2003 NMR User Training Course

National Program for Genomic Medicine High-Field NMR Core Facility,
The Genomic Research Center, Academia Sinica
09/29-09/30, 2003

09/30, 2003 Course Handout

Useful Topics for NMR Methodologies

by

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Lecture I:

- **Water suppression techniques**

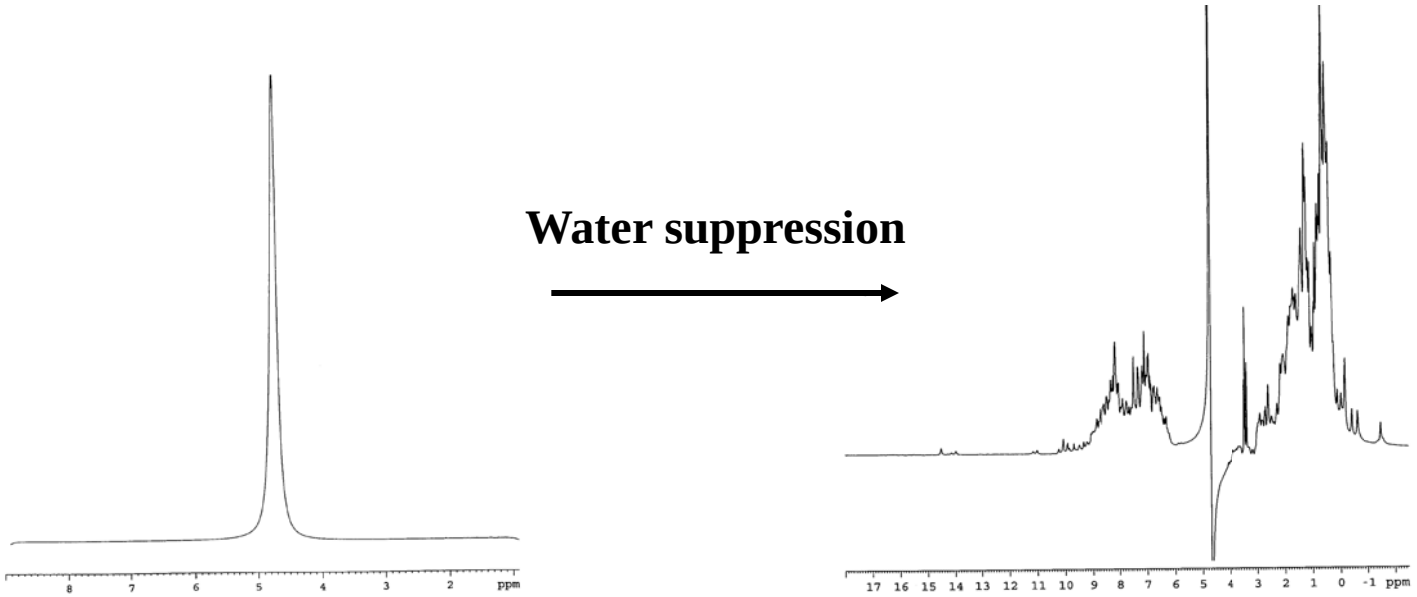
Lecture II:

- **Variant HSQC experiments**
- **Pulsed field gradient NMR**

Water Suppression Techniques

[H_2O]=55,000 mM **[Protein]< 5 mM**

[H_2O]/[Protein]>11,000

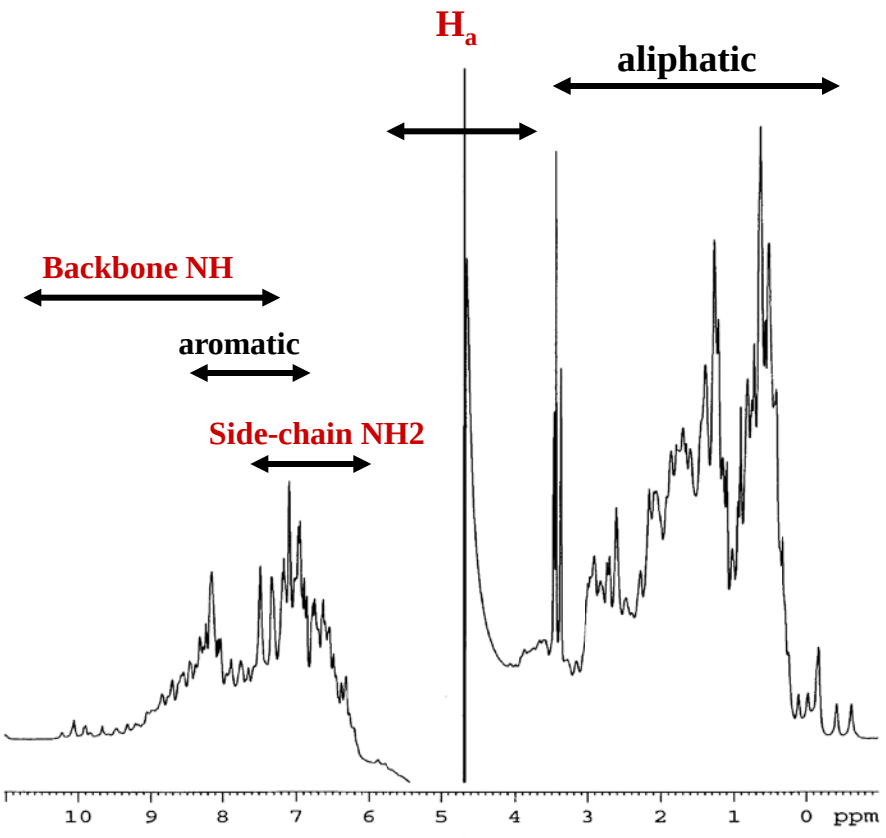


Sample used throughout this lecture: 1 mM TEP-I in 90% H_2O/10%D_2O, pH 6.0, 290 K.

Water Suppression Technique

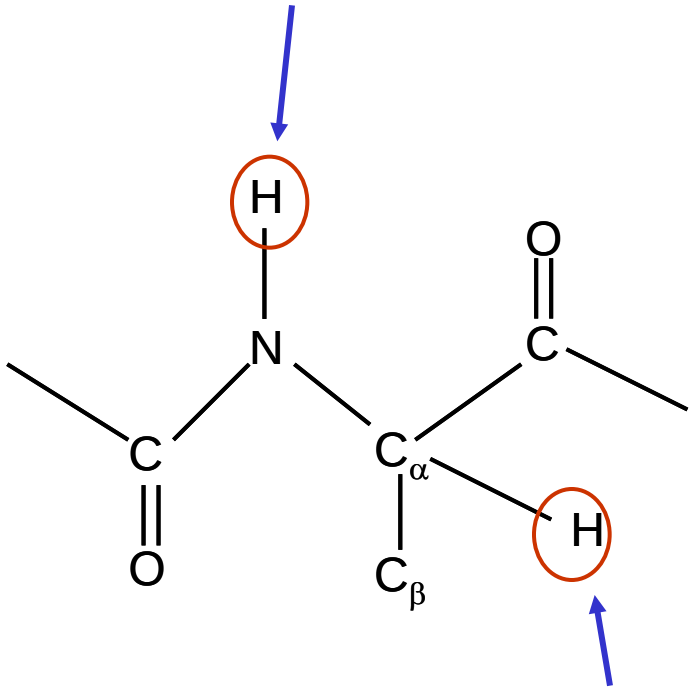
- Presaturation
- Watergate
- Water flip-back
- Jump and return, 1-1, 1331
- Suppression by coherence pathway rejection

Water Suppression Technique in Protein NMR



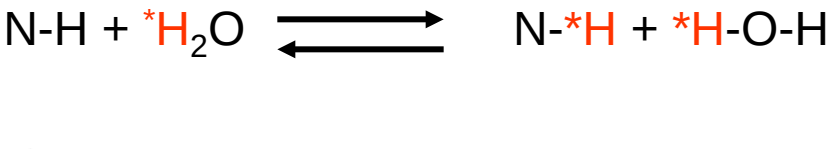
^1H

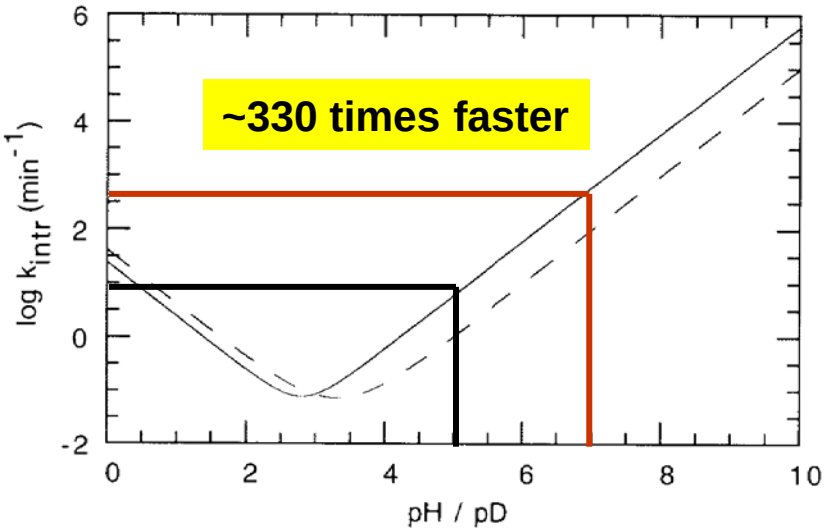
Labile, exchange with water (pH, structure, temperature dependent)



Resonates near the water frequency

pH Dependence of Amide Proton Exchange Rates





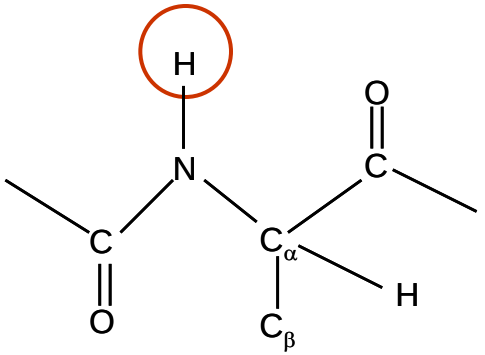
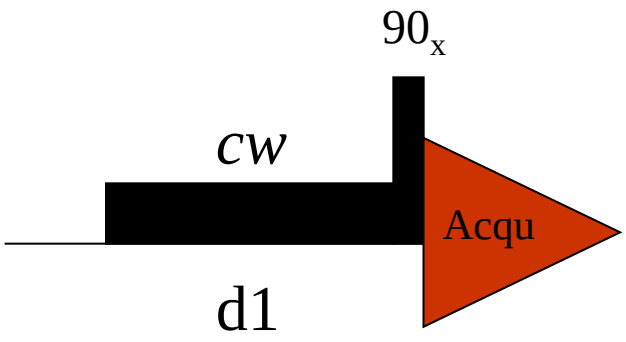


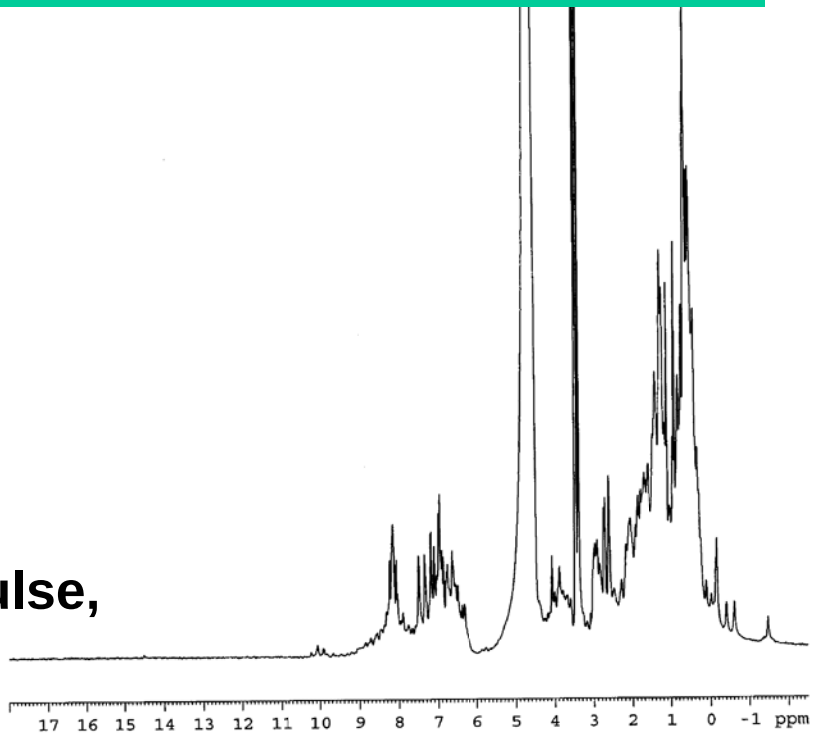
FIGURE 3.26 Intrinsic backbone amide proton exchange rates calculated according to Connelly *et al.* (*63*). The intrinsic exchange rate, k_{intr}, is shown for exchange of a backbone amide proton with (—) H_2O or (- - -) D_2O as a function of pH or pD. The pD values are corrected for isotope effects; uncorrected pH meter readings would be 0.4 units smaller.

Figure modified from p154 of John Cavanagh et al.,"Protein NMR Spectroscopy: Principles and Practice", Academic Press (1995)

In practice: the pH value for a protein sample for NMR studies is kept below 7.5 to avoid fast exchange rates.

Presaturation





1D ^{1}H spectra of TEP-I in 90%H_2O/10%D_2O , pH 6, 290 K.

- **Apply a low power C.W. irradiation on water before the first 90 degree pulse, usually during the relaxation delay**

Parameter adjustment:

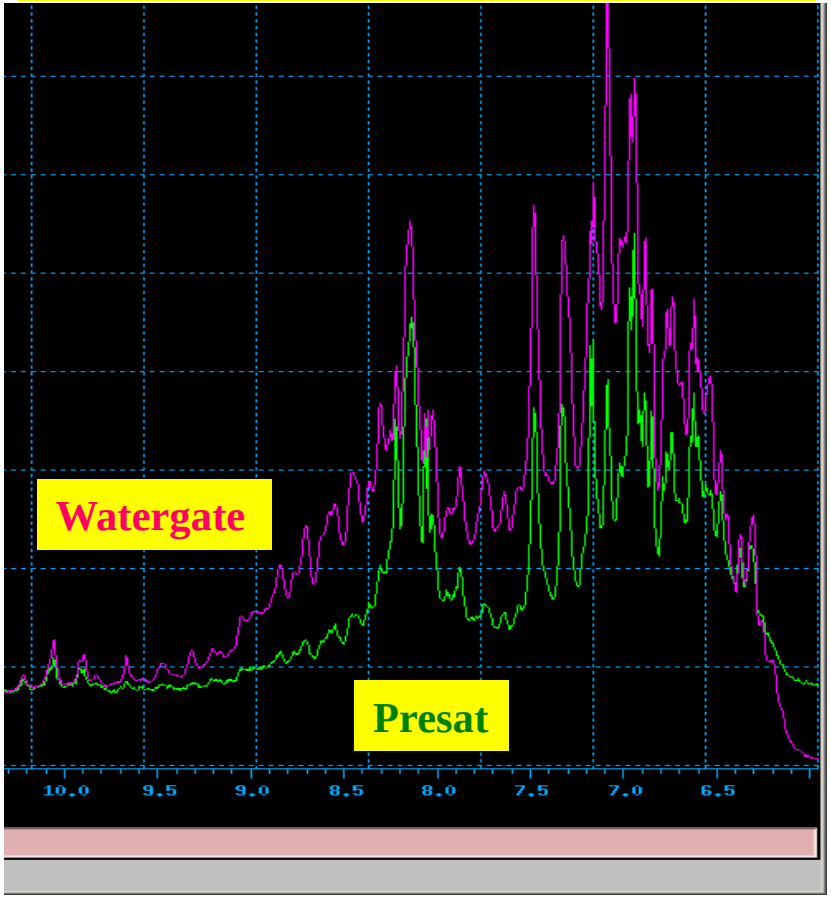
Pulprog=zgpr

Adjustment: pl9; power level for presaturation

Drawback of Presaturation

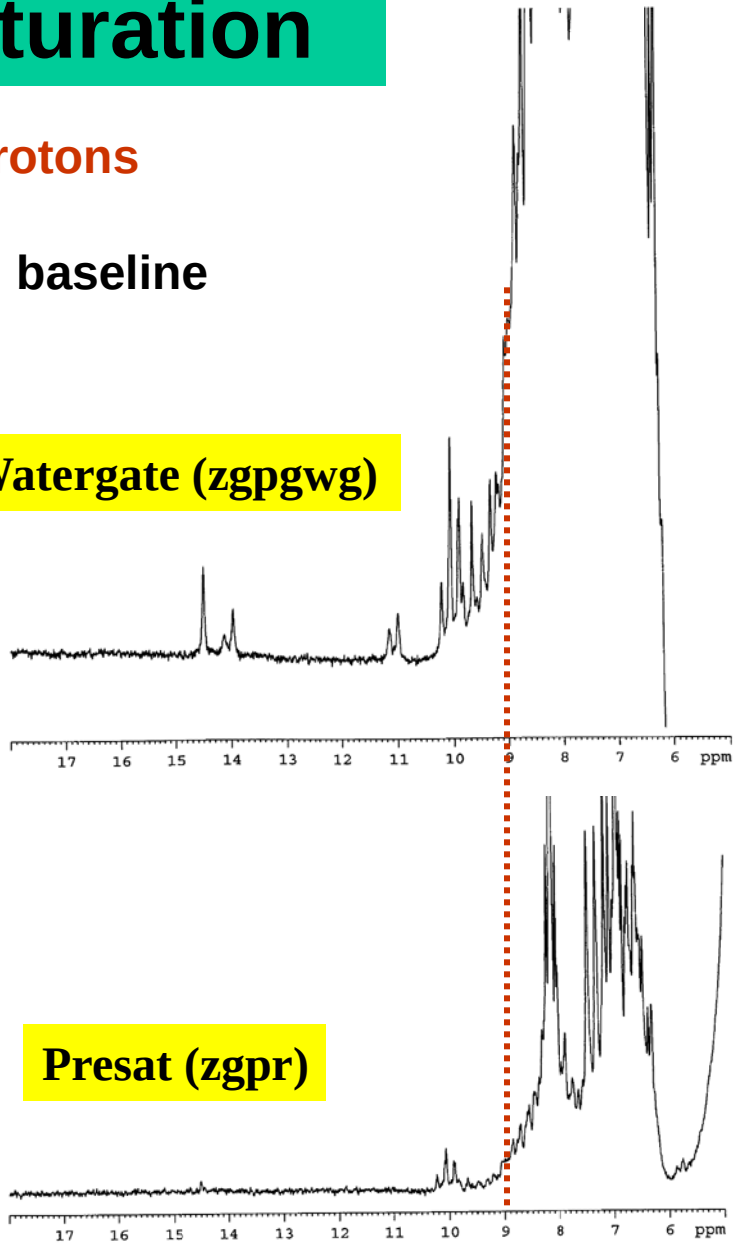
- **Saturation transfer to exchangeable NH protons**
- **Bleaching of signals near water**
- **Large dispersive tail of water signal: tilted baseline**

1D ^{1}H spectra of TEP-I, pH 6, 290 K.



Watergate (zgpgwg)

Presat (zgpr)



Pulsed Field Gradient (PFG)

- **A field-gradient pulse is a pulse or a period during which the magnetic field is made deliberately inhomogeneous.**

$B=B_0+B_g(z)$

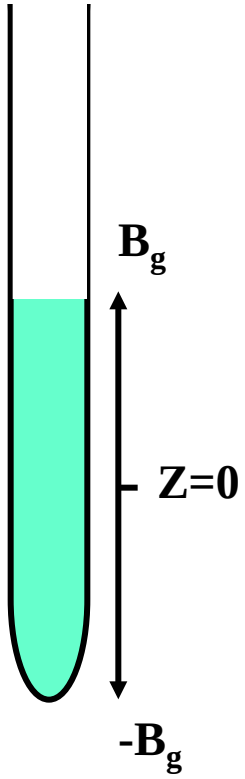
- **The magnetic field, generated by a gradient pulse, $B_g(z)$ varies linearly along the Z-axis**

$B_g(z)=zG_z$, where

G_z: gradient strength (G/cm), Z: z-axis position

- **Viewing on the rotating frame, spins at different z-position acquire different phase (Larmor frequencies):** $\phi(z)=\gamma z G_z \tau$,

where ϕ=phase, γ: gyromagnetic ratio, τ: gradient duration



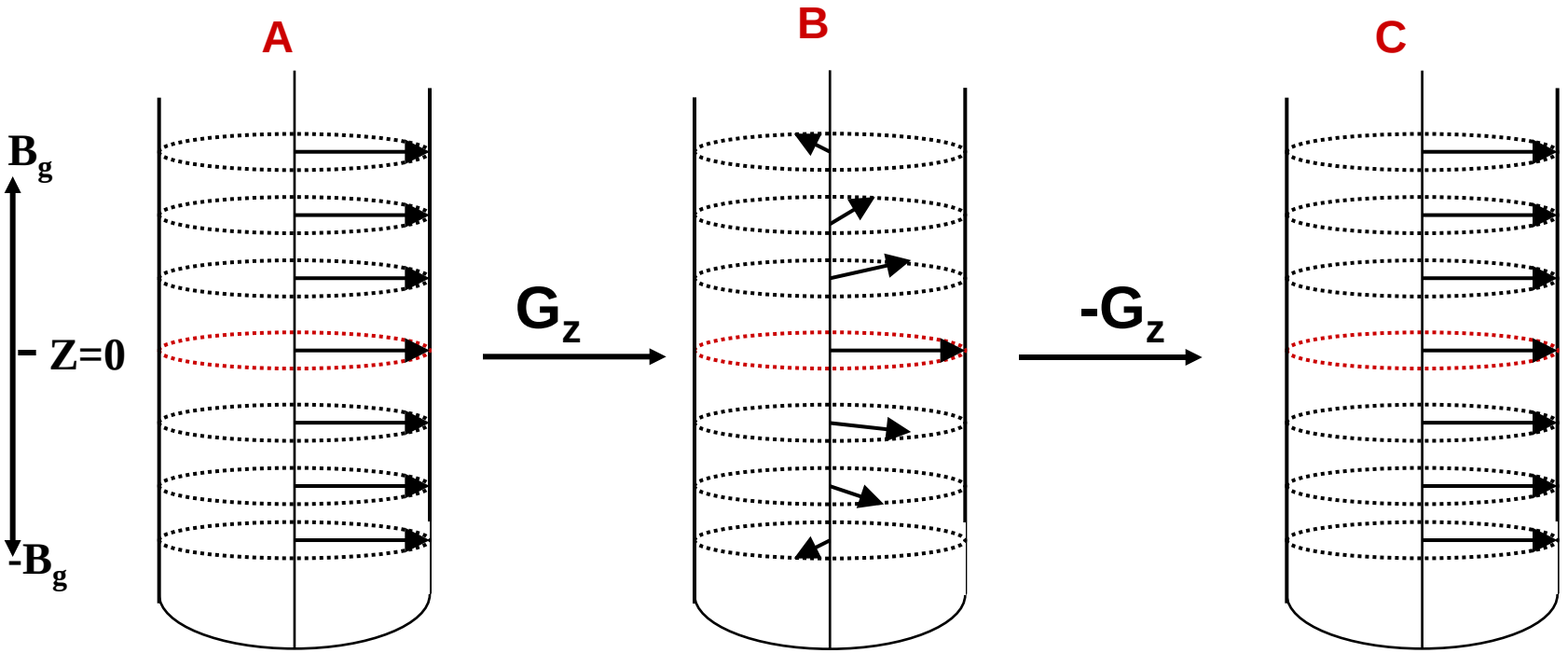
- ***Actively shielded gradient coil reduces eddy current, and is now popular in multidimensional NMR spectroscopy.***

Pulsed Field Gradient (PFG)

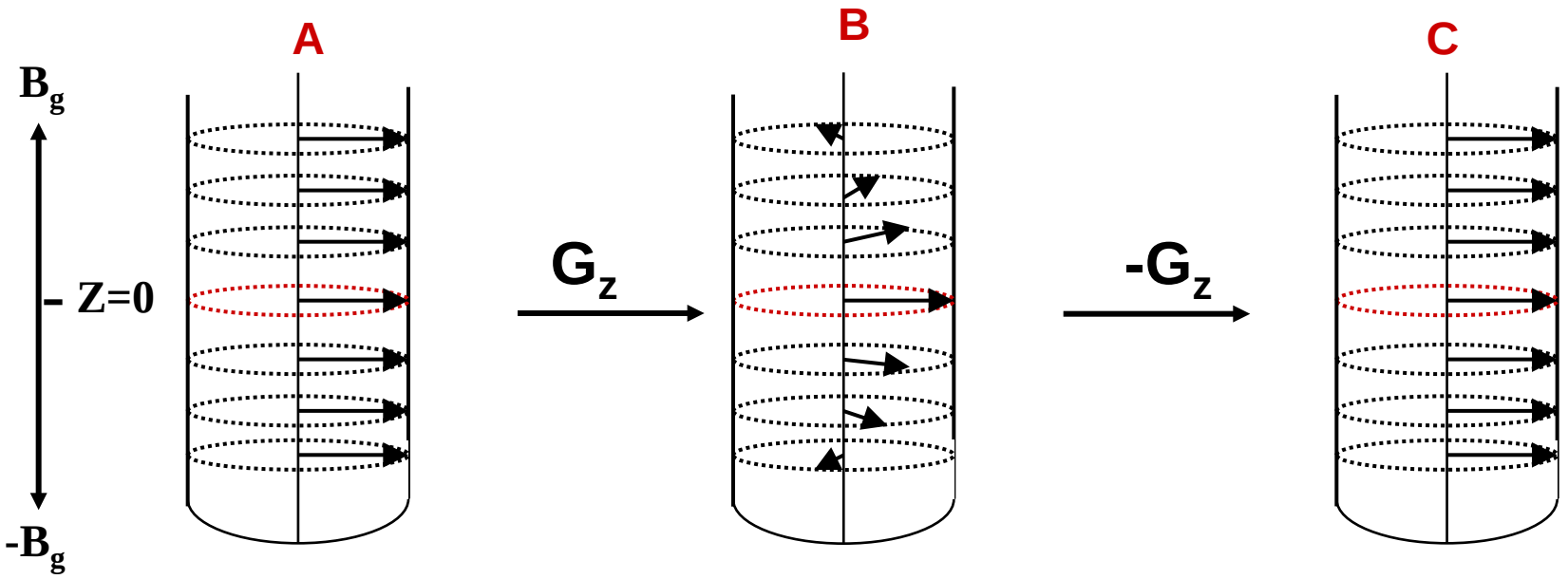
A. Initially spins in each slice (isochromat) are “phase-coherence”.

B. After a field-gradient pulse, the spins at different slice experience different magnetic field strength, and acquire different Larmor frequencies. The “phase-coherence between slices is now lost due to Larmor precession.

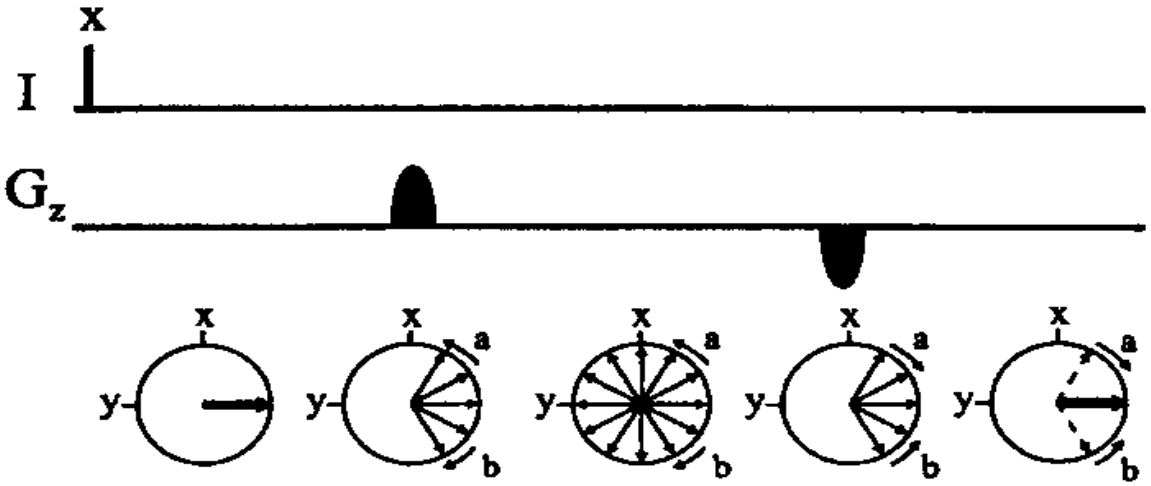
C. The coherence can be refocused by another gradient pulse (gradient echo).



Pulsed Field Gradient (PFG)

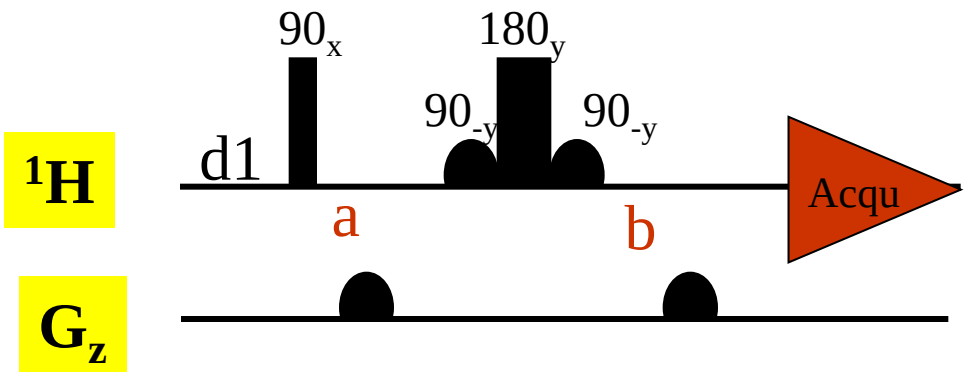


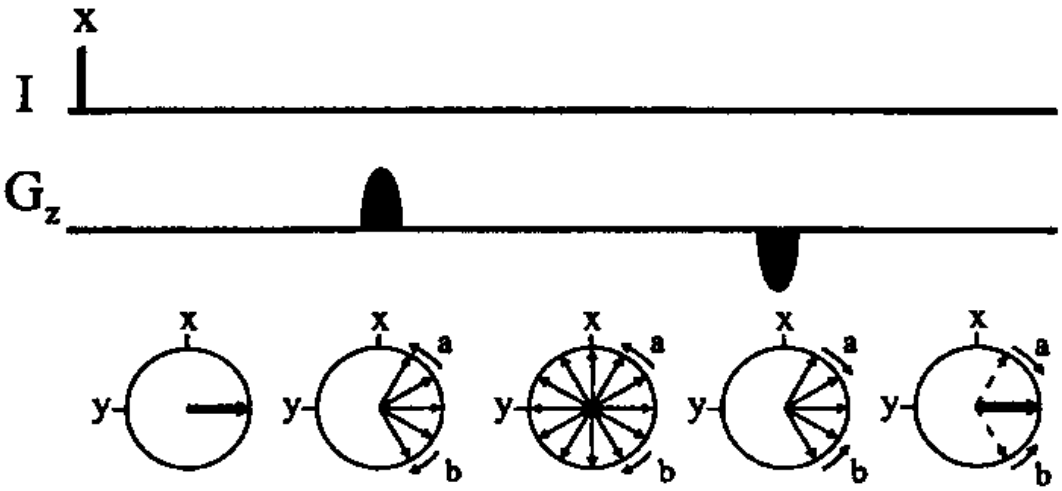
Viewing from the Z-axis:



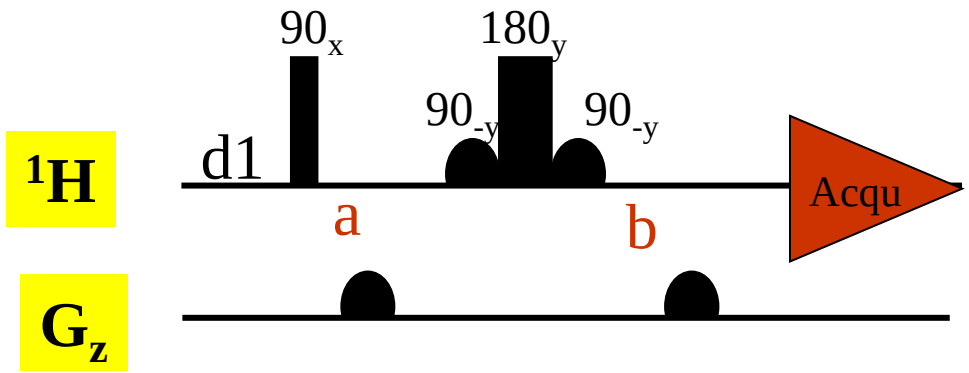
(figure from p106 of Sattler et al. Prog. In Nucl. Mag. Reson. Spect. 34 (1999)

WATERGATE





WATERGATE



•A strong Z-gradient pulse can be used to destroy transverse magnetization.

