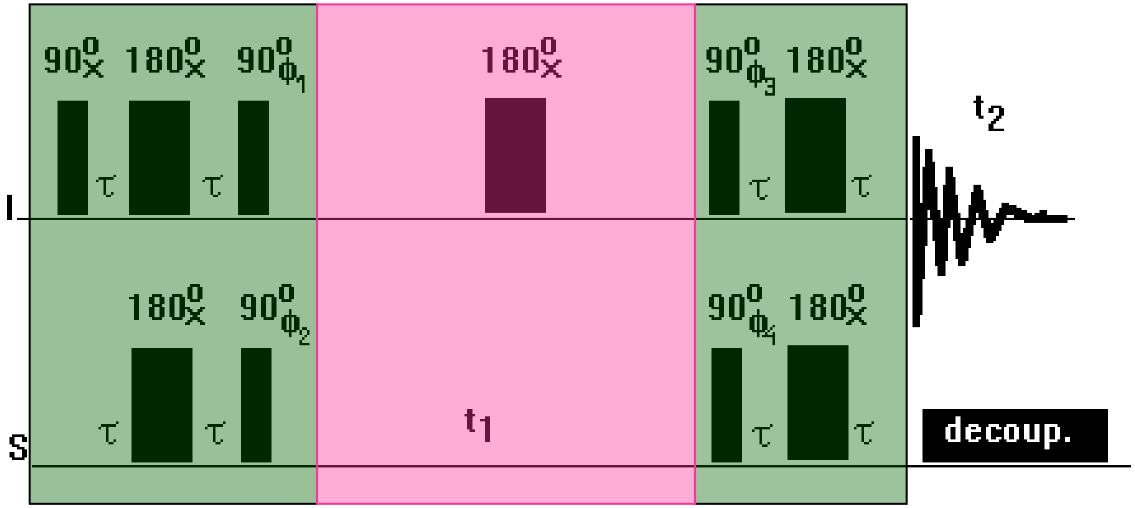
HSQC = Heteronuclear Single-Quantum Coherence

The pulse sequence:
Consider: Ω_I, Ω_S and J_{IS}
$\tau = 1/(4J_{IS})$ in order to maximize the coherence transfer from I to S



The overal pulse sequence can be subdivided as follows: **INEPT** + **echo** + **INEPT.**

1) The first INEPT is to improve the sensitivity of the low magnetogyric ratio nuclei (^{15}N, ^{13}C).

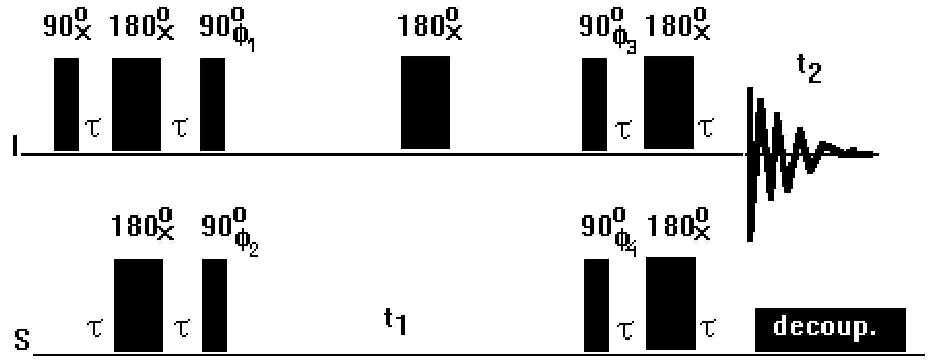
2) During the defocused delay (echo), t_1, only Ω_S evolves (coupling is refocused).

3) The final INEPT-type unit is to perform inverse detection
(observing the frequency of the low magnetogyric ratio nuclei as ^1H magnetization.)

σ[eq.] $\mathbf{I}_z$

$\hat{H} = \pi/2\ (\hat{I}_x)$ $\downarrow 90°_x$

σ[0] $-\mathbf{I}_y$



this is a „homo" type echo:

$\hat{H} = \hat{I}_z(\Omega_I\tau)$ and $S_z(\Omega_S\tau)$

$\hat{H} = 2\hat{I}_z\check{S}_z(J_{IS}\pi\tau)$

$\hat{H} = \pi\hat{I}_x$

$\hat{H} = \pi S_x$

$\hat{H} = \hat{I}_z(\Omega_I\tau)$ and $S_z(\Omega_S\tau\ )$ $\downarrow$

$\hat{H} = 2\hat{I}_z\check{S}_z(J_{IS}\pi\tau)$

the chemical shift is refocused but coupling evolves,

thus the effective $\hat{H}$ is composed of 3 terms: $\hat{H} = 2\hat{I}_z\check{S}_z(J_{IS}\pi 2\tau)$, $\hat{H} = \pi\hat{I}_x$ and $\hat{H} = \pi S_x$

σ[0] $-\mathbf{I}_y$

$\hat{H} = 2\hat{I}_z\check{S}_z(J_{IS}\pi 2\tau)$ $\downarrow$

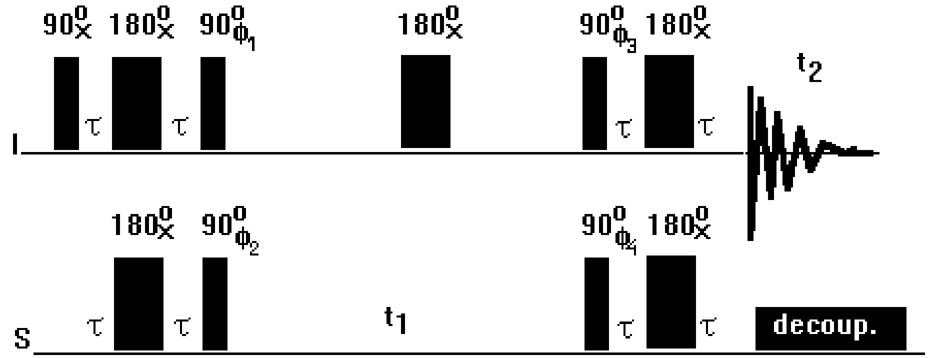
$-\mathbf{I}_y \cos(\pi J_{IS}2\tau) + 2\mathbf{I}_x\mathbf{S}_z \sin(\pi J_{IS}2\tau)$

