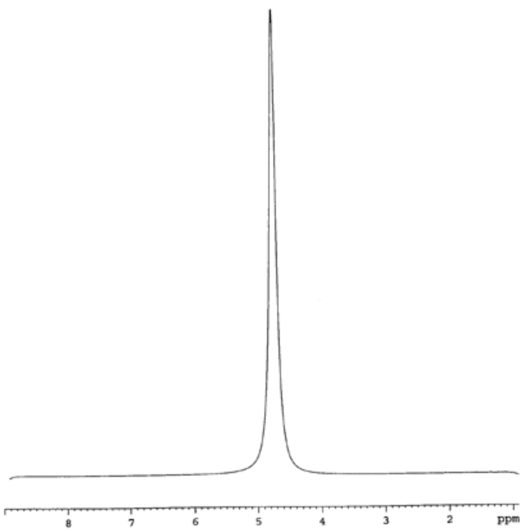
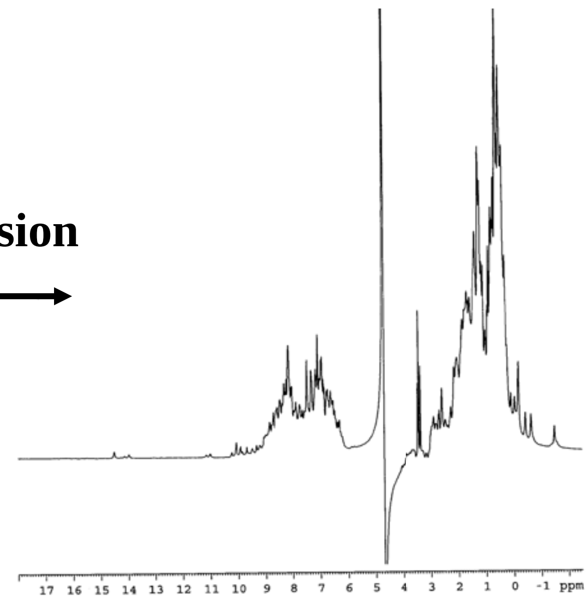
Water Suppression Techniques