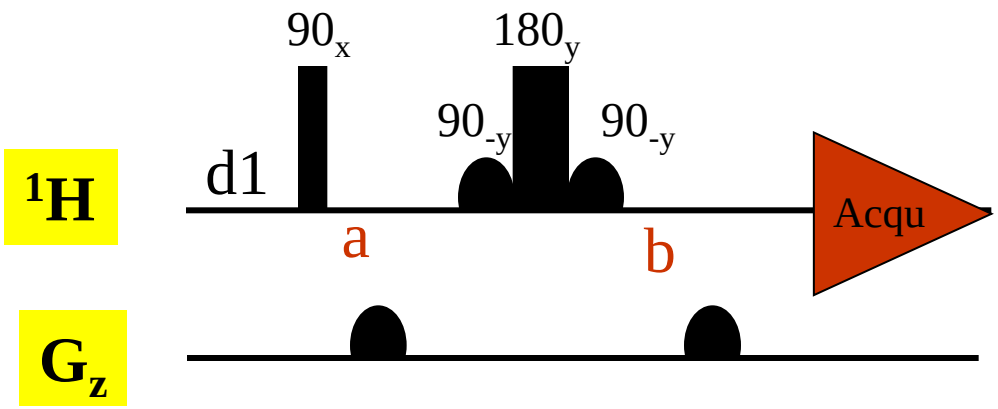
•A destroyed (dephased) magnetization can be refocused by another z-gradient pulse of the same amplitude but of opposite phase. (or use a 180 pulse in between the two identical z-gradient pulse).

•H_2O: the two extra selective 90 pulse on water makes the 2nd z-gradient pulse act as another defocus gradient pulse.

•Protein signals: the 180 pulse makes the 2nd Z-gradient act as a refocus gradient.

Ref: M. Piotto, V. Saudek & V. Sklenar, J. Biomol. NMR 2, 661 - 666 (1992)

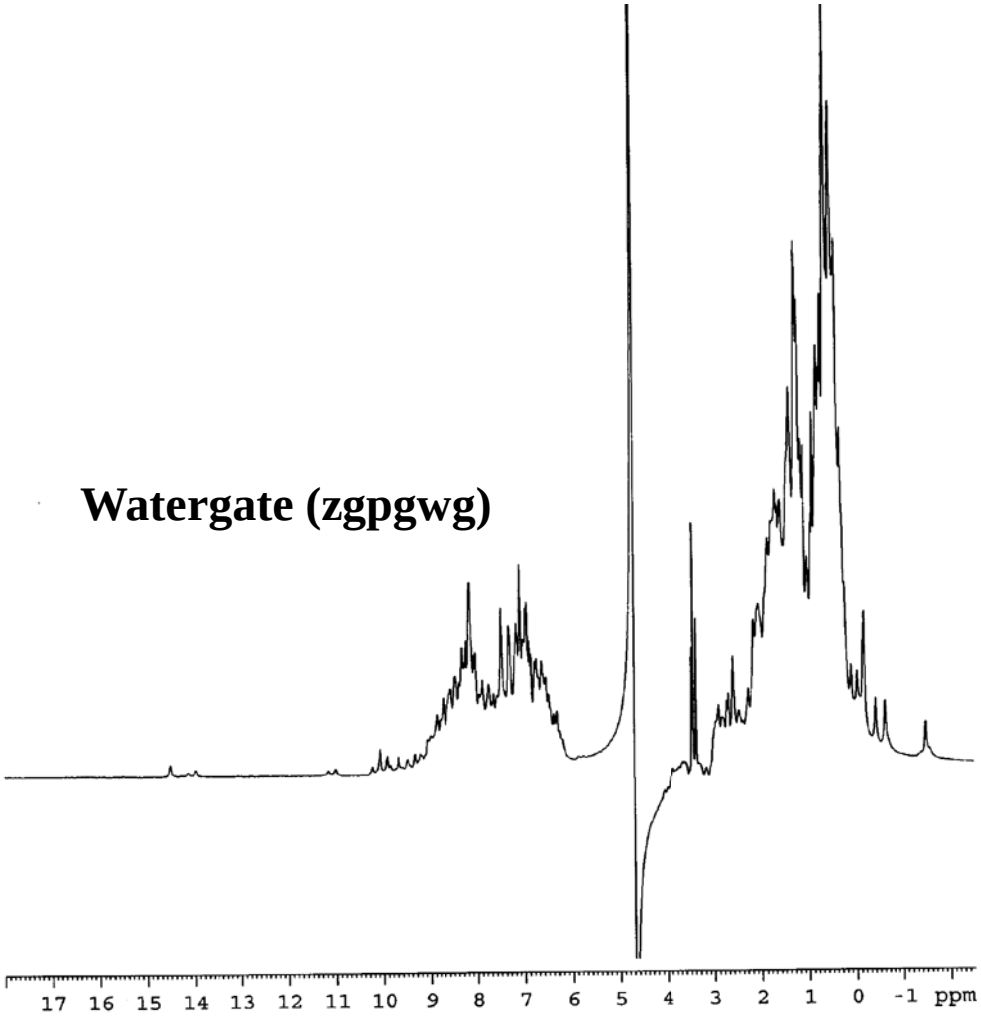
WATERGATE



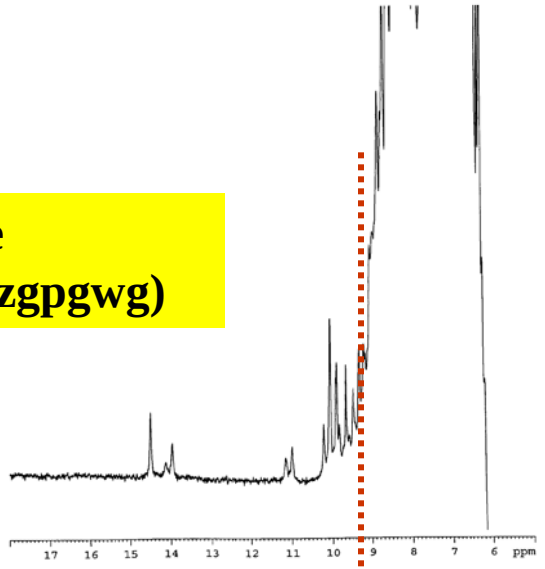
- **Parameter adjustment, Pulprog=zgpgwg**
- **p11: pulse length for 90 degree shaped pulse**
- **sp1: power level for 90 degree shaped pulse**
- **spnam1: name of shaped pulse**

For example: set spnam1=Sinc1.1000, p11=1 msec,
Minimize the water fid by adjusting sp1 in the “gs” utility.

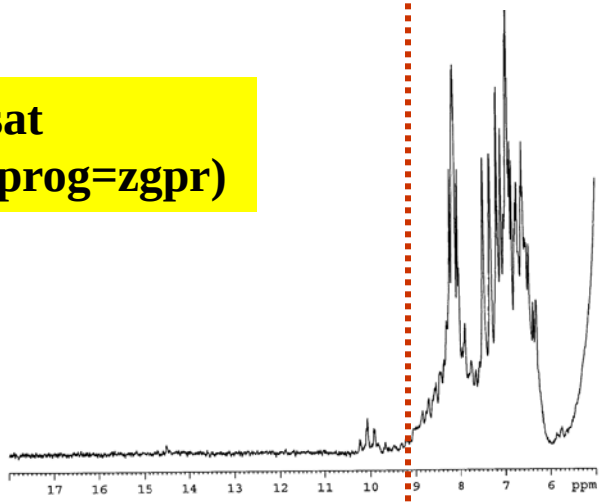
WATERGATE V.S. Presaturation



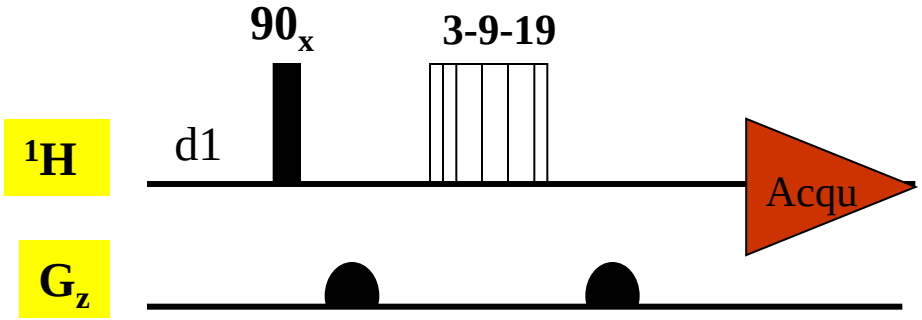
Watergate (pulprog=zgpgwg)



Presat (pulprog=zgpr)

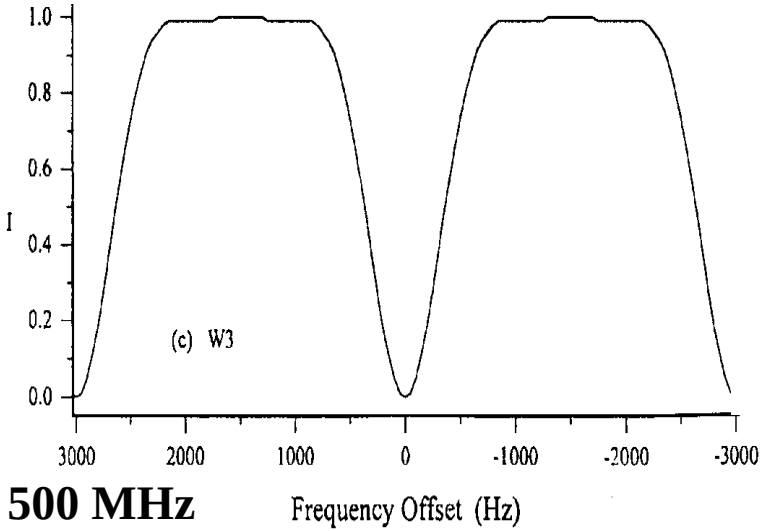


3-9-19 WATERGATE



Sklenar et al., J. Magn. Reson., A102, 241-245 (1993)

- **Off resonance DANTE excitation technique.**
- **3-9-19: 3α-τ-9α-τ-19α–τ-19α-τ-3α, where 26α=180, τ=delay.**
  (This is also referred as "W3".)



Delay $\tau = 1/(4\,\Delta\upsilon_{max})$,
where $2\Delta\upsilon_{max}$=distance of next null (Hz).
(The delay τ is field-dependent !!)

3-9-19 WATERGATE

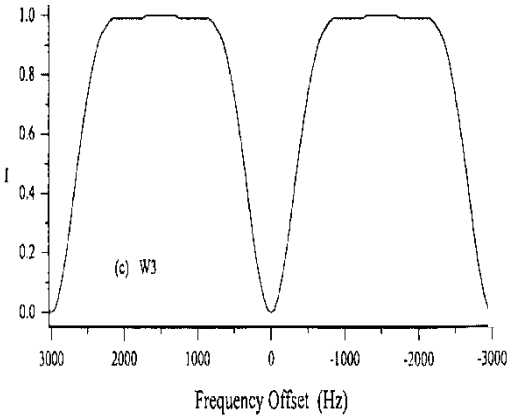
Delay τ =1/(4 $\Delta\upsilon_{max}$), where 2 $\Delta\upsilon_{max}$= distance of next null (Hz).
(The delay τ is field-dependent !!)

For example: Have the center of NH region (i.e. 8.2 ppm) to be the center of maximal excitation region:

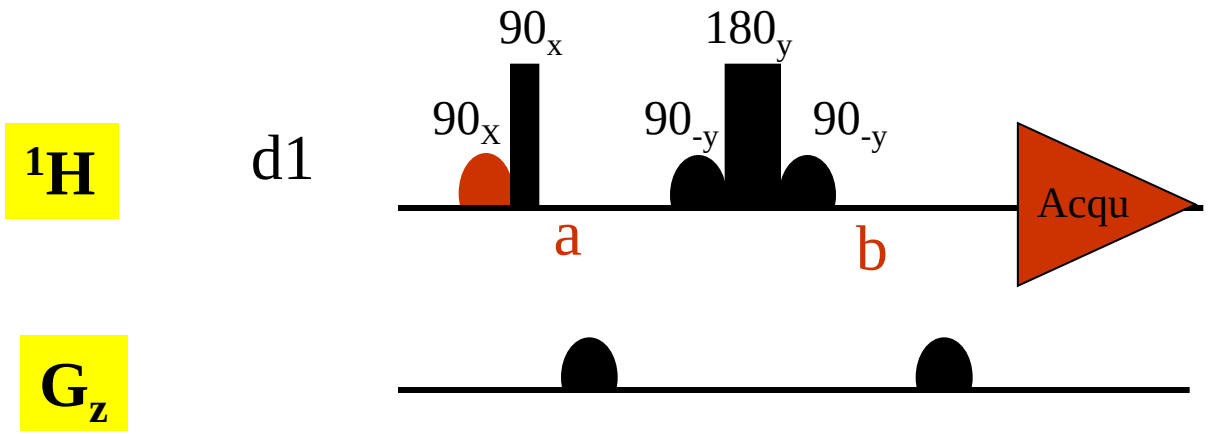
τ=1/[4*(8.2-4.75)*600.13]=121 usec @600 MHz machine
τ=1/[4*(8.2-4.75)*500.13]=145 usec @500 MHz machine

Parameter adjustment:
Pulprog=p3919
Set pl18=pl1, p27=p1, p0=p1
;d19: delay for binomial water suppression
;d19 = (1/(2*d)), d = distance of next null (in Hz)

Adjust d19 according to the magnetic field strength and where you want the center of maxima excitation to be.



Water Flip-back WATERGATE



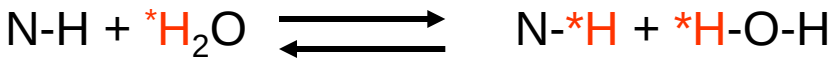
S. Grzesiek and A. Bax, J. Am. Chem. Soc., 115, 12593-12594 (1993)

- **Water is aligned along the z axis before any z-gradient pulse (point "a"). So, it is not destroyed by the z-gradient pulse.**

- **This reduces the signal loss of exchangeable protons due to attenuation of water signal (saturation transfer).**

Parameter adjustment:
Pulprog=*fp*, i.e. "hsqcetfpf3gp" calibrate the shaped pulse as describe in WATERGATE.