$\hat{H} = \pi\hat{I}_x$ $\downarrow$

$+\mathbf{I}_y \cos(\pi J_{IS}2\tau) + 2\mathbf{I}_x\mathbf{S}_z \sin(\pi J_{IS}2\tau)$

$\hat{H} = \pi S_x$ $\downarrow$

$+\mathbf{I}_y \cos(\pi J_{IS}2\tau) - 2\mathbf{I}_x\mathbf{S}_z \sin(\pi J_{IS}2\tau)$



$+\mathbf{I}_y \cos(\pi J_{IS}2\tau)$ $-2\mathbf{I}_x\mathbf{S}_z \sin(\pi J_{IS}2\tau)$

$\tau = 1/(4J_{IS})$ in order to maximise the coherence transfer from spin I to S

if $\tau = 1/(4J_{IS})$, then after 2τ $\sin(\pi J_{IS}2\tau) = 1$ and $\cos(\pi J_{IS}2\tau) = 0$

$-2\mathbf{I}_x\mathbf{S}_z$ (anti-phased magnet. on I)

$\hat{H} = \pi/2\hat{I}_y$ $\downarrow$

$+2\mathbf{I}_z\mathbf{S}_z$

$\hat{H} = \pi/2S_x$ $\downarrow$

$-2\mathbf{I}_z\mathbf{S}_y$ (anti-phased magnet. on S)

$\hat{H} = \hat{I}_z(\Omega_I[1/2t_1])$

$\hat{H} = 2\hat{I}_z\check{S}_z(J_{IS}\pi[1/2t_1])$

$\hat{H} = \pi\hat{I}_x$

$\hat{H} = \hat{I}_z(\Omega_I[1/2t_1])$

$\hat{H} = 2\hat{I}_z\check{S}_z(J_{IS}\pi[1/2t_1])$

hetero echo:

1) coupling is refocused

2) chemical shift of I doesn't evolve.

Thus, the only term to be considered is:

$\hat{H} = \pi\hat{I}_x$ effecting spin S

$\hat{H} = S_z(\Omega_S t_1)$ $\downarrow$

Coupling is refocused during a hetero type echo and the chemical shift of I doesn't evolve.

$-2\mathbf{I}_z\mathbf{S}_y$

$\hat{H} = \pi\hat{I}_x$ $\downarrow$

$+2\mathbf{I}_z\mathbf{S}_y$

$\hat{H} = S_z(\Omega_S t_1)$ $\downarrow$

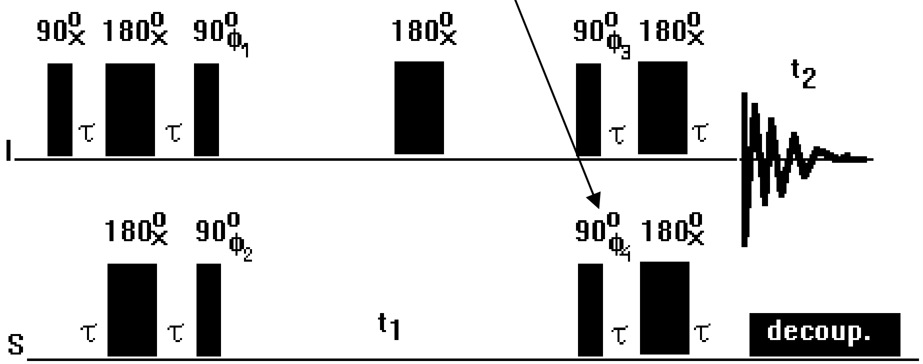
$+2\mathbf{I}_z\mathbf{S}_y \cos(\Omega_S t_1) -2\mathbf{I}_z\mathbf{S}_x \sin(\Omega_S t_1)$

$\hat{H} = \pi/2\hat{I}_x$ $\downarrow$

$-2\mathbf{I}_y\mathbf{S}_y \cos(\Omega_S t_1)+2\mathbf{I}_y\mathbf{S}_x \sin(\Omega_S t_1)$

$\hat{H} = \pi/2S_x$ $\downarrow$

$-2\mathbf{I}_y\mathbf{S}_z \cos(\Omega_S t_1)+2\mathbf{I}_y\mathbf{S}_x \sin(\Omega_S t_1)$



this is a „**homo**" type echo:

$\hat{H} = \hat{I}_z(\Omega_I\tau)$ and $S_z(\Omega_S\tau)$

$\hat{H} = 2\hat{I}_z\check{S}_z(J_{IS}\pi\tau)$

$\hat{H} = \pi\hat{I}_x$

$\hat{H} = \pi S_x$

$\hat{H} = \hat{I}_z(\Omega_I\tau)$ and $S_z(\Omega_S\tau)$

$\hat{H} = 2\hat{I}_z\check{S}_z(J_{IS}\pi\tau)$

the chemical shift is refocused so the effective $\hat{H}$ is: $\mathbf{\hat{H} = 2\hat{I}_z\check{S}_z(J_{IS}\pi 2\tau)}$, $\boldsymbol{\pi}\mathbf{I_x}$ and $\boldsymbol{\pi}\mathbf{S_x}$

so:

$\hat{H} = 2\hat{I}_z\check{S}_z(J_{IS}\pi 2\tau)$

$-2\mathbf{I}_y\mathbf{S}_z \cos(\Omega_S t_1)$	$+2\mathbf{I}_y\mathbf{S}_x \sin(\Omega_S t_1)$	(multiple quantum term)

$\downarrow$

$-2\mathbf{I}_y\mathbf{S}_z \cos(\Omega_S t_1)\cos(\pi J_{IS}2\tau) \quad +\mathbf{I}_x \cos(\Omega_S t_1)\sin(\pi J_{IS}2\tau)$

$\hat{H} = \pi\hat{I}_x$

$\downarrow$

$-2\mathbf{I}_z\mathbf{S}_z \cos(\Omega_S t_1)\cos(\pi J_{IS}2\tau) \quad +\mathbf{I}_x \cos(\Omega_S t_1)\sin(\pi J_{IS}2\tau)$

