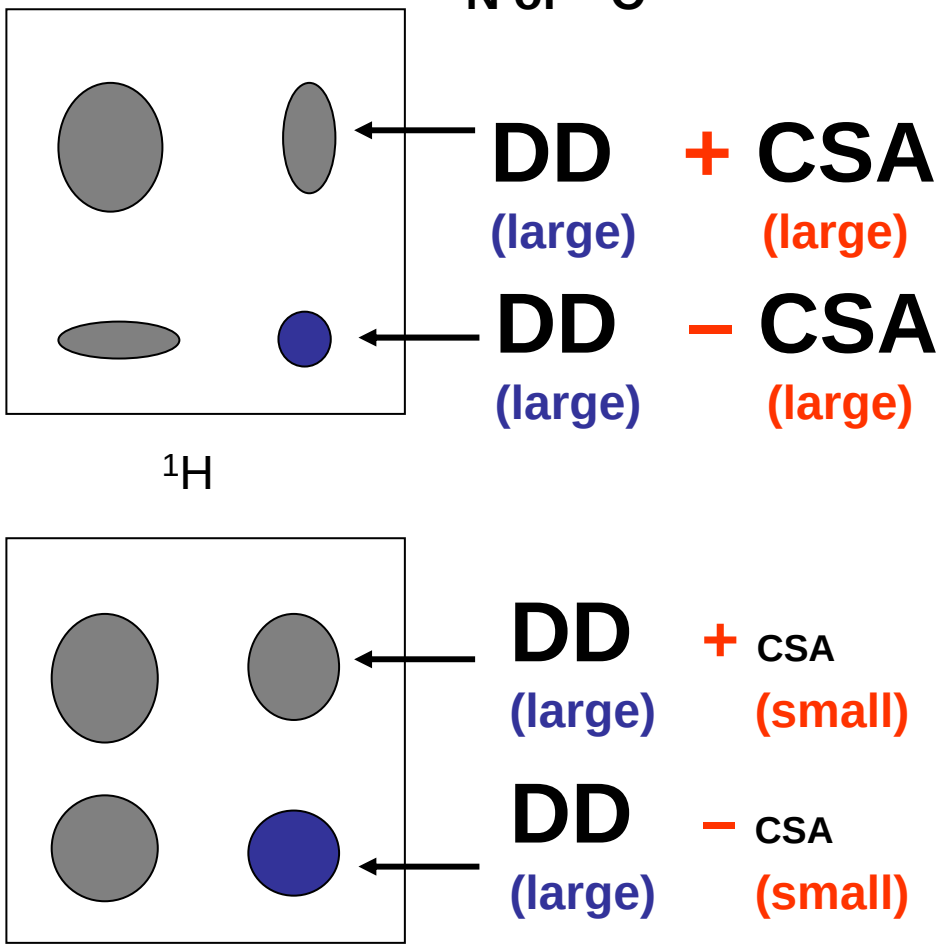
Transverse Relaxation-Optimized Spectroscopy (TROSY)

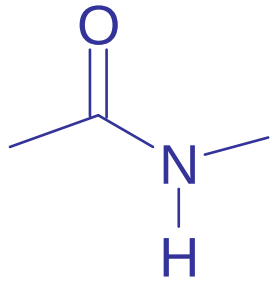


1. **Main relaxation source for ^{1}H and ^{15}N: dipole-dipole (DD) coupling and, at high magnetic fields, chemical shift anisotropy (CSA).**
2. **Different relaxation rates (line width) for each of the four components of ^{15}N-^{1}H correlation.**
3. **The narrowest peak (the blue peak) is due to the constructive canceling of transverse relaxation caused by chemical shift anisotropy (CSA) and by dipole-dipole coupling at high magnetic field.**
4. **TROSY selectively detect only the narrowest component (1 out of 4).**

Pervushin et al. *PNAS USA,* v94, p12366 (1997).