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

$[H_2O]$/[Protein]>11,000



Water suppression →

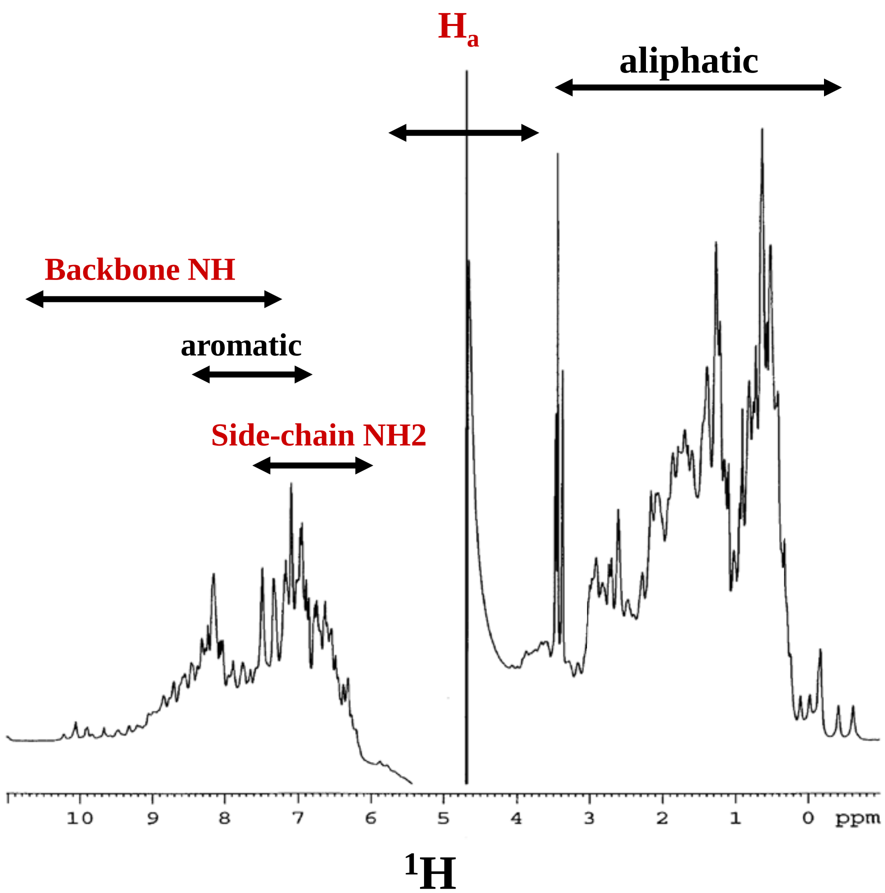


Sample used throughout this lecture: 1 mM TEP-I in 90% H_2O/10%D_2O, pH 6.0, 290 K.

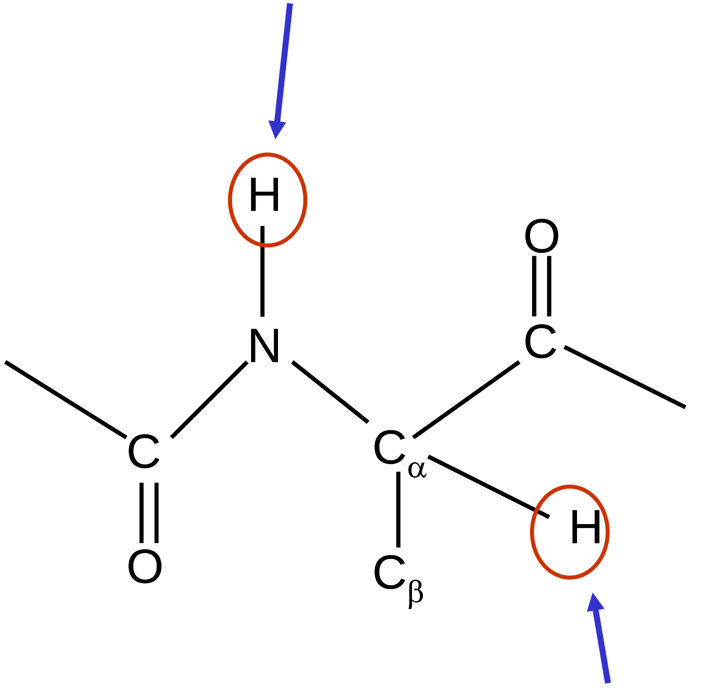
Water Suppression Technique

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

Water Suppression Technique in Protein NMR



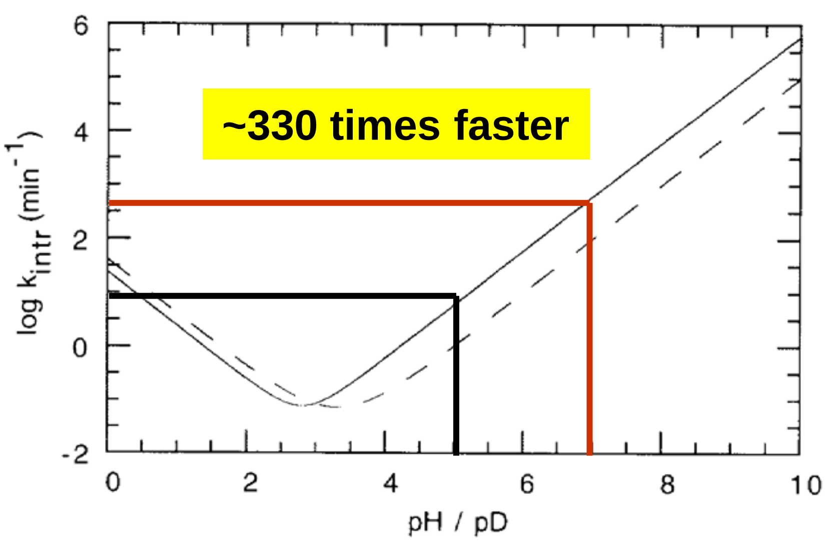
Labile, exchange with water (pH, structure, temperature dependent)



Resonates near the water frequency

pH Dependence of Amide Proton Exchange Rates

$$\text{N-H} + {}^{*}\text{H}_2\text{O} \rightleftharpoons \text{N-}{}^{*}\text{H} + {}^{*}\text{H-O-H}$$