$\hat{H} = \pi S_x$

$\downarrow$

$+2\mathbf{I}_z\mathbf{S}_y \cos(\Omega_S t_1)\cos(\pi J_{IS}2\tau) \quad +\mathbf{I}_x \cos(\Omega_S t_1)\sin(\pi J_{IS}2\tau)$

$\tau = 1/(4J_{IS})$ to maximise the coherence transfer from I to S

(if $\tau = 1/(4J_{IS})$ then after 2τ $\sin(\pi J_{IS}2\tau) = 1$ and $\cos(\pi J_{IS}2\tau) = 0$)

So before ACQ the following term remains: $+\mathbf{I}_x \cos(\Omega_S t_1)$

ACQ

$\hat{H} = \hat{I}_z(\Omega_I[t_2])$ and $S_z(\Omega_S[t_2])$

$\hat{H} = 2\hat{I}_z\check{S}_z(J_{IS}\pi[t_2])$

$\downarrow$

$+\mathbf{I}_x \cos(\Omega_S t_1)\cos(\Omega_I t_2)\cos(\pi J_{IS} t_2)$

$+2\mathbf{I}_y\mathbf{S}_z \cos(\Omega_S t_1)\cos(\Omega_I t_2)\sin(\pi J_{IS} t_2)$

$+\mathbf{I}_y \cos(\Omega_S t_1)\sin(\Omega_I t_2)\cos(\pi J_{IS} t_2)$

$-2\mathbf{I}_x\mathbf{S}_z \cos(\Omega_S t_1)\sin(\Omega_I t_2)\sin(\pi J_{IS} t_2)$

memo 1: put the receiver on x

therefore only the single x term remains : $+\mathbf{I}_x \cos(\Omega_S t_1) \sin(\Omega_I t_2) \cos(\pi J_{IS} t_2)$

memo 2: sin(A)cos(B) = 1/2[sin(A+B)+sin(A-B)]

therefore:

$-1/2\mathbf{I}_x \cos(\Omega_S t_1) [+\sin\{(\Omega_I+\pi J_{IS})t_2\}+ \sin\{(\Omega_I-\pi J_{IS})t_2\}]$

the following term can be found $- \mathbf{I}_x$ [+ .. + ..] at Ω_I , Ω_S

if one sets the phase that *cos is absorptive (a) in* t_1

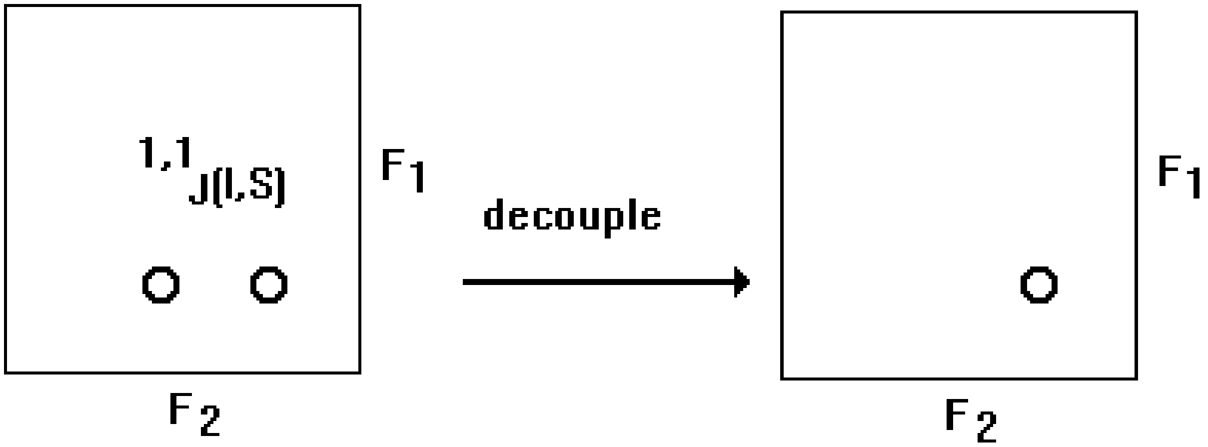
sin is absorptive (d) in t_2

$\mathbf{I}_x$ [+a .. +a ..] at Ω_I , Ω_S

Singulet in F1 and the in phased doublet in F2. ($^{1,1}J[H,N]$ ≅90 Hz, $^{1,1}J[H,C]$ ≅150 Hz)

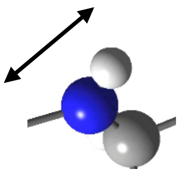
if we decouple during t_2 ($\hat{H} = 2\hat{I}_z\check{S}_z(J_{IS}\pi[t_2])$ is not active) then the spectrum is