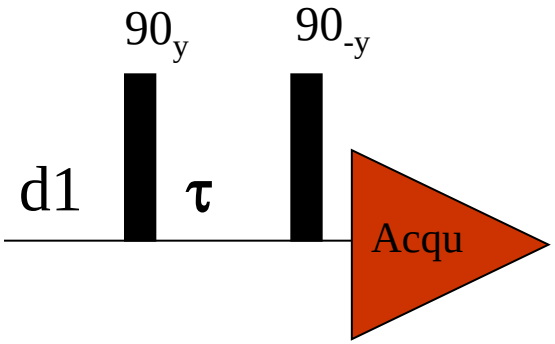
Pulse Sequence for Observing Fast-Exchanging Protons



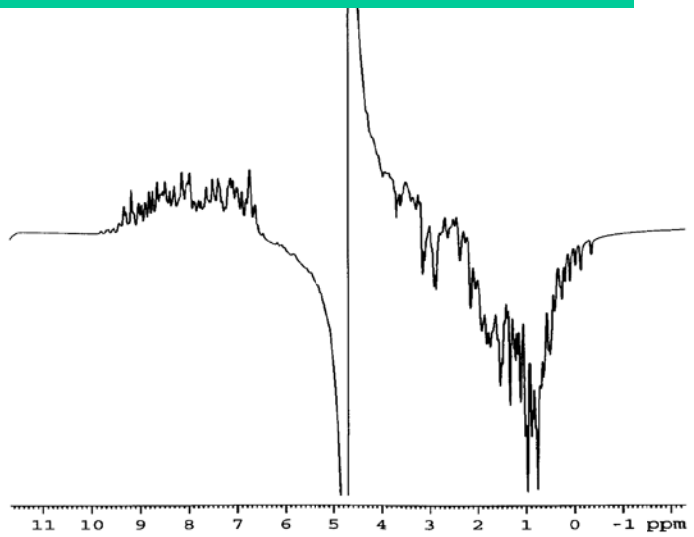
Imino protons in DNA, hydroxyl protons (-OH), Histidine side chain protons in proteins are usually in a fast exchange process with water.

- **Flip-back WATERGATE (marginal performance)**
- **Jump and return 1-1**
- **1-3-3-1**

Jump and Return: 1-1



P. Plateau et and M. Gueron, al., J. Am. Chem. Soc. ***1982****, 104, 7310-7311*



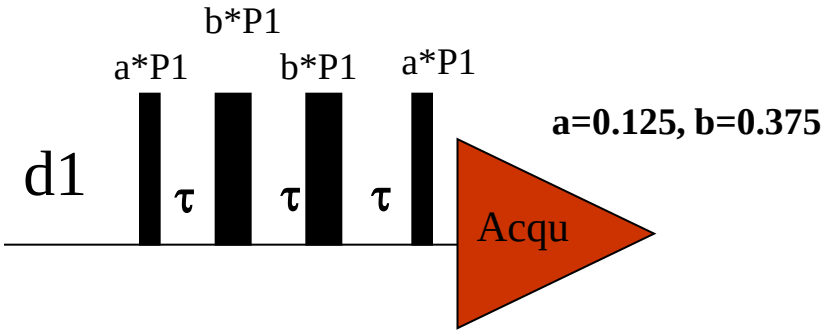
- **Water signal: "on resonance", aligned to the "z" axis,**
- **Protein signals: free to precess on the transverse plan**
- **Peak Intensity: I_x Sin(Ωτ)**
- **Delay $\tau = 1/(4\Delta\upsilon_{max})$, $\Delta\upsilon_{max}$=distance of maxima intensity**
- **For example: To observe a peak at 14 ppm at 600 MHz, τ=1/[4*(14-4.75)*600.13]=45 usec**

Parameter adjustment: Pulprog=p11

p1: 90 pulse, p0: 90 degree "return" pulse, adjust p0 to be slightly shorter than p1 (0.1-0.3 usec).

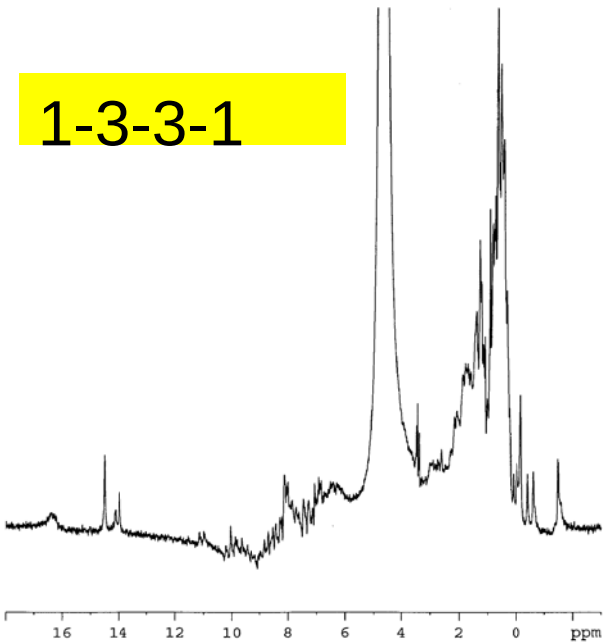
d19: d19= (1/(2*d)), d = distance of next null (in Hz)

Binominal: 1-3-3-1



a=0.125, b=0.375

P.J. Hore, J. Magn. Reson., 55, 283-300 (1983)



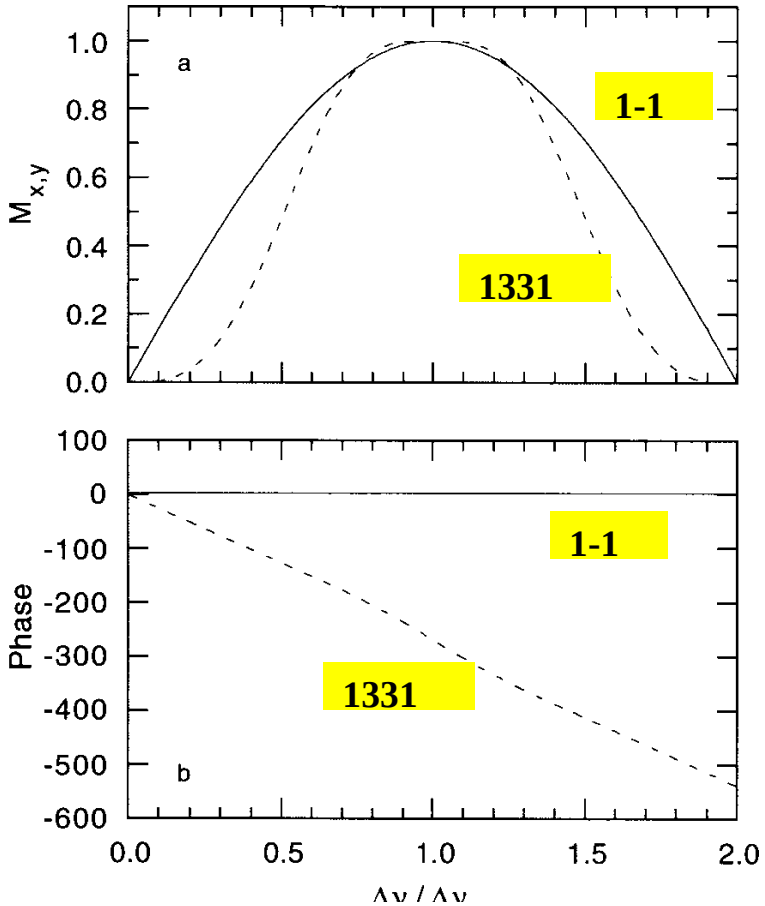
- **Delay $\tau = 1/(2\Delta\upsilon_{max})=1/d$,**
  $\Delta\upsilon_{max}$=distance of maximal intensity
  d=distance of next null

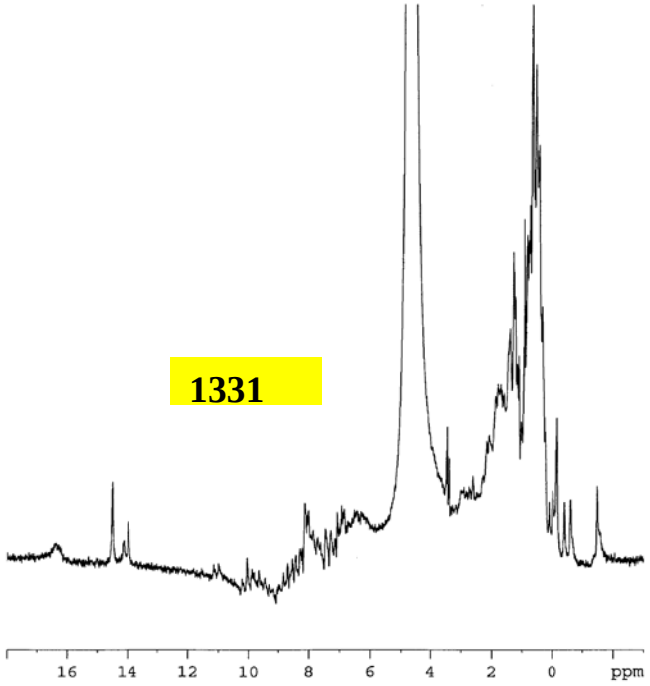
- **For example: To observe a peak at 14 ppm at 600 MHz,**
  τ=1/[2*(14-4.75)*600.13]=90 usec

Parameter adjustment:

- **Pulpro=p1331**
- **d19: delay for binomial water suppression**
- **d19 = (1/d), d = distance of next null (in Hz)=2*distance of maximal intensity**
- **d19=τ as defined above**

Jump-Return 1-1 and Binominal 1-3-3-1





Binomial excitation profiles of 1-1 and 1-3-3-1.

John Cavanagh et al., page 154, “Protein NMR Spectroscopy: Principles and Practice”, Academic Press (1995)

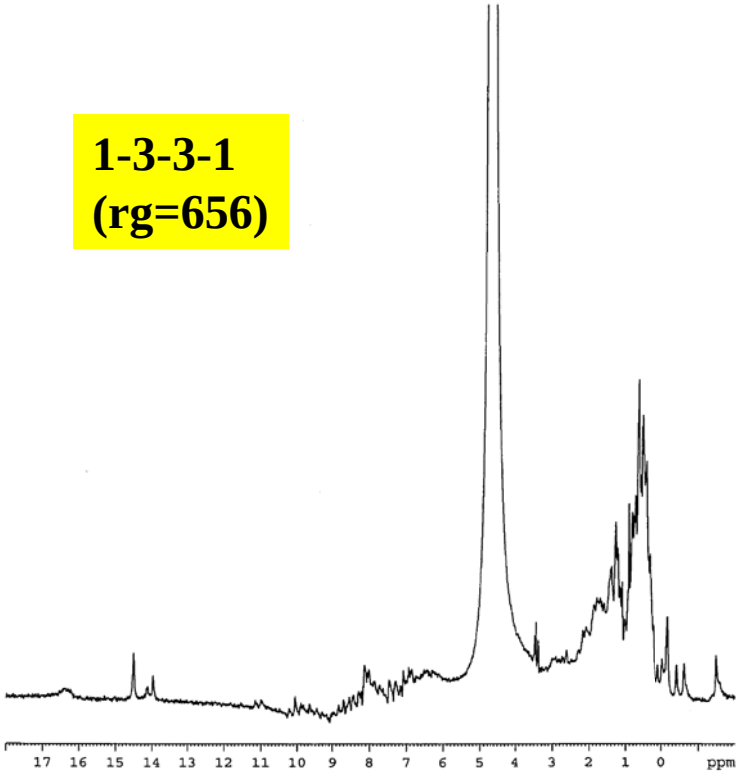
Jump-Return 1-1 and Binominal 1-3-3-1

Both are for observing fast exchanging protons.

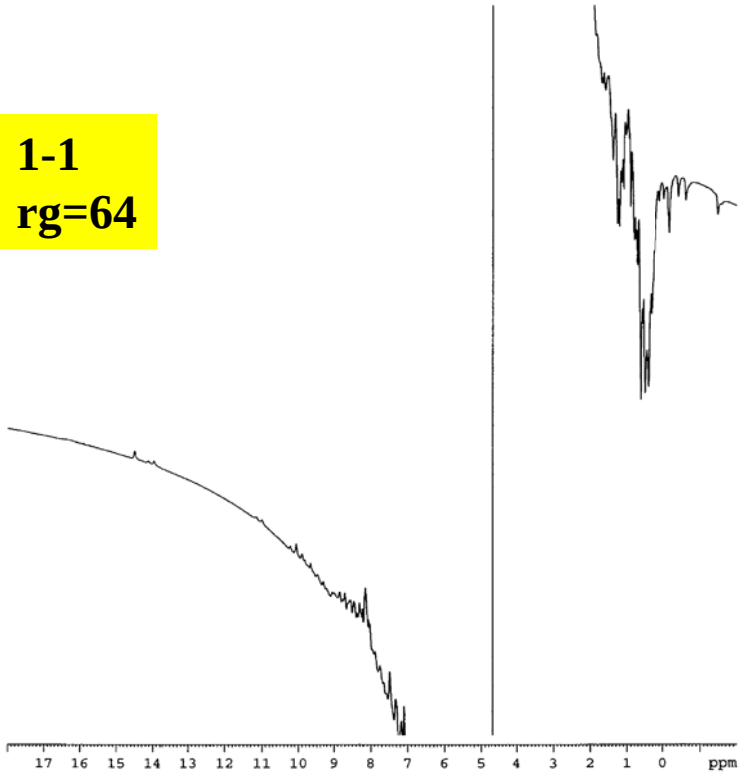
- **1-3-3-1: Better water suppression (higher receiver gain), but with offset-dependent phase distortion**

- **1-1: low receiver gain, the dispersive tail of water interferes with the signals of interest.**

1-3-3-1 (rg=656)

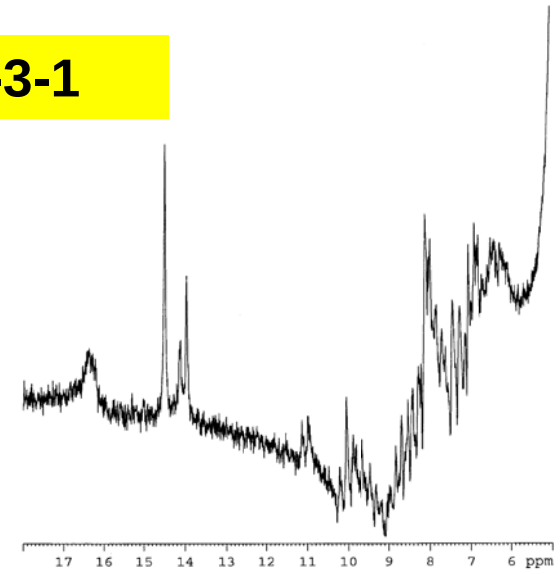


1-1 rg=64

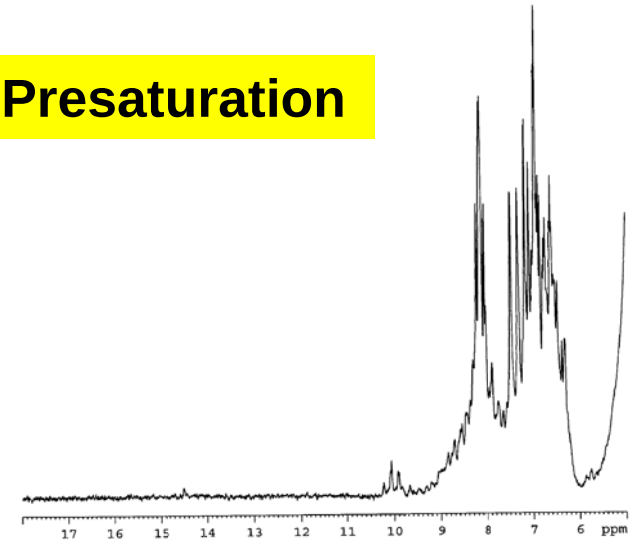


What are you trying to detect ?

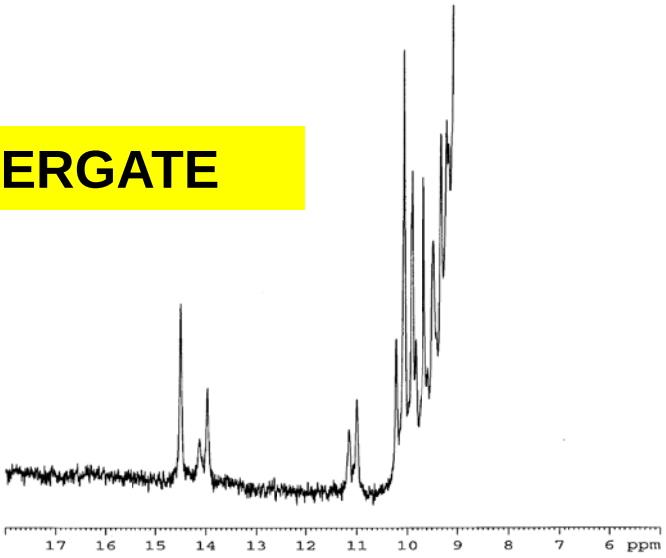
1-3-3-1



Presaturation



WATERGATE



Water Suppression via Coherence Pathway Rejection

Coherence pathway selected by gradients:

In a gradient selection experiment (echo/antiecho), the water coherence is not "refocused" by the refocus gradient (therefore, is not selected), this naturally suppression the water signal.