Interference between DD and CSA Relaxation



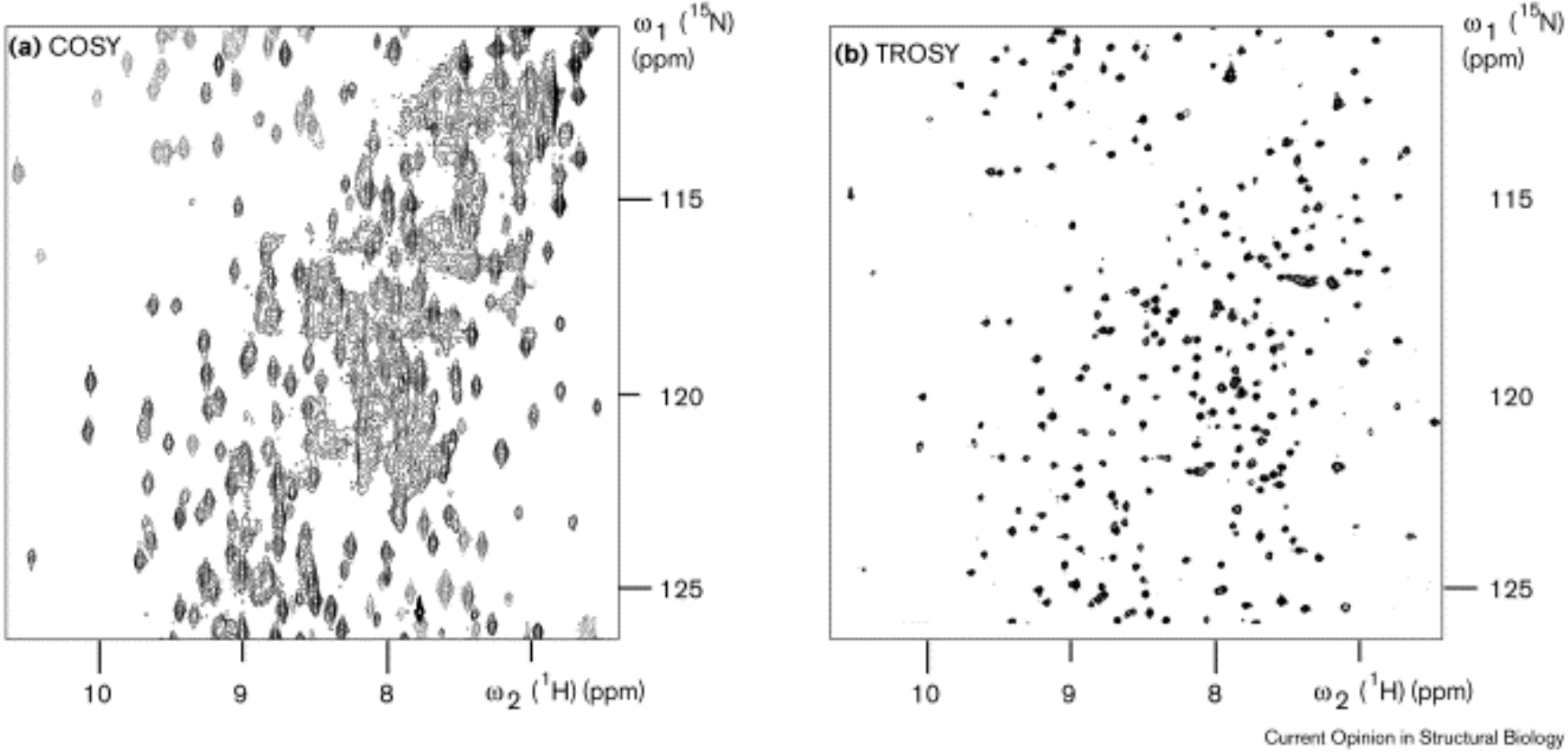


(A) At High Magnetic Field (TROSY line-narrowing effect)

(B) At Low Magnetic Field (almost no TROSY line-narrowing effect)

•DD relaxation is field-independent. However, CSA relaxation $\propto B_0^2$, therefore at high magnetic fields, CSA relaxation can be comparable to DD relaxation, and the interference effect on relaxation can be observed.