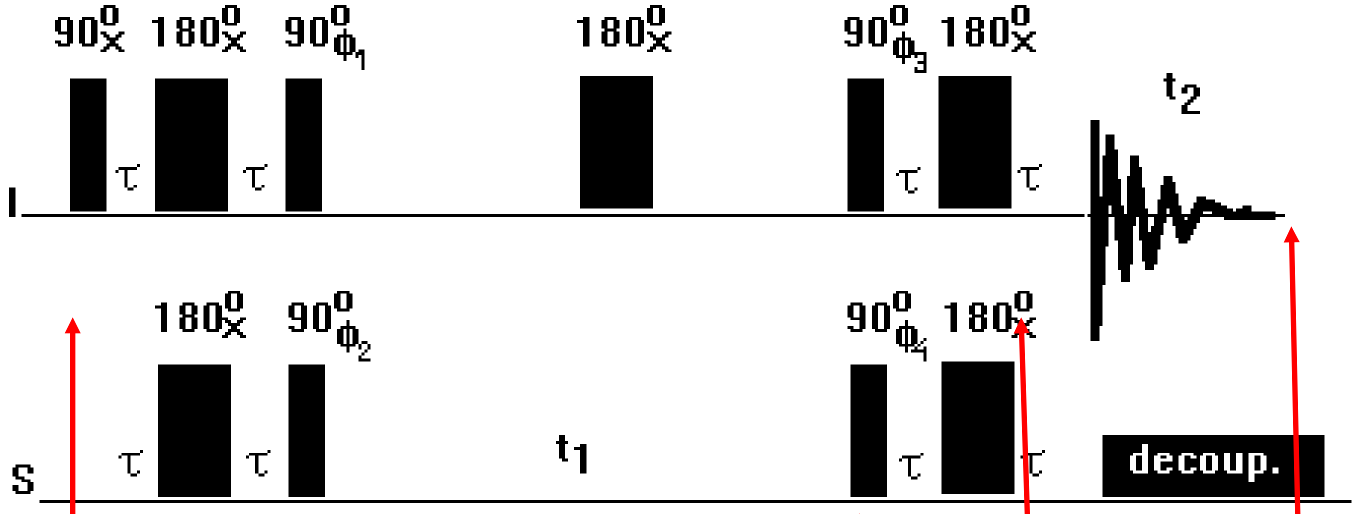
$\mathbf{I}_x$ [+a] at Ω_I , Ω_S



Heteronukleáris egyszeres-kvantum koherencia spektrum
HSQC = Heteronuclear Single-Quantum Coherence

$^{1,1}J \sim 90$Hz





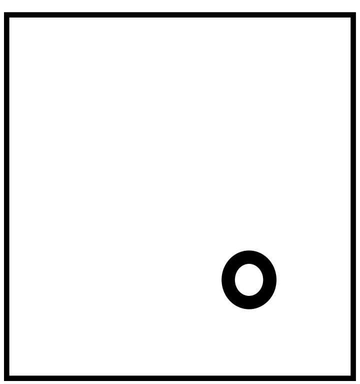
$\mathbf{H}_z$

$-2\mathbf{H}_z\mathbf{N}_y$

$+2\mathbf{H}_z\mathbf{N}_y \cos(\Omega_N t_1)$

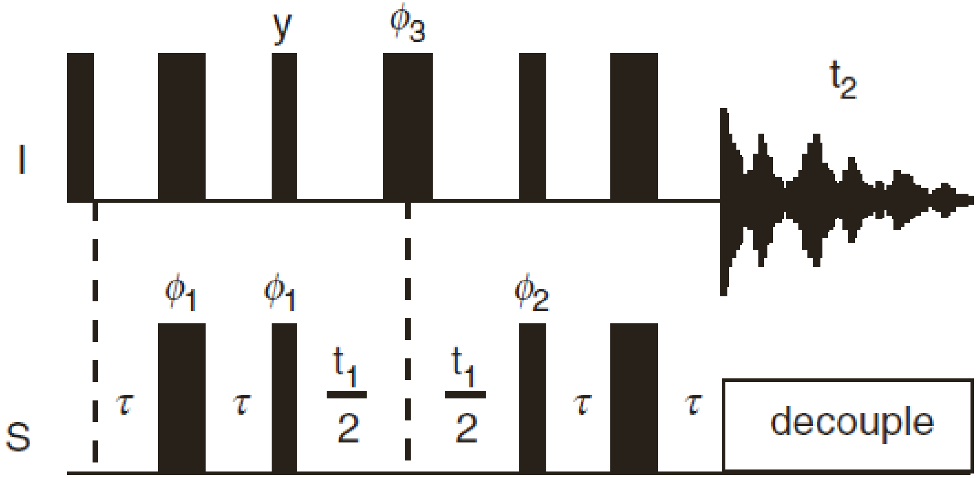
$\mathbf{H}_x \cos(\Omega_N t_1)$

$\mathbf{H}_x \cos(\Omega_N t_1)\cos(\Omega_H t_2)$

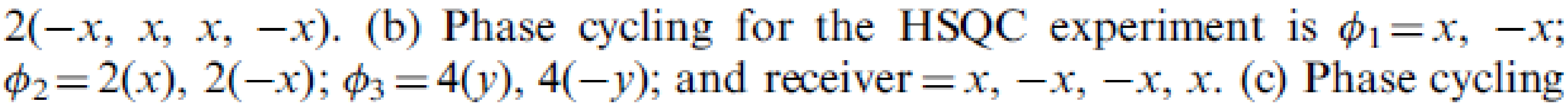


HSQC-CT = Heteronuclear Single-Quantum Coherence with constant time

Cavanagh II / 536 o.



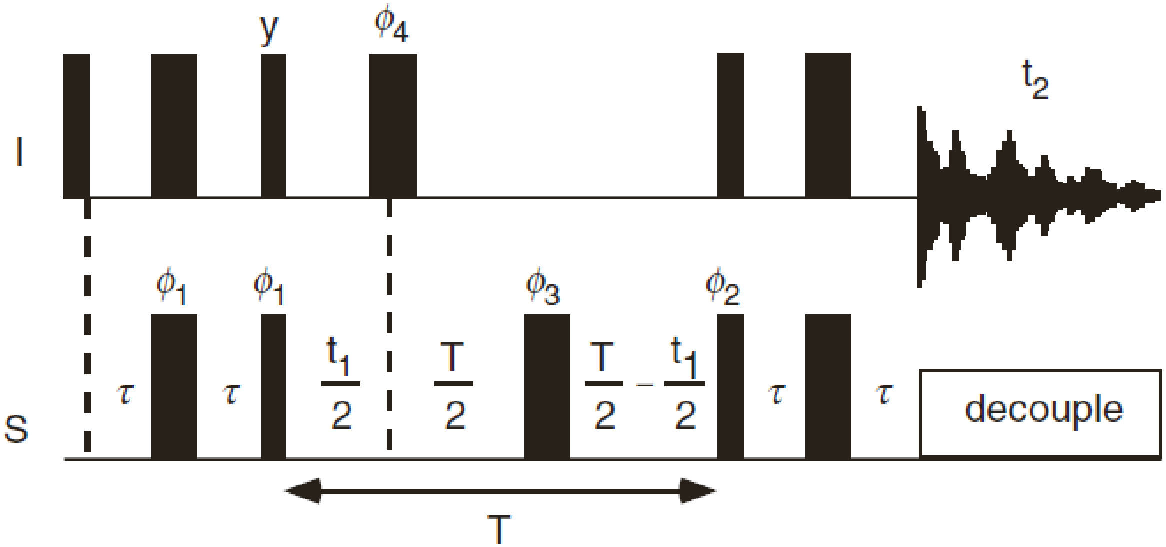
Phase cycling:



2($-x$, x, x, $-x$). (b) Phase cycling for the HSQC experiment is $\phi_1 = x, -x$; $\phi_2 = 2(x), 2(-x)$; $\phi_3 = 4(y), 4(-y)$; and receiver $= x, -x, -x, x$. (c) Phase cycling

HSQC-CT = Heteronuclear Single-Quantum Coherence with constant time

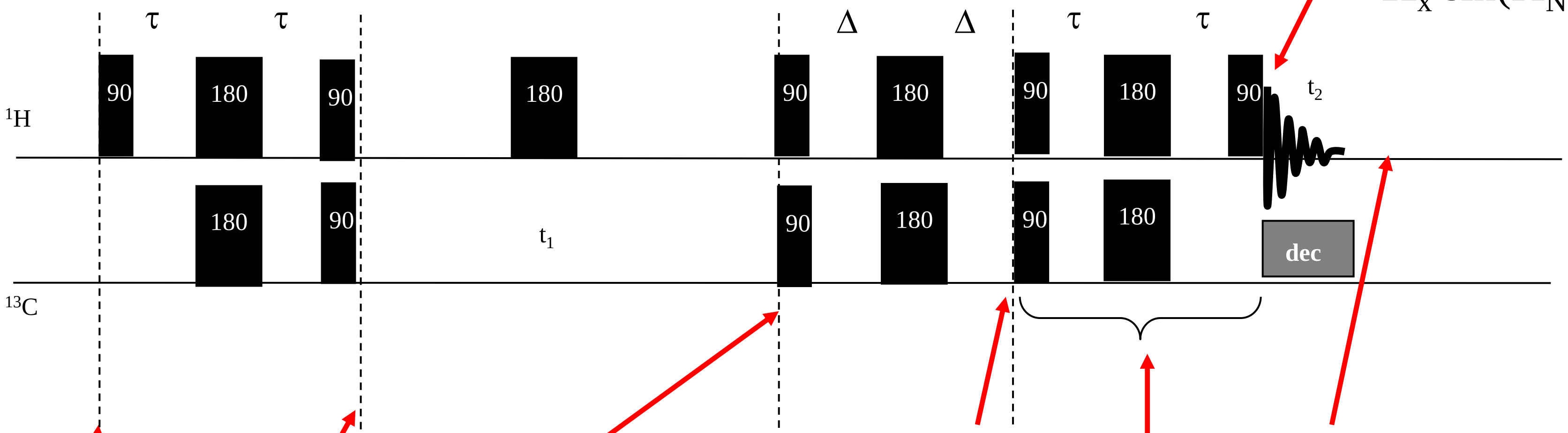
Cavanagh II / 536 o.



$\phi_2 = 2(x)$, $2(-x)$; $\phi_3 = 4(y)$, $4(-y)$; and receiver $= x, -x, -x, x$. (c) Phase cycling for the constant-time HSQC experiment is $\phi_1 = x, -x$; $\phi_2 = 8(x)$, $8(-x)$; $\phi_3 = 2(x)$, $2(y)$, $2(-x)$, $2(-y)$; $\phi_4 = 16(y)$, $16(-y)$; and receiver $= 2(x, -x, -x, x)$, $2(-x, x, x, -x)$. If desired, this 32-step phase cycle can be reduced to 8 steps

HSQC-SE = Heteronuclear Single-Quantum Coherence with sensitivity enhancement

$-/+\mathbf{H}_y \cos(\Omega_N t_1)$
$+\mathbf{H}_x \sin(\Omega_N t_1)$



$-/+\mathbf{H}_y \cos(\Omega_N t_1)\cos(\Omega_H t_2)$
$-\mathbf{H}_x \sin(\Omega_N t_1)\sin(\Omega_H t_2)$

REVERSE INEPT:

from $\mathbf{H}_z\mathbf{N}_y$ to $\pm\mathbf{H}_x$ and

form $\mathbf{H}_z\mathbf{N}_x$ to $\mathbf{H}_y\mathrm{N}_x$

Both coherence pathways are considered

Frequency labeling:

$\mathbf{H}_z\mathbf{N}_y \cos(\Omega_N t_1)$

$\mathbf{H}_z\mathbf{N}_x \cos(\Omega_N t_1)$

Both anti-phase N coherences are to be selected

$\mathbf{H}_z$

SE module:

from $+/-\mathbf{H}_x$ to $-/+\mathbf{H}_y$

form $\mathbf{H}_y\mathrm{N}_x$ to $\mathbf{H}_x$

(phase modulated signal with the contribution of both coh. trans. pathways: gain a factor of √2.

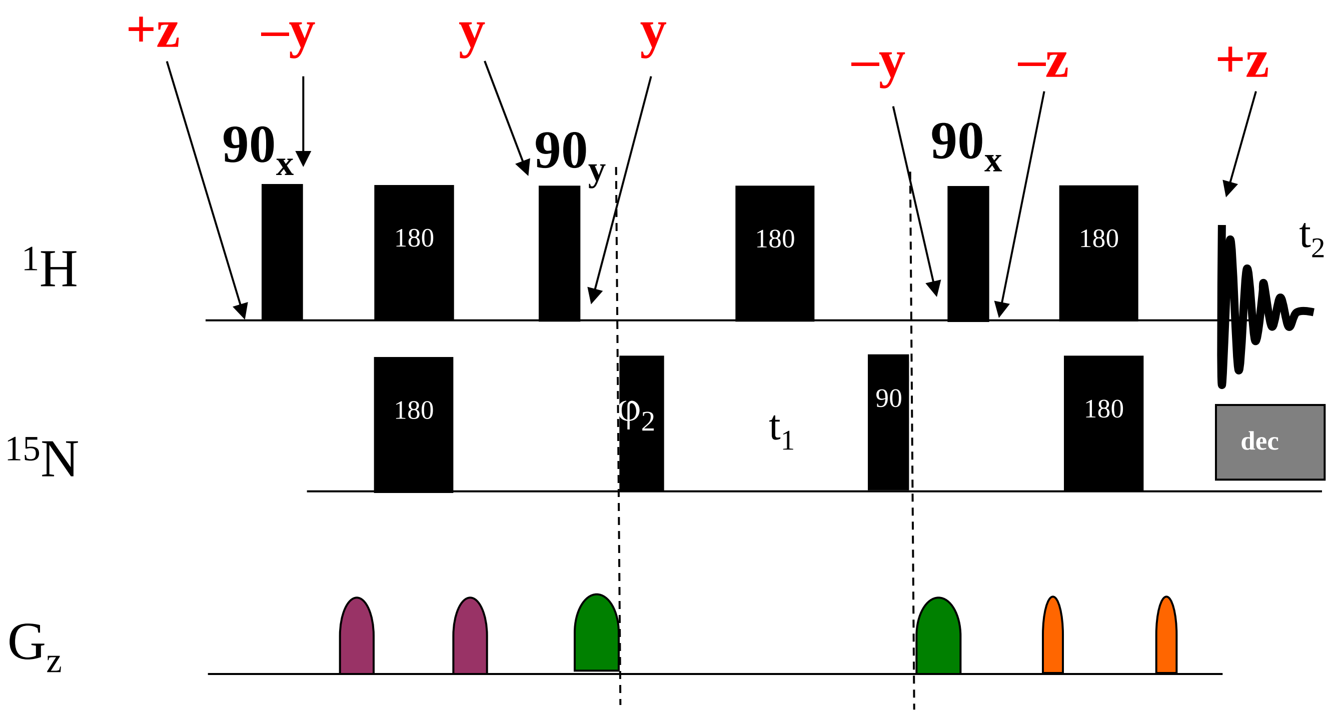
INEPT:

from $-\mathbf{H}_y$ coherence is transferred to the less sensitive nuclei resulting in anti-phase coherence on N: $\mathbf{H}_z\mathbf{N}_y$

F.J.M van de Ven 259-261
Palmer et al. J.Magn.Reson. 1991, 93, 151-170
Cavanagh & Rance J.Magn.Reson. 1990, 88, 72-85

HSQC = Heteronuclear Single-Quantum Coherence

Monitoring H_2O along the sequence:



What is happanining with the balk water during the HSQC pulses?

$+I_z$ —$(90°_x)$→ $-I_y$—$(180°_x)$→ $+I_y$ —$(90°_y)$→ $+I_y$ —$(180°_x)$→ $-I_y$ —$(90°_x)$→ $-I_z$ —$(180°_x)$→ $+I_z$

Water before acquisition is aligned along +z. Thus, most of the water is not detected during t_2. Unfortunately, that part of the water which is not parallel to z will be detected as residual water. The latter amount of water should be minimized, (dynamic range problem of the detectors).

^{1}H-^{13}C HSQC-SE:

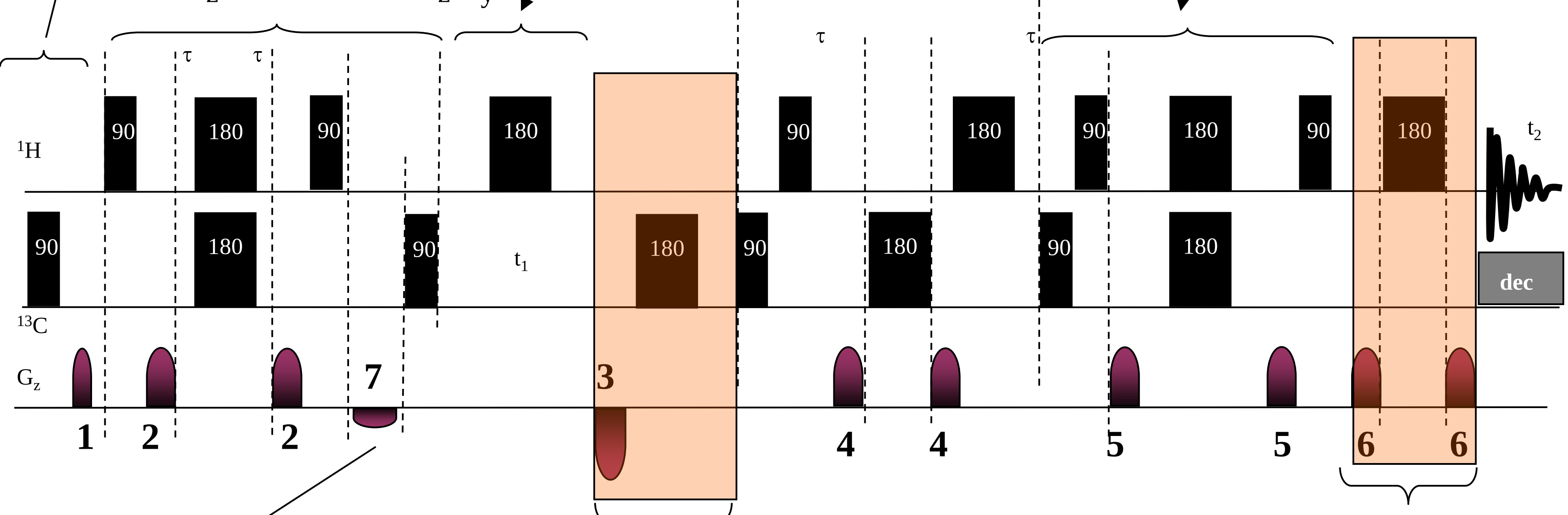
Destroy all residual $^{13}C_z$: Put it into the transverse plane and defocused by grad.

INEPT: from $\mathbf{H_z}$ generates $+2\mathbf{H_z C_y}$

Labeling by ^{13}C c$\hat{H}$ shift: from $2\mathbf{H_z C_y}$ generates $2\mathbf{H_z C_y}\cos(\Omega_N t_1)$

REVERSE INEPT: from $\mathbf{H_z C_y}$ to $\pm\mathbf{H_x}$ and form $\mathbf{H_z C_x}$ to $\mathbf{H_y}C_x$ (in a no-SE version the latter "path" is ignored)

SE module: from $+/-\mathbf{H_x}$ to $-/+\mathbf{H_y}$ form $\mathbf{H_y}C_x$ to $\mathbf{H_x}$ (phase modulated signal with the contribution of both coh. trans. pathways: gain a factor of √2.