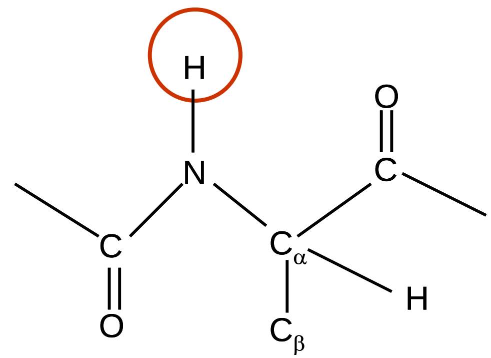
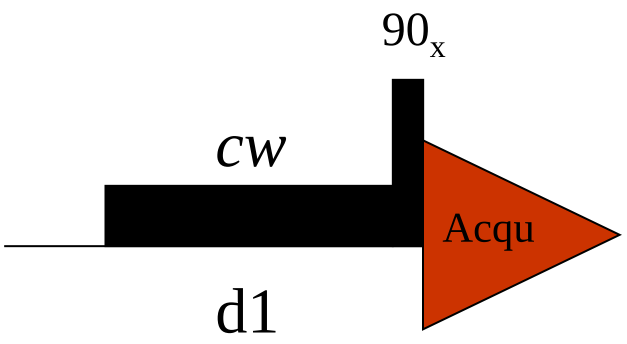


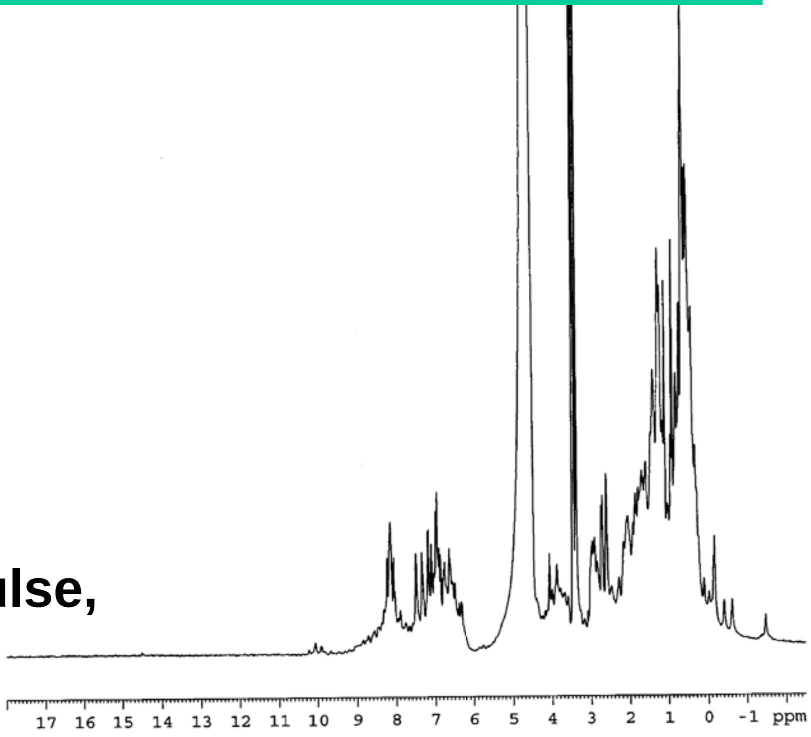
FIGURE 3.26 Intrinsic backbone amide proton exchange rates calculated according to Connelly *et al.* (63). The intrinsic exchange rate, k_{intr}, is shown for exchange of a backbone amide proton with (—) H_2O or (- - -) D_2O as a function of pH or pD. The pD values are corrected for isotope effects;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