Example: cosydfetgp.1, hsqcetf3gp

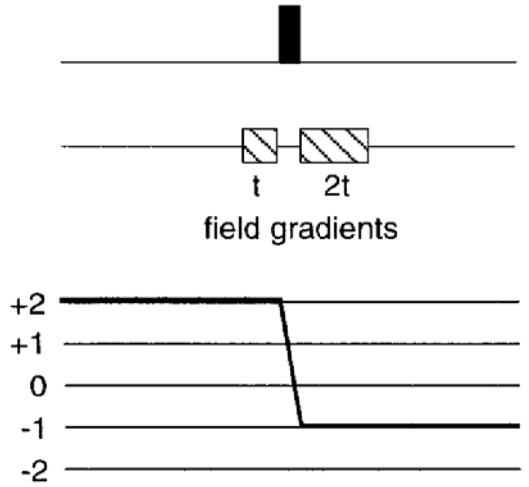


Figure from John Cavanagh et al.,"Protein NMR Spectroscopy: Principles and Practice", Academic Press (1995)

Practical Implementation: 1D, 2D and 3D

Fast exchangeable proton (His sidechain, -OH): 1-1 (good for 2D), 1-3-3-1 (not suitable for 2D, 3D).

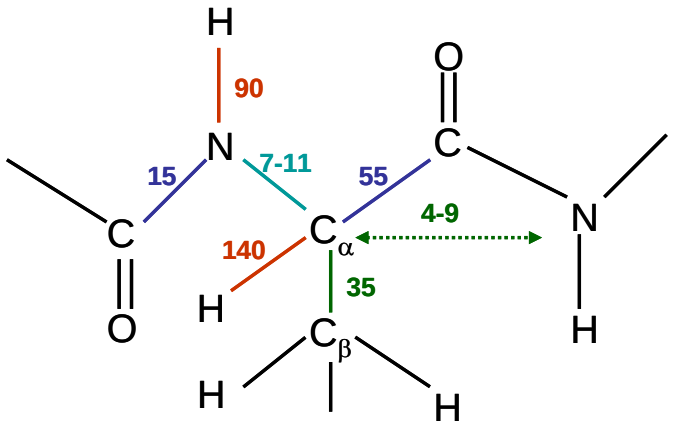
Exchangeable NH: Water-flip-back HSQC, Fast-HSQC.

Signals (H_α) near water: (i.e. TOCSY, COSY) WATERGATE with selective pulse, echo-antiecho PFG.

Variant HSQC Experiments

by
Wen-Jin (Winston) Wu

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

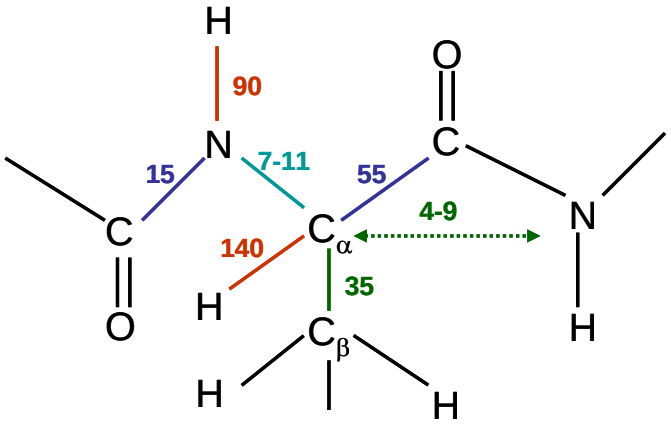


Coherence Order

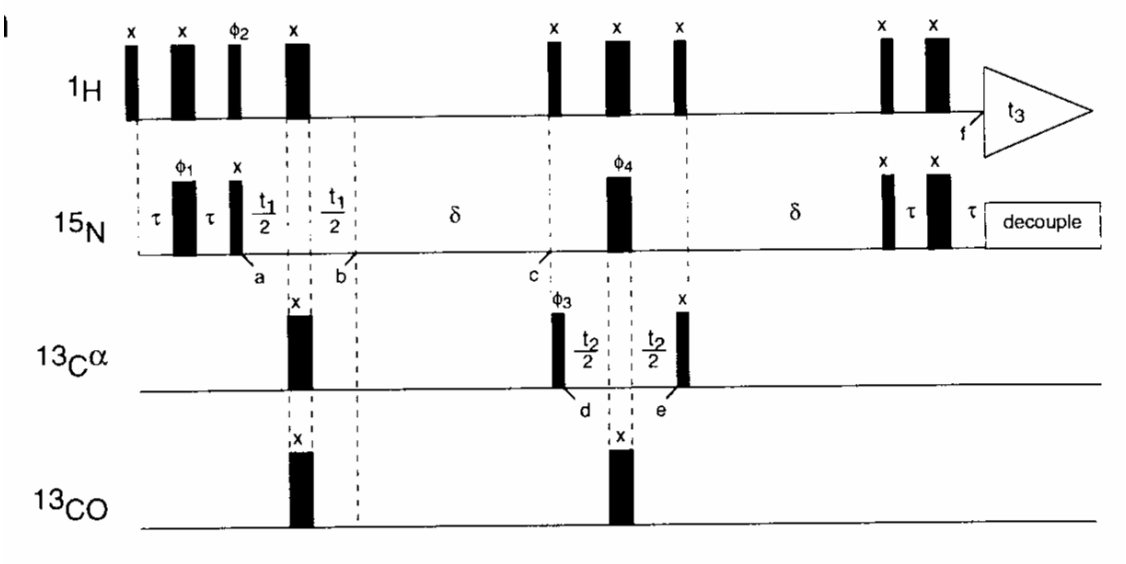
- **Zero quantum coherence: I_zS_z…etc**
- **Single quantum coherence: I_xS_z, I_yS_z, I_zS_y …etc**
- **Double quantum coherence: I_xS_x, I_xS_y…etc**
- **Triple quantum coherence: $I_xK_xS_x$, $I_xK_xS_y$ …etc**

- **HSQC: Hetero-nuclear single quantum coherence.**
- **HMQC:Hetero-nuclear multiple quantum coherence.**

Scalar Coupling (J-Coupling)



Coupling Constant in proteins.



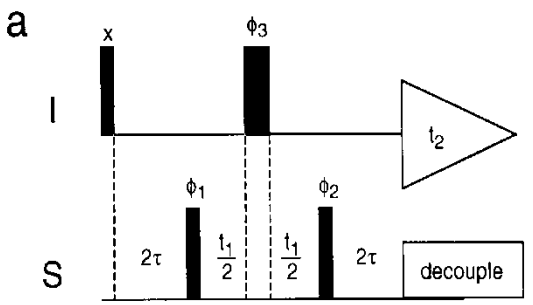
- Scalar couplings (J-couplings) are used as basic magnetization transfer in correlation spectroscopy.

- The efficiency of transfer depends on the magnitude of J-coupling constants.

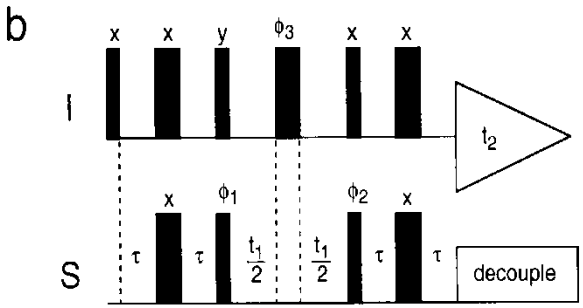
HMQC VS HSQC

- **HMQC:Hetero-nuclear multiple quantum coherence. Schem a.**
- **$2\tau=2*(1/4*J_{IS})$**
- **N-H: 2*[1/(4*90)]=5.5 ms**
- **C-H: 2*[1/(4*140)]=3.6 ms**

- **HSQC: Hetero-nuclear single quantum coherence. Scheme b.**
- **Intensity: $2I_zS_y\sin(2\pi J_{IS}t)$.**
- **Maximal intensity with $t=1/4J_{IS}$**
- **$\tau=1/4*J_{IS}$**
- **N-H: 1/(4*90)=2.7 ms**
- **C-H: 1/(4*140)=1.8 ms**

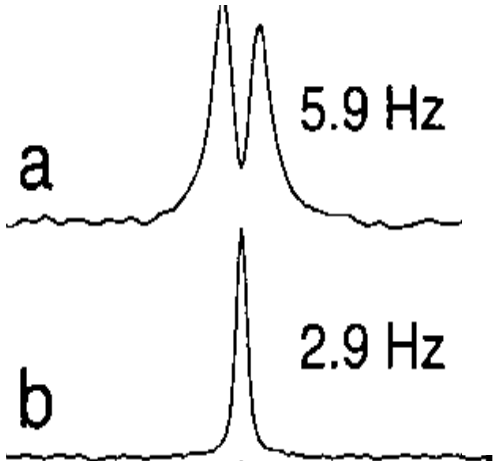


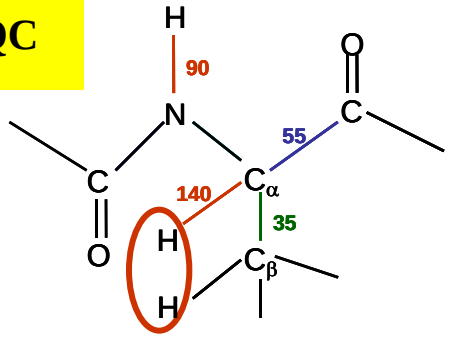
HMQC



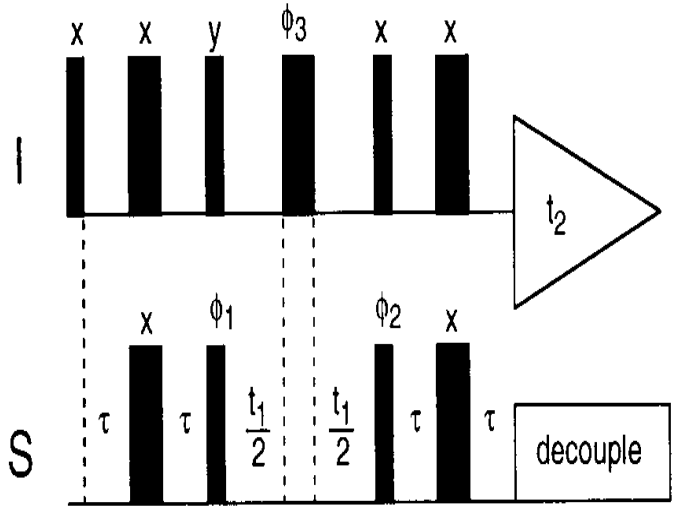
HSQC

Passive H-H coupling in HMQC



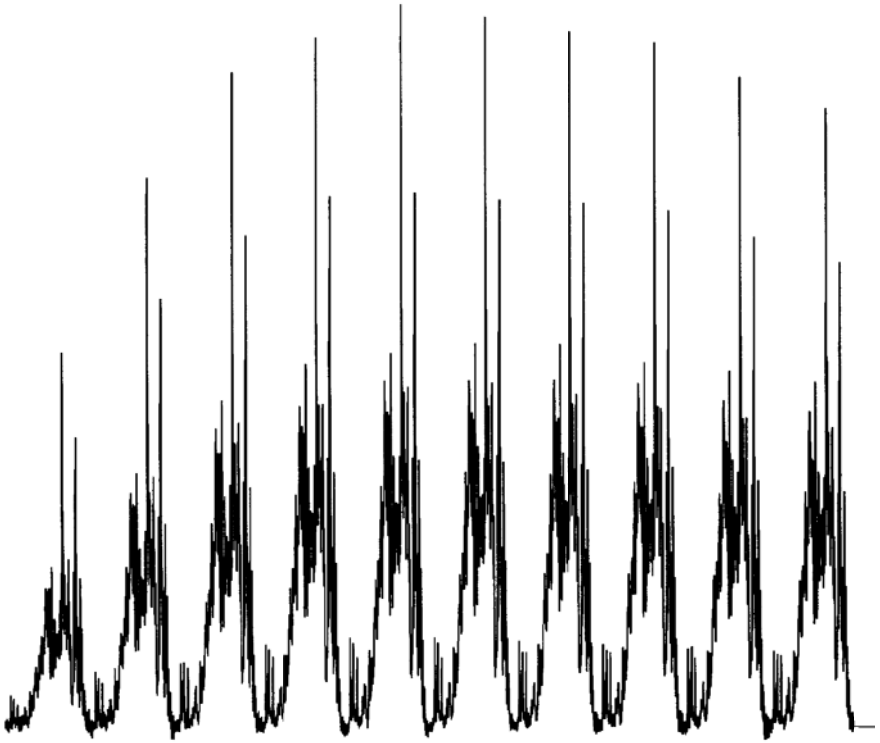


HSQC: Adjustment of J-Evolution Time



Page 412, John Cavanagh et al., "Protein NMR Spectroscopy: Principles and Practice", Academic Press (1995)

$\tau=(1/4J_{IS})$

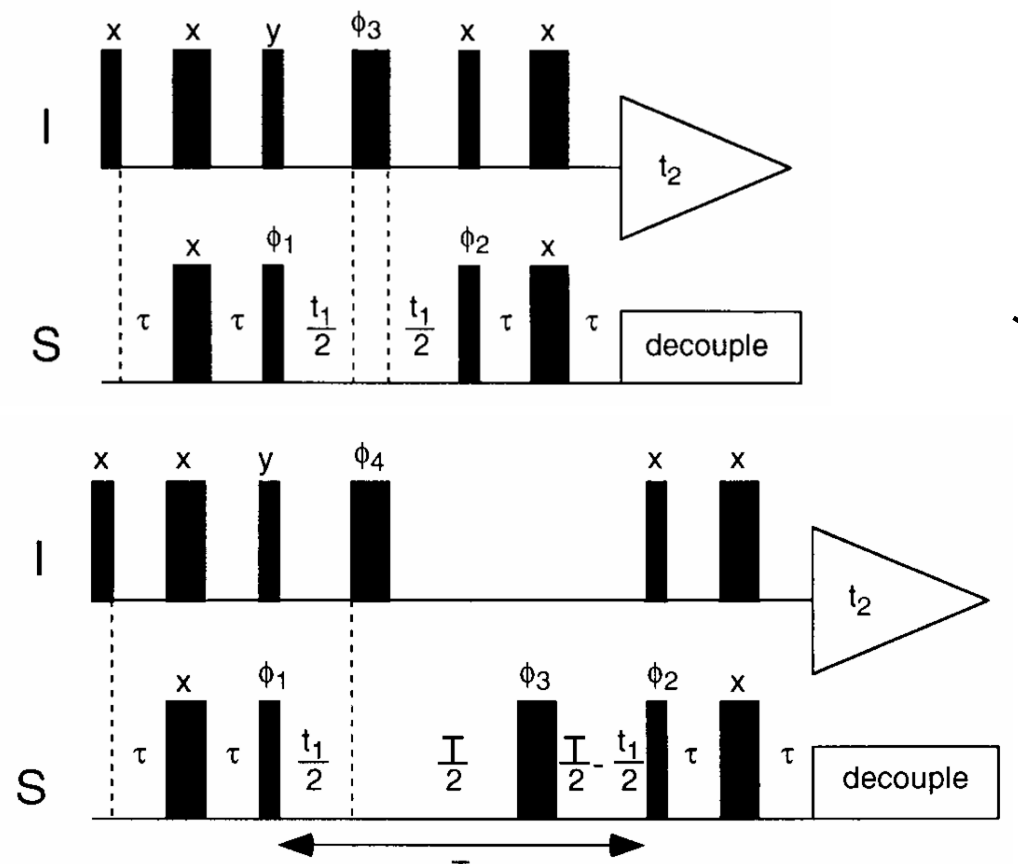


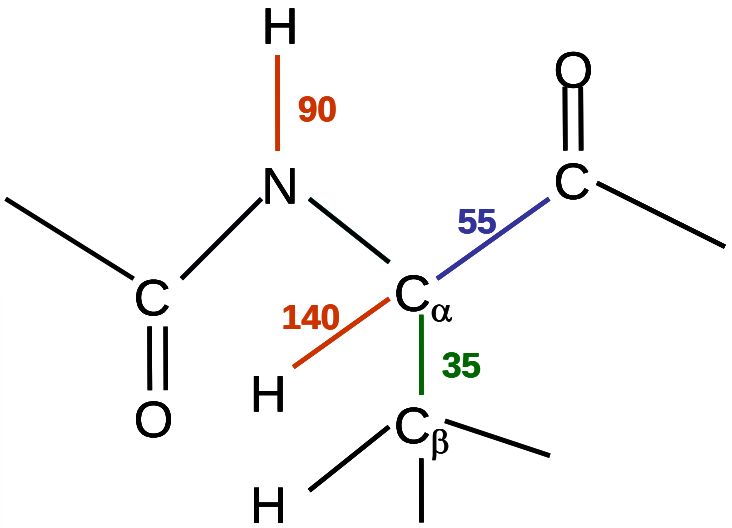
J=cnst4 = 60, 70, 80, 90, 100, 110, 120, 130, 140, 150 Hz

(The fist t1 of a HSQC for the protein TEP-I (~20 KDa), pH 6.0, 290 K, 600 MHz proton field strength. Pulprog=hsqcf3etgpsi2)

To compensate for relaxation loss, adjust the length of delay according to correlation time (i.e. molecular weight) of your proteins.