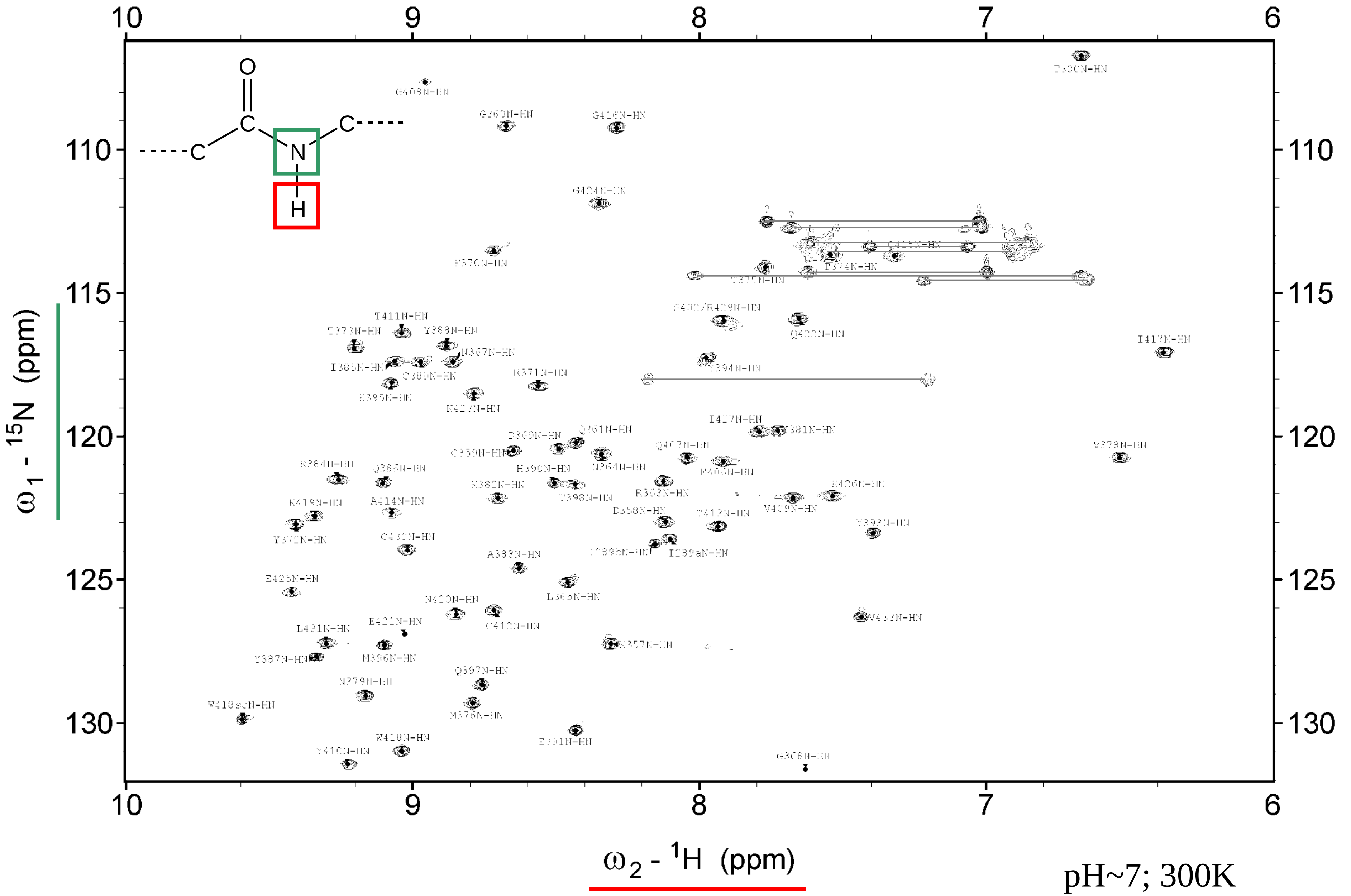


Grad.7: before converting $-2\mathbf{H_z C_z}$ into $-2\mathbf{H_z C_y}$ defocuses any other transverse coherences present (kills everything not coupled to ^{13}C)

Coh. selection by grad.: $2\mathbf{H_z C_x}$ and $2\mathbf{H_z C_y}$ are selected (SE experiment) only. (180 refocuses evolution occurring during the "long" gradient 3: a hetero nuclear echo.)