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

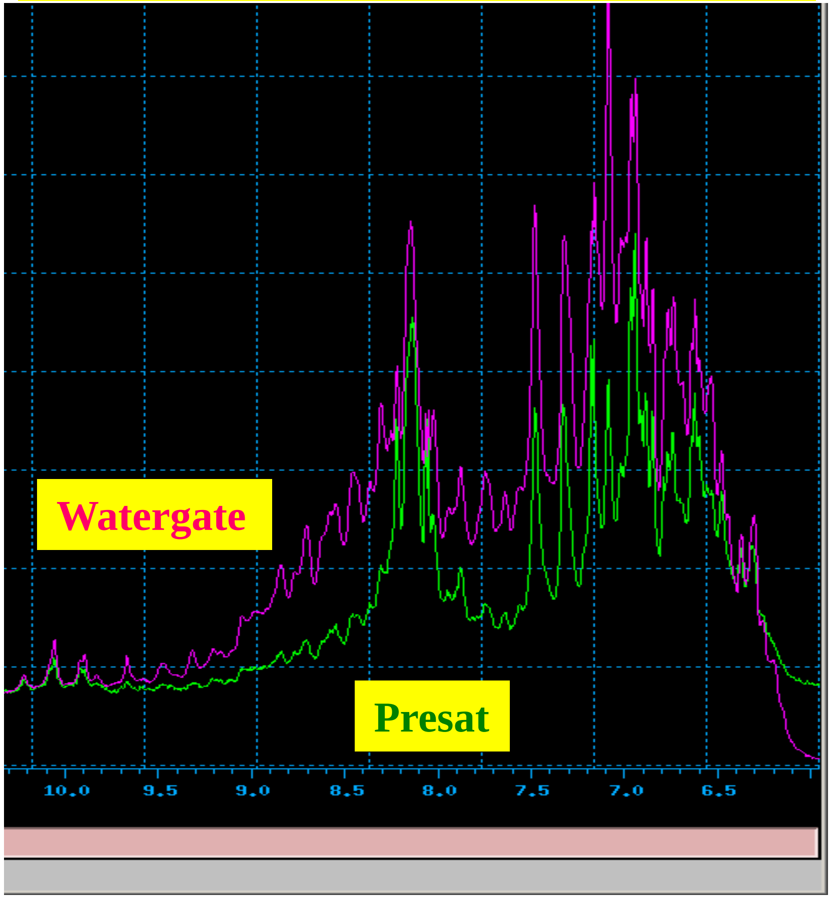
1D [1]H spectra of TEP-I in 90%H_2O/10%D_2O , pH 6, 290 K.

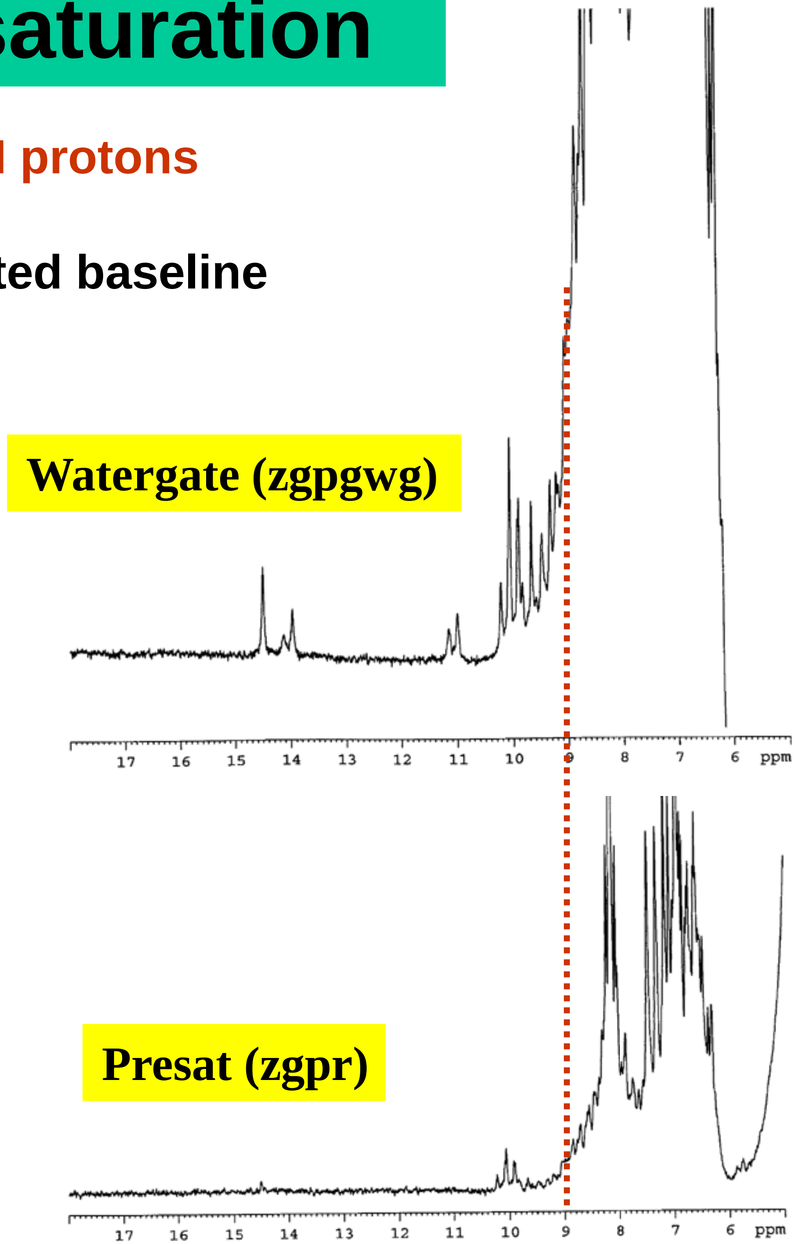
Parameter adjustment:
Pulprog=zgpr
Adjustment: pl9; power level for presaturation