Constant-Time HSQC





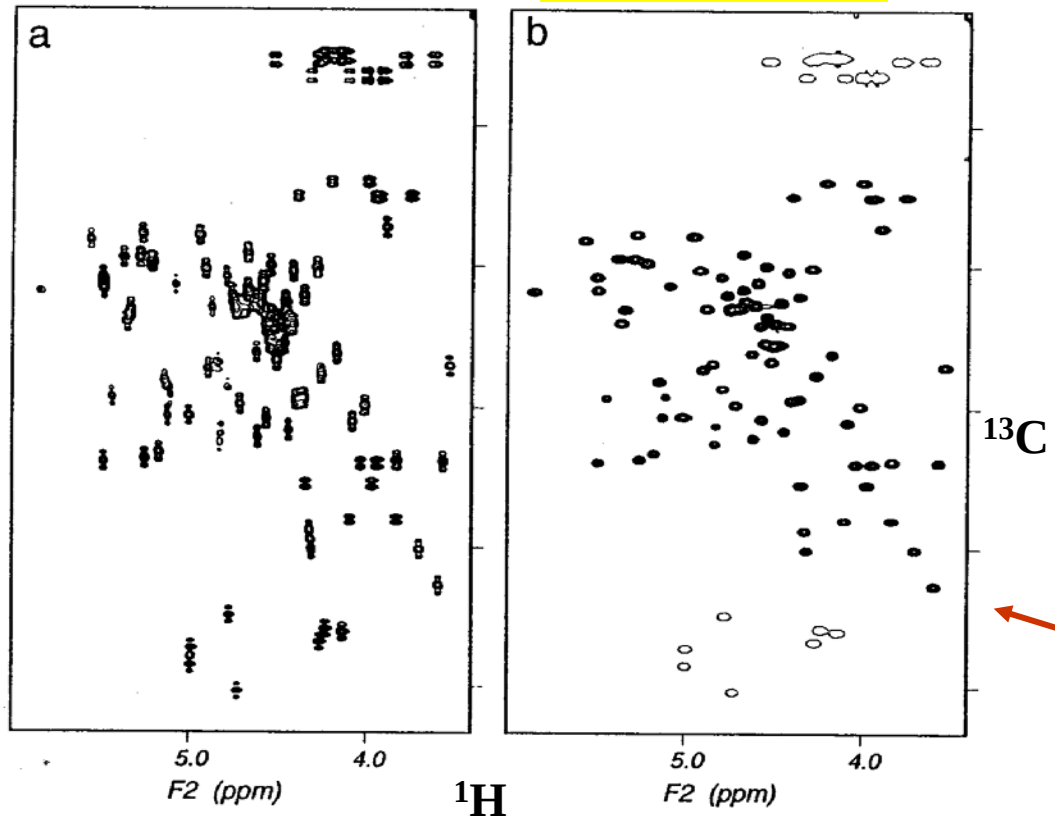
Page 412, John Cavanagh et al., "Protein NMR Spectroscopy: Principles and Practice", Academic Press (1995)

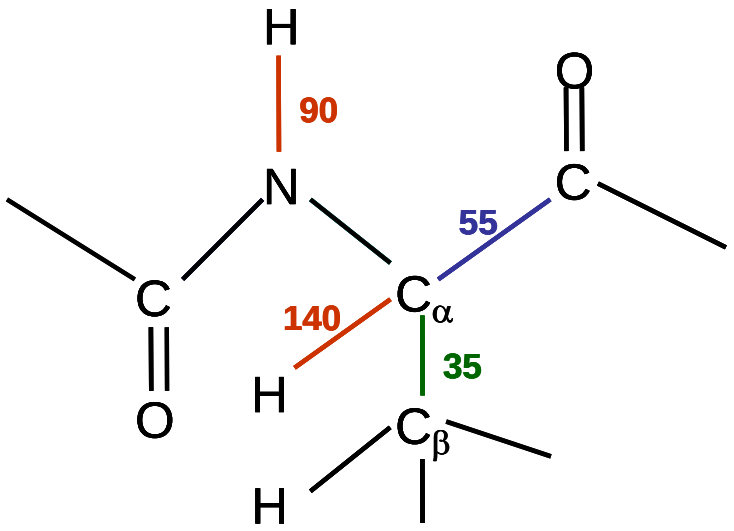
- **Spectral resolution can be compromised by passive J-coupling.**
- **The resolution of a ^{1}H, ^{13}C correlation (i.e. HSQC) can be enhanced by removing the passive C-C J-coupling (35 Hz) using a "constant time" scheme.**

Resolution Enhancement by the Constant Time Scheme

^{1}H, ^{13}C-HSQC

Constant time ^{1}H, ^{13}C-HSQC



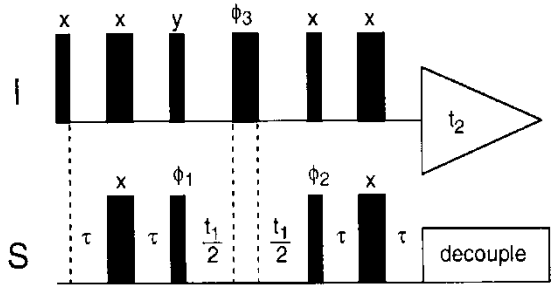


No C-C J-coupling

Page 439, John Cavanagh et al., "Protein NMR Spectroscopy: Principles and Practice", Academic Press (1995)

Sensitivity Improvement in HSQC

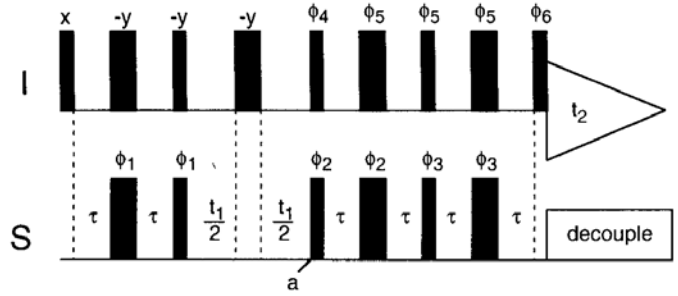
A.



HSQC

Only either $2I_xS_y$ or $2I_zS_x$ is refocused. Pulprog=hsqcf3ph

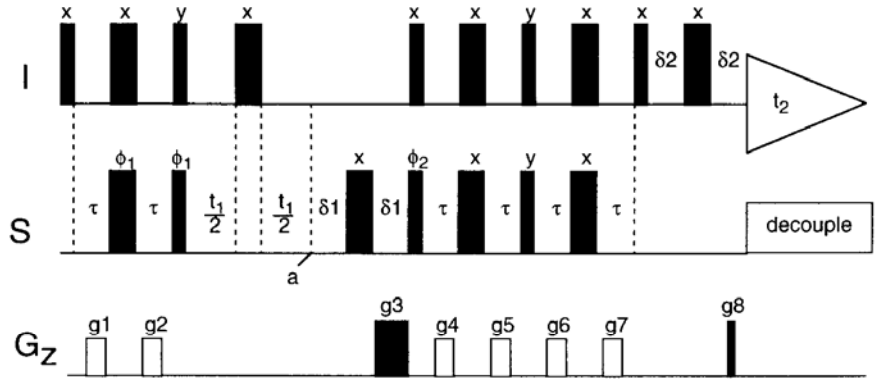
B.



PEP-HSQC

(PEP: preservation of equivalent pathway)
Both $2I_xS_y$ and $2I_zS_x$ are refocused.
$(2)^{1/2}$ increase in sensitivity without considering relaxation

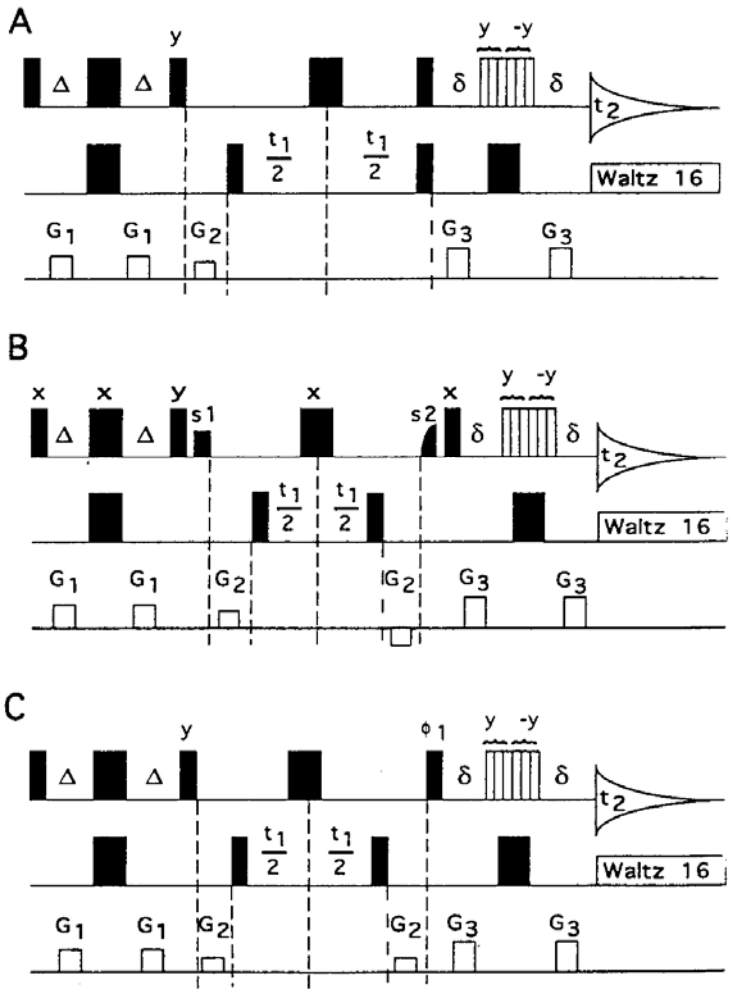
C.



PFG-PEP-HSQC:
Pulprog=hsqcetf3gpsi
Echo-antiecho:
gpz3: 80%
gpz8: 8.1% for N-15, 20.1% for C-13
Relaxation will compromise sensitivity of this experiment.

Page 441 of John Cavanagh et al., page 154, "Protein NMR Spectroscopy: Principles and Practice", Academic Press (1995)

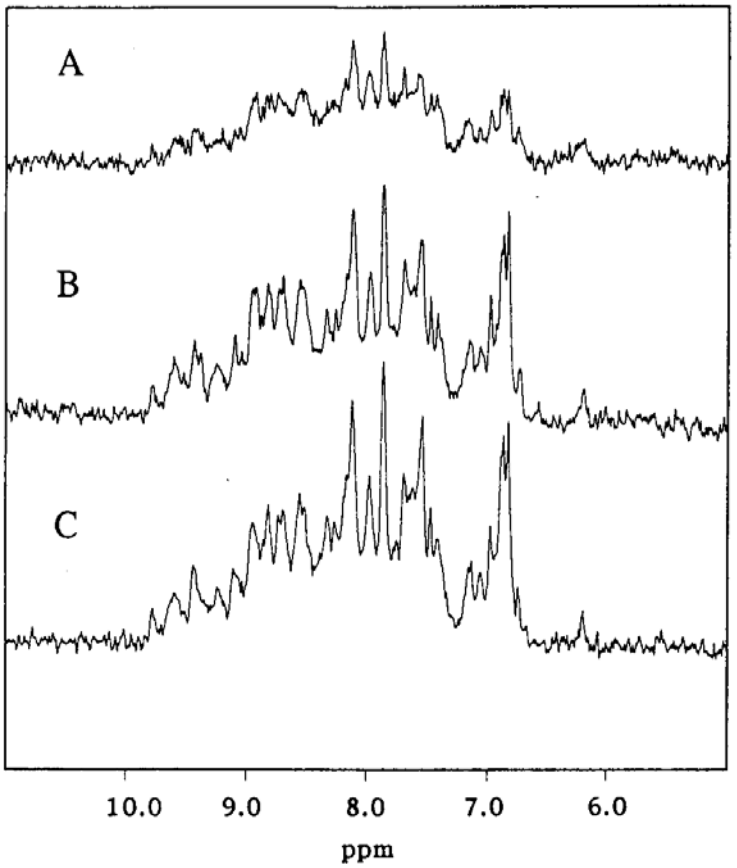
HSQC With Different Water Suppression Schemes



WATERGATE-HSQC (pulprog= hsqcf3gpph19)

Flip-back WATERGATE-HSQC (similar Pulprog =hsqcetfpf3gp)

Fast-HSQC (Pulprog= fhsqcf3gpph)

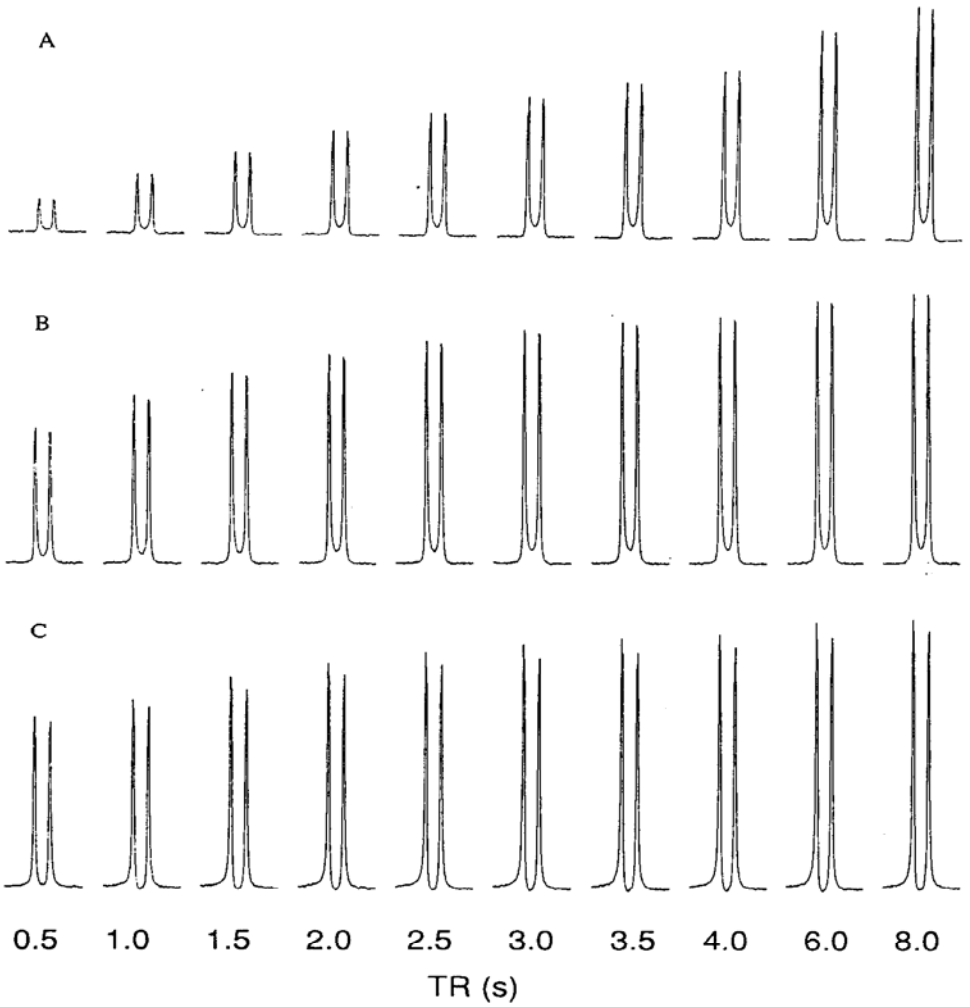


1D HSQC of 1.5 mM SNase (pH 7.4). 1 sec recycling delay

Water is kept along the Z-axis in both B and C before the final dephasing sequence to avoid saturation transfer.