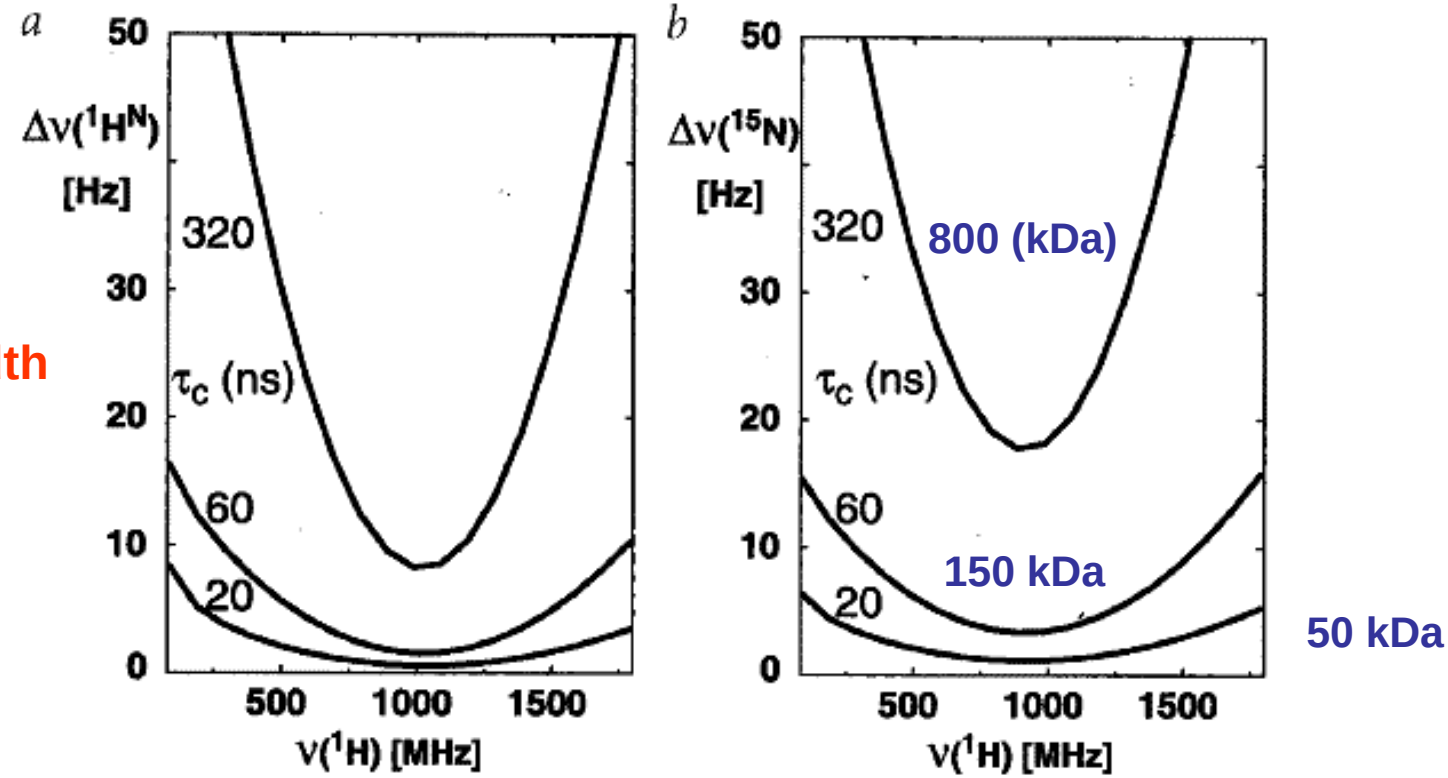
The Sensitivity and Resolution Gain by TROSY and Deuteration



u-^{2}H,^{15}N-Gyrase-45 (45 kDa), 750 MHz

Wider and Wuthrich, *Current Opinion in Structural Biology*, 1999, 9:594-601

TROSY Effect is Field Dependent and Motion Dependent



Linewidth

Magnetic field strength

•**Optimal field strength:** 1 GHz for amide NH;
600 MHz for CH in aromatic moieties (500-800 MHz applicable).