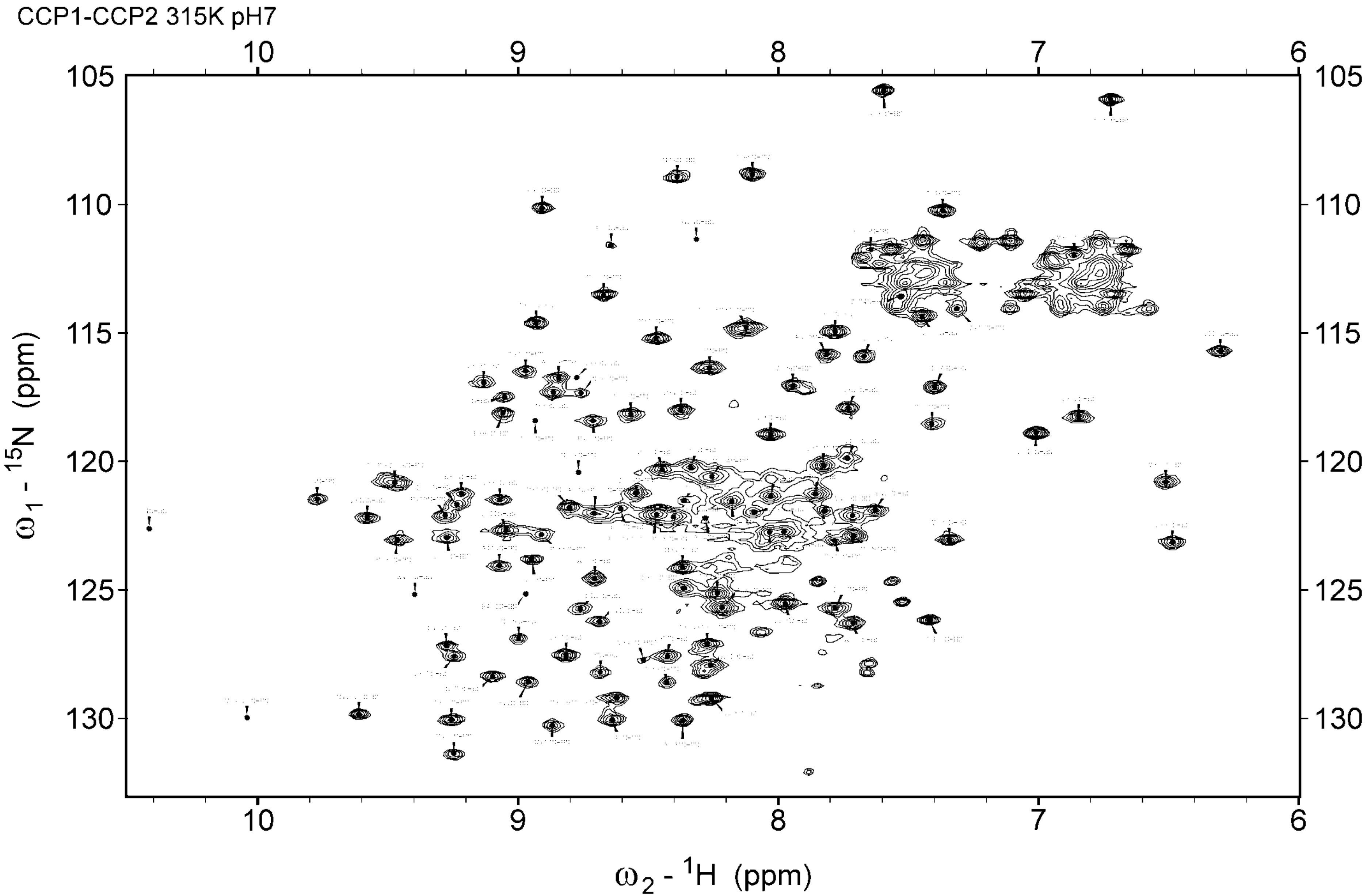
Coh. selection by grad.: refocuses on proton $(\mathbf{H_y})$ what was defocused on carbon $(2\mathbf{H_z C_x})$.

1) ^{1}H-^{15}N HSQC: CCP2



pH~7; 300K

Az azonosított gerinc NH jelek a modulpárból

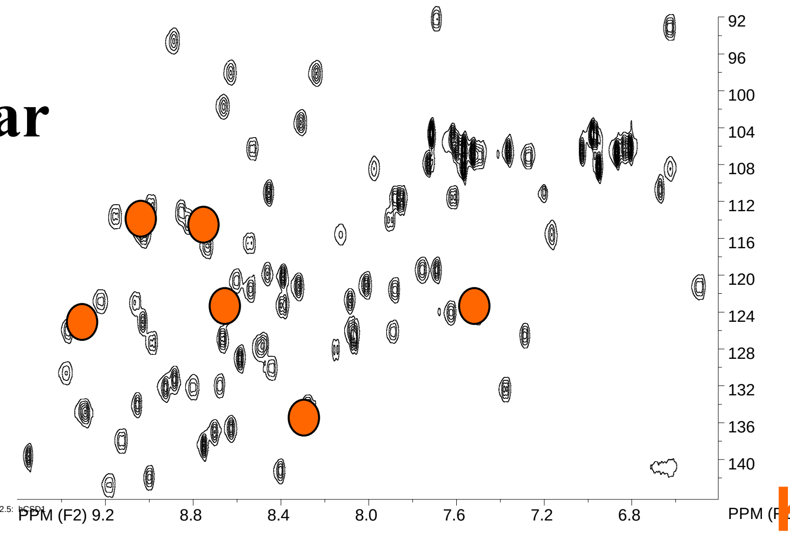


Signal dispersion of a globular and an IUP

Due to the averaged environment, the ^{1}H dispersion of IUPs is low!

globular

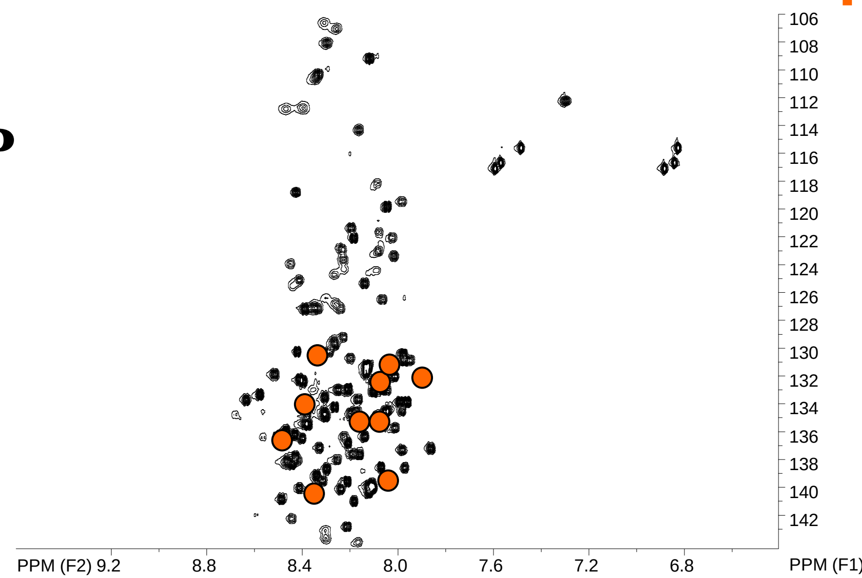
K ●

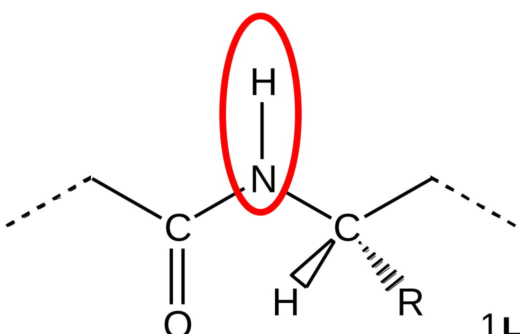


K = Lys

IUP

K ●





^{1}H - ^{15}N correlation spectra of a globular and of an IUP.