Figures from Mori et al., J. Mag. Reson. B 108, 94-98 (1995)

HSQC With Different Water Suppression Schemes



WATERGATE-HSQC

Flip-back WATERGATE-HSQC

FHSQC (Fast-HSQC)

Series of 1D HSQC spectra for 10 mM ^{15}N-N-acetylalanine (pH 9.2) with different recycling delay.

Figures from Mori et al., J. Mag. Reson. B 108, 94-98 (1995)

Practical Usage of Experiments Containing HSQC

- **Saturation Transfer: avoid saturating water signal, especially at neutral pH.**

- **Relaxation: Extra pulse sequence length in "sensitivity enhanced" HSQC can cause sensitivity loss due to T_2 relaxation. This is particularly serious for large proteins (>20 kDa).**

Transverse Relaxation-Optimized Spectroscopy (TROSY)

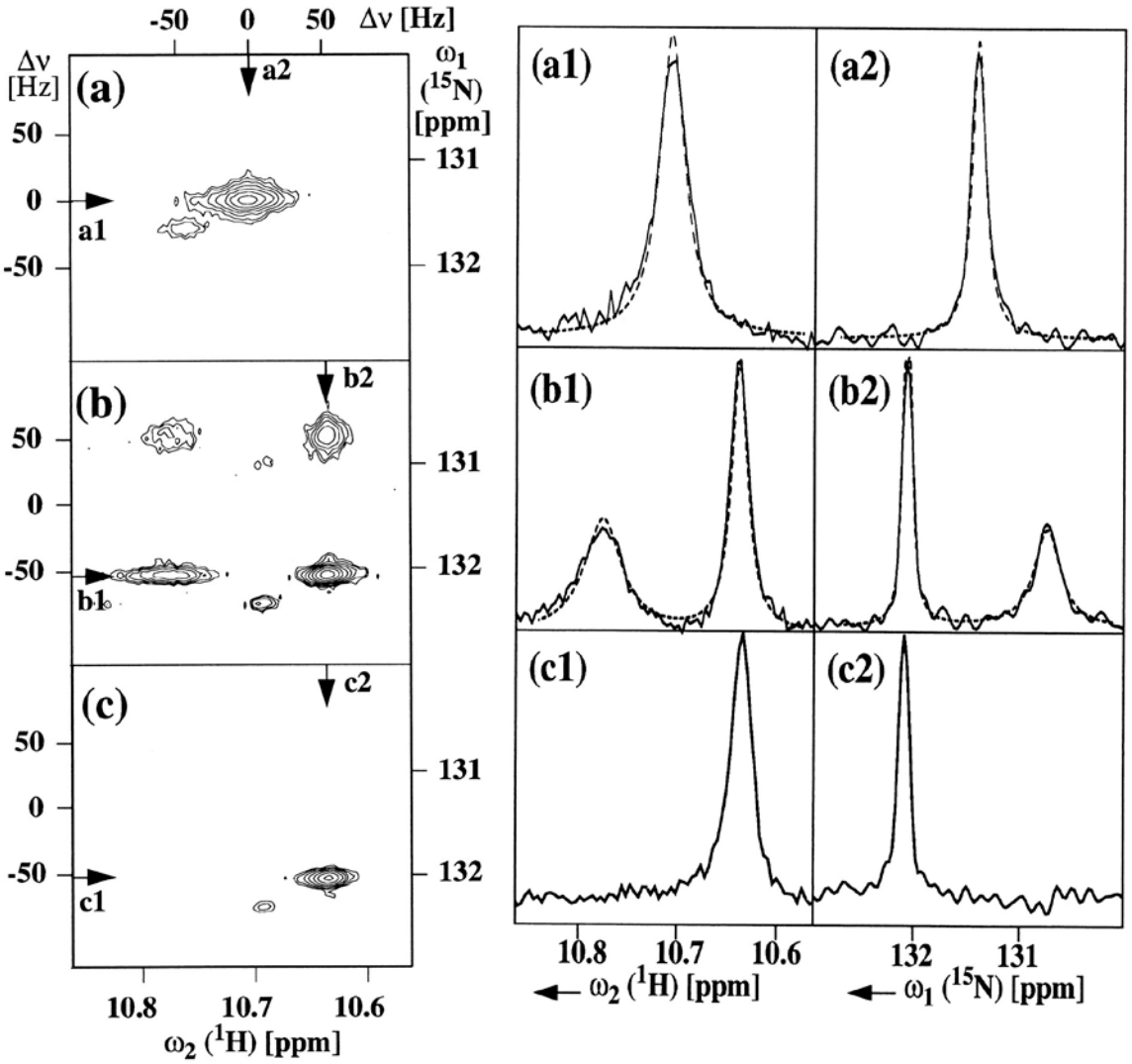
- **Constructive canceling of transverse relaxation caused by chemical shift anisotropy (CSA) and by dipole-dipole coupling at high magnetic field.**
- **Each of the four multiplet components of ^{15}N-^{1}H correlation components has different relaxation rates (line width).**
- **Select only the narrowest component (1 out of 4).**

TROSY at 750 MHz

Decoupled HSQC (during t1 &t2)

None-decoupling HSQC

TROSY-HSQC



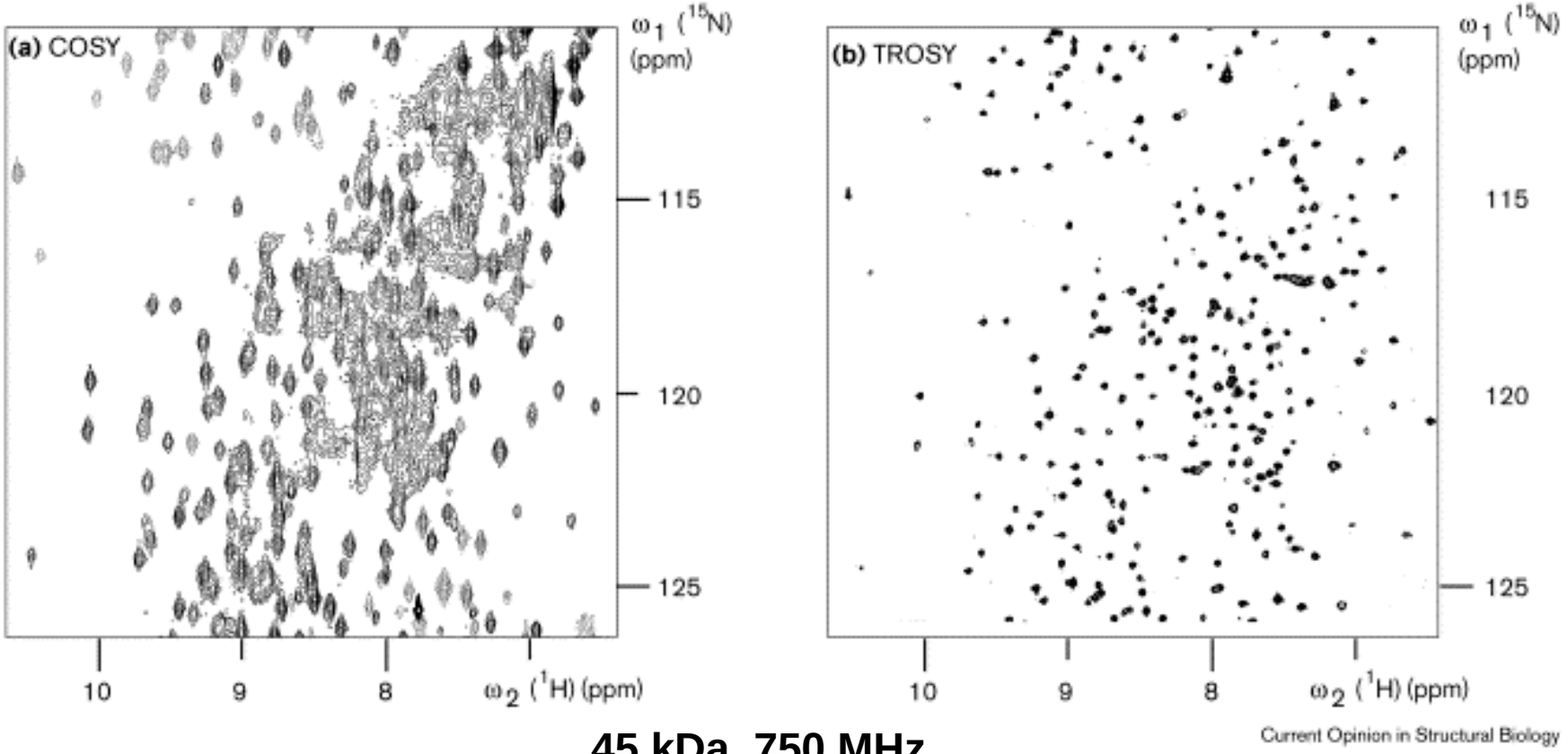
Linewidrh:
60% reduction in ^{1}H, 40%reduction in ^{15}N

If perdeuterated:
Expected reduction 40-fold for ^{1}H & 10-fold for ^{15}N

M.W~17 KDa

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

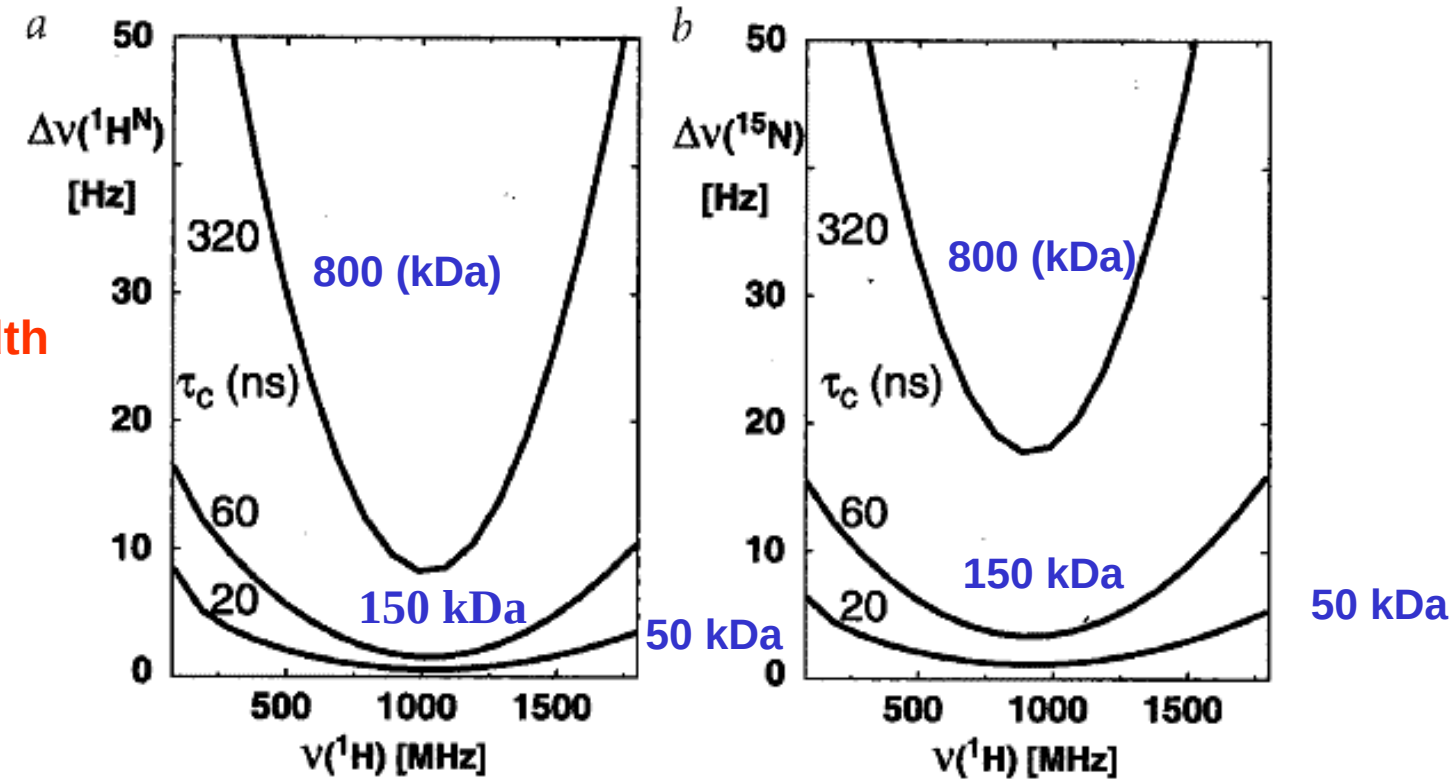
The Resolution Power of TROSY



45 kDa, 750 MHz

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

TROSY Effect is Field-Dependent



Linewidth

Magnetic field strength

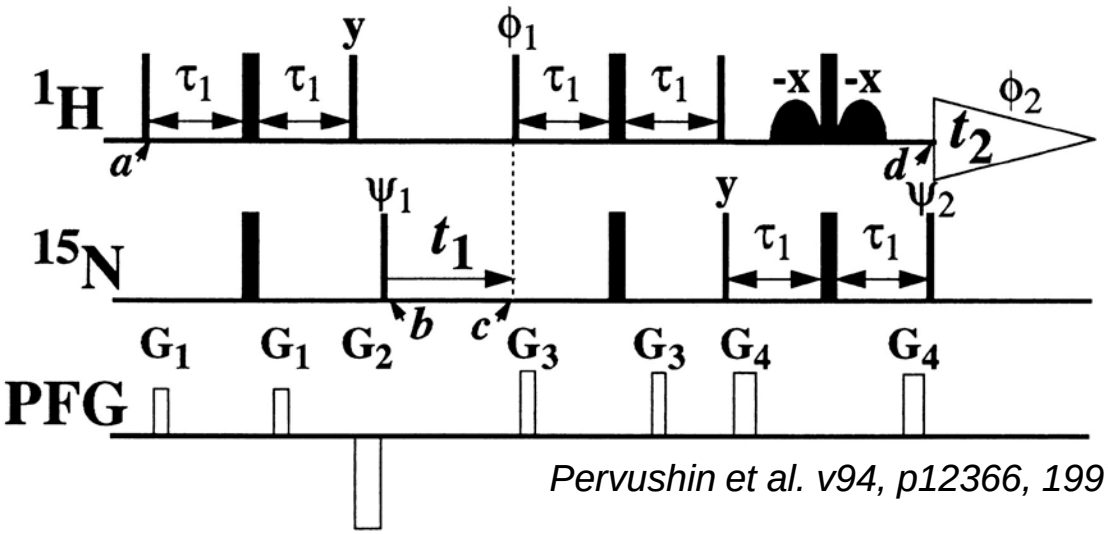
Optimal field strength: 1 GHz for NH;
600 MHz for aromatic moieties (500-800 MHz).

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

TROSY

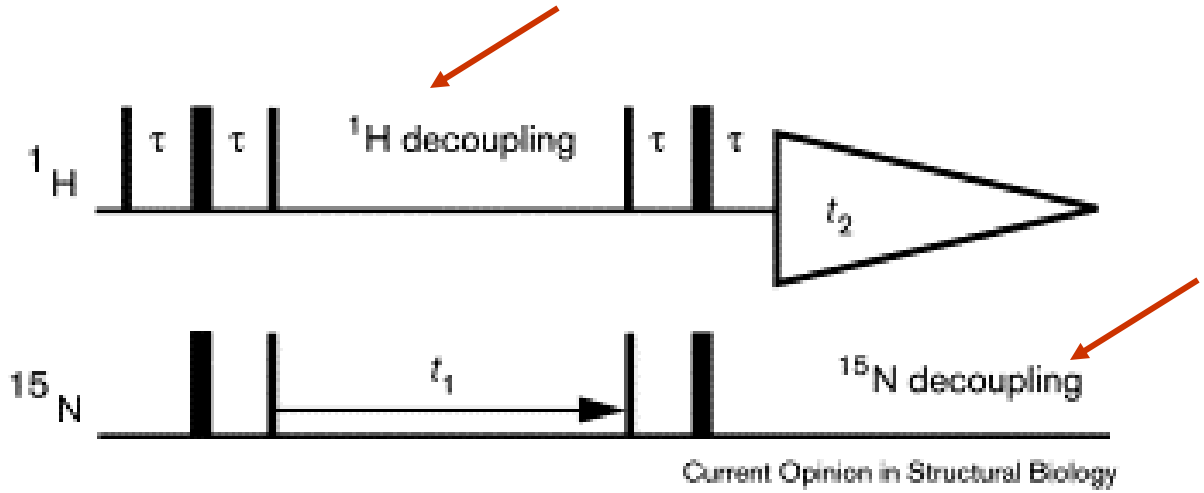
TROSY-HSQC

Pulprog=trosyf3gpph19, trosyf3gpphsi19



Pervushin et al. v94, p12366, 1997 PNAS

HSQC



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

Some Notes on TROSY

- **Intrinsic sensitivity loss by just selecting ¼ component. (worth doing it when T_2 relaxation is fast for large proteins).**
- **At the present time, at least 8-step of phase cycling is required to achieve coherence pathway selection.**
- **TROSY effect is field strength-dependent. Optimal field strength: 1 GHz for NH; 600 MHz for aromatic moieties (500-800 MHz).**
- **TROSY effect is well suited for large molecule.**

"NMR analysis of a 900K GroEL-GroES complex." Flaux et al., Nature V. 418, 11, p207 (2003)

Pulsed Field Gradient (PFG) NMR

- **Solvent suppression.**

- **Artifact suppression.**

Have the coherence of interested align along the z-axis, then destroy any unwanted signals left on the transverse plan.

- **Coherence pathway selection.**

Select coherence pathway of interested in one single scan instead of 8 or 16 as in phase cycling.
More number of increments can be used in 3D, 4D experiment: higher resolution.

- **Diffusion measurement to study aggregation.**

Pulsed Field Gradient

- **A field-gradient pulse is a pulse or a period during which the magnetic field is made deliberately inhomogeneous.**

$B=B_0+B_g(z)$

- **The magnetic field, generated by a gradient pulse, Bg(z) varies linearly along the Z-axis**

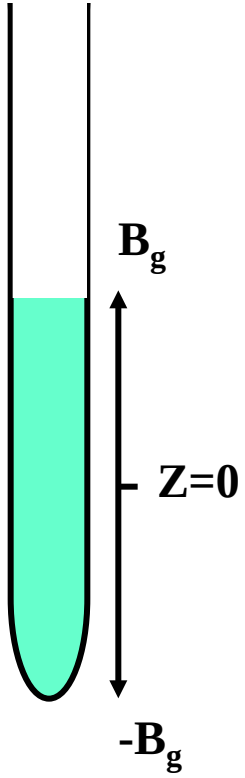
$B_g(z)=zG_z$, where

G_z: gradient strength (G/cm), Z: z-axis position

- **Viewing on the rotating frame, spins at different z-position acquire different phase (Larmor frequencies):**

$\phi(z)=\gamma z G_z t$,

where γ: gyromagnetic ratio, τ: gradient duration

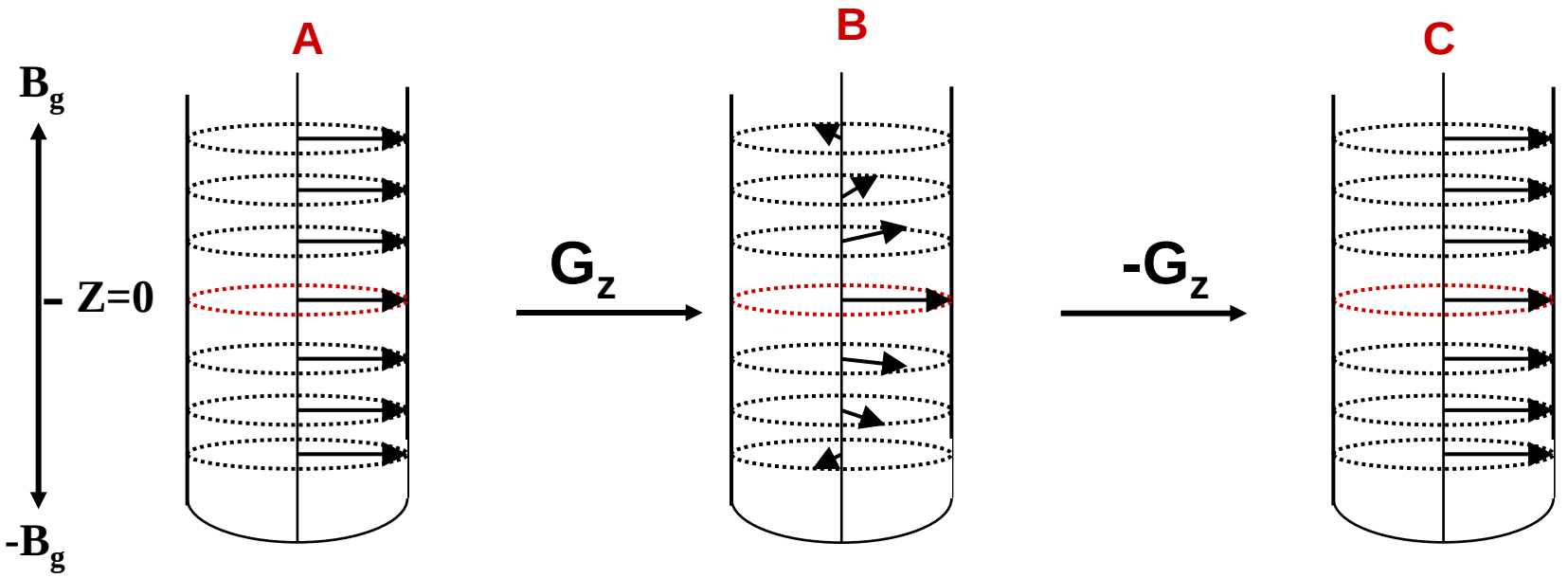


- **A coherence can be dephased by a strong pulse field gradient.**
- **A dephased coherence can be refocused by a refocus-gradient providing the "overall phase change " is zero.**

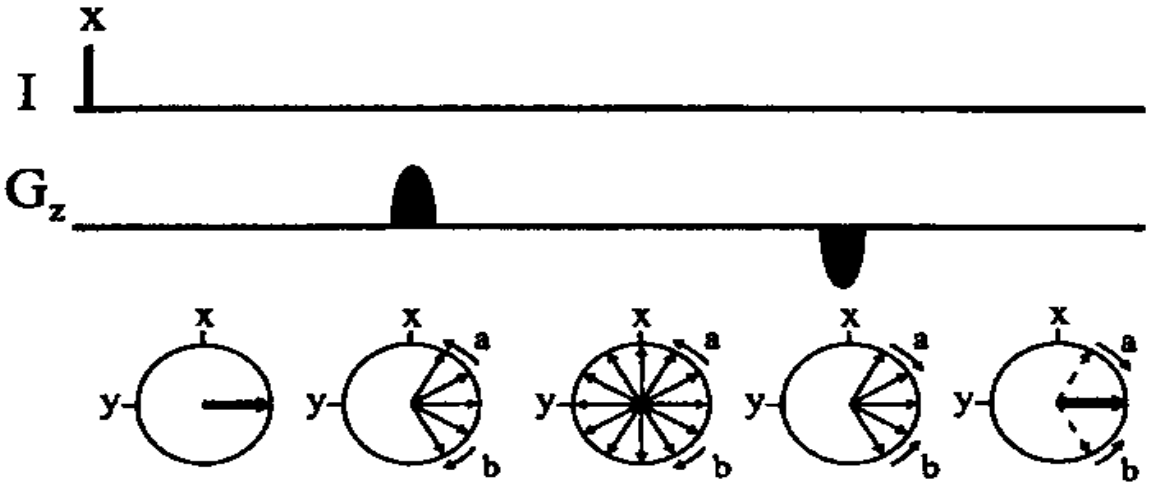
$\phi_i+\phi_f=0$ **;Coherence refocused.**

$\phi_i+\phi_f\neq 0$ **;Coherence dephased.**

Pulsed Field Gradient (PFG)



Viewing from the Z-axis:

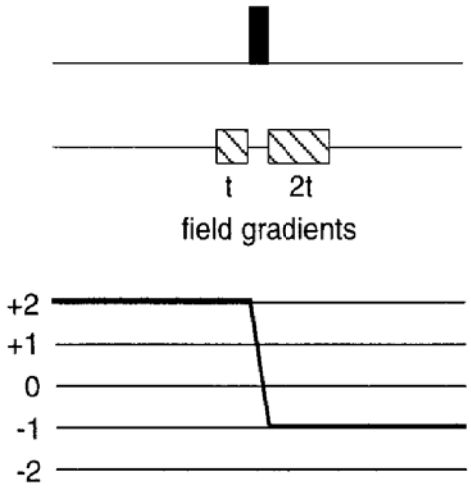


(figure from p106 of Sattler et al. Prog. In Nucl. Mag. Reson. Spect. 34 (1999)

Coherence Pathway Pathway Selection by PFG

$\Phi_i = G_i P_i \gamma_i$ (phase acquired after the first gradient)
$\Phi_f = G_f P_f \gamma_f$ (phase acquired after the second gradient)

$G_i = s_i B_g \tau_i$ (gradient term) P_i: coherence order, γ:gyromagnetic ratio τ_i:gradient pulse length, S_i: shape factor of a gradient pulse (what kind of shaped pulse).



Coherence Selection by Gradient:

If $\Phi_i + \Phi_f = 0$;Coherence is refocused (selected). (gradient echo)
If $\Phi_i + \Phi_f \neq 0$;Coherence is dephased (rejected).

Example: Selection of the coherence pathway p=2 to p=-1 by PFG.
$\Phi_i = 1*2*1 + 2*(-1)*1 = 0$

Figure from Page 225 of John Cavanagh et al., page 154, "Protein NMR Spectroscopy: Principles and Practice", Academic Press (1995)

PFG-NMR: Coherence Selection, Artifact Suppression

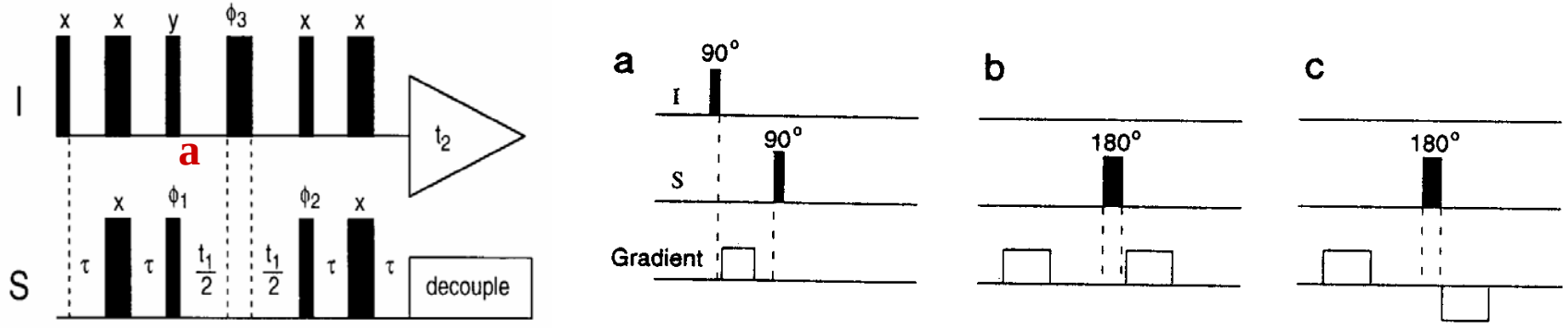


FIG. 1. Examples of different applications of pulsed field gradients in heteronuclear NMR. (a) Selection of an I_zS_z intermediate, (b) selection of transverse S-spin magnetization which is being refocused by a 180° pulse, and (c) elimination of transverse S-spin components caused by an imperfect 180° (S) decoupling pulse.

$$I_xS_z \xrightarrow{90^\circ_y(I)} -I_zS_z \xrightarrow{90^\circ_y(S)} -I_zS_x. \qquad [1a]$$

Unwanted magnetization is associated with terms such as

$$I_yS_z \xrightarrow{90^\circ_y(I)} I_yS_z \xrightarrow{90^\circ_y(S)} I_yS_x \qquad [1b]$$

$$I_y \xrightarrow{90^\circ_y(I)} I_y \xrightarrow{90^\circ_y(S)} I_y \qquad [1c]$$

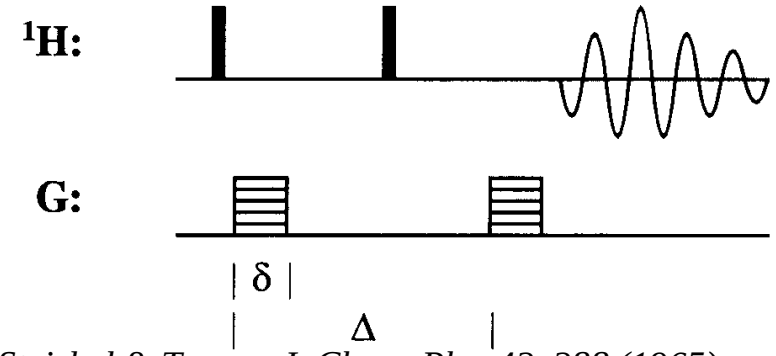
or

$$I_x \xrightarrow{90^\circ_y(I)} -I_z \xrightarrow{90^\circ_y(S)} -I_z. \qquad [1d]$$

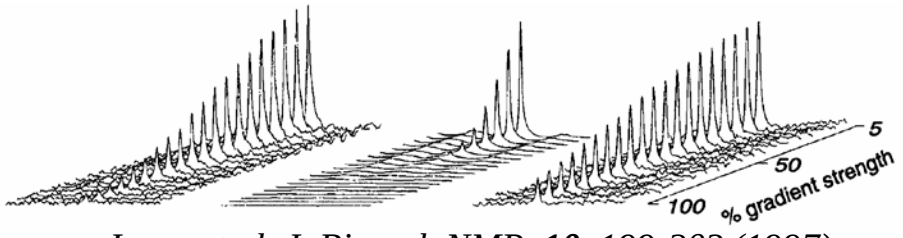
Example "a": Destroy the "unwanted" components with a Z-gradient pulse when they are on the transverse plan and the component of interest is a long the z-axis.

A. Bax & S. Pochapsky, J. Magn. Reson. 99, 638-643 (1992)

Diffusion by PFG-NMR



Stejskal & Tanner, J. Chem. Phy. 42, 288 (1965)



*Jones et al. J. Biomol. NMR, **10**, 199-203 (1997)*

$D_s = kT/6\pi\eta r_s$ **r_s: hydrodynamic radius**

Different size of molecules have different self-diffusion coefficients D_s.

$$I_{(2\tau)} = I_0 * \exp[-(\gamma\delta G)^2(\Delta - \delta/3)D_s$$

γ: gyromagnetic ratio; δ: gradient duration;
G: gradient strength, Δ: time between gradient pulse,
I_0: signal intensity in the absence of gradient

Monitor signal intensity as a function of either gradient duration or gradient strength. D_s can then be obtained by nonlinear-squares fits to the above equation.