Drawback of Presaturation

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

1D ¹H spectra of TEP-I, pH 6, 290 K.





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**

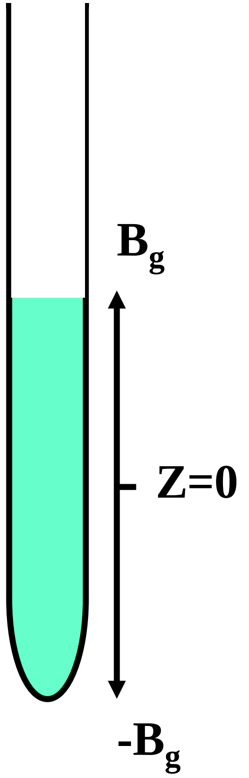
$B_g(z)=zG_z$, where

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.***

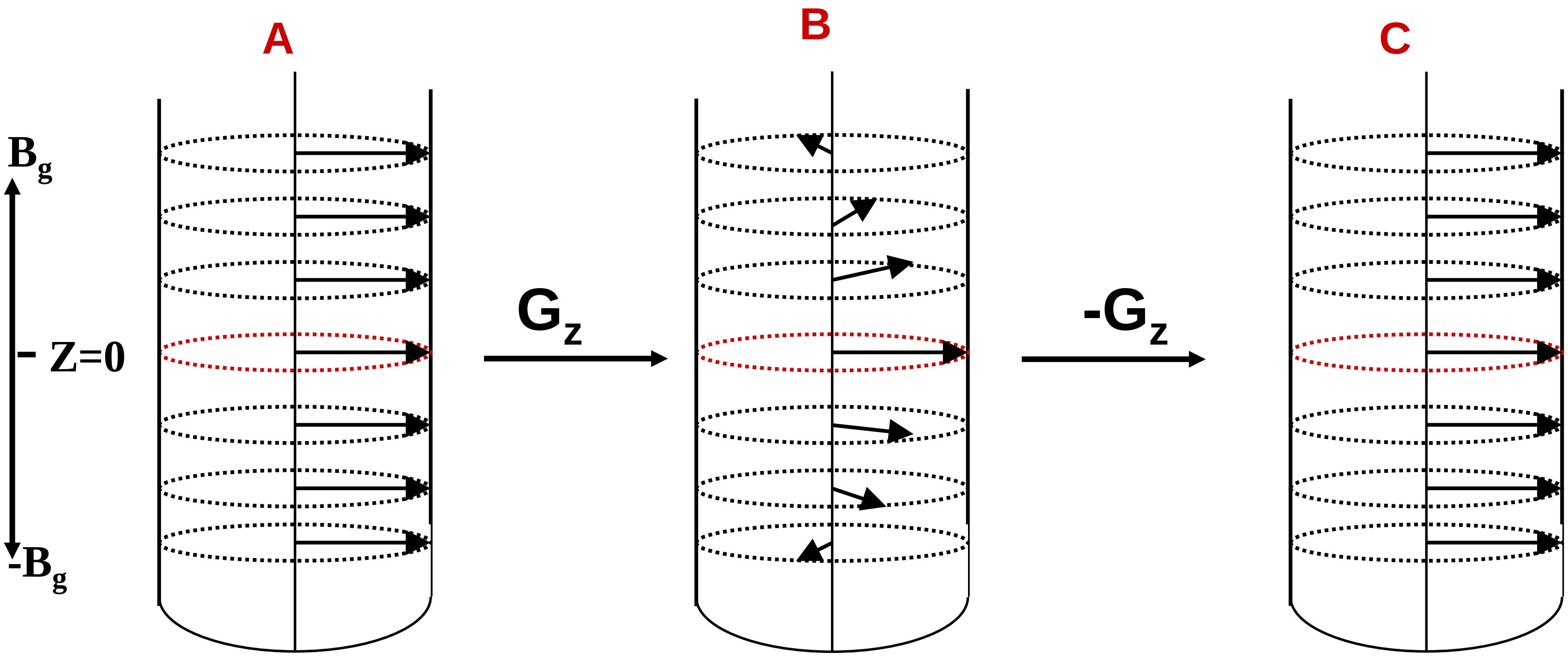


Pulsed Field Gradient (PFG)

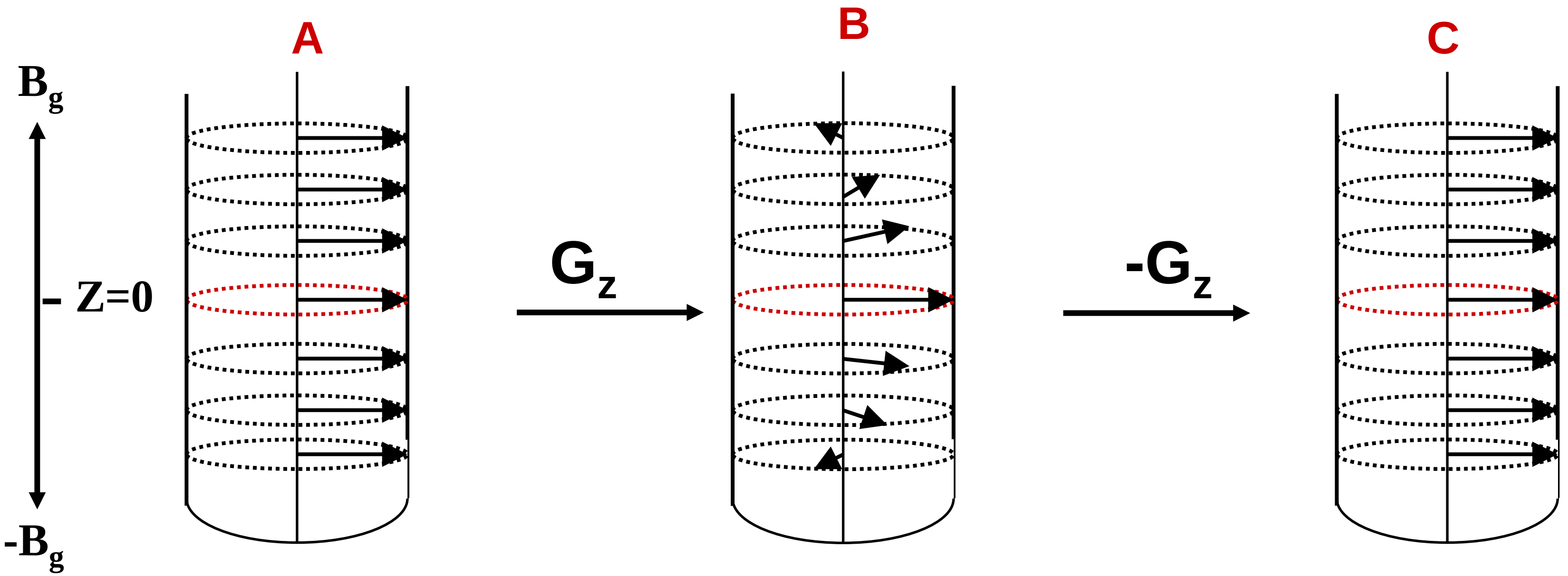
A. **Initially spins in each slice (isochromat) are "phase-coherence".**

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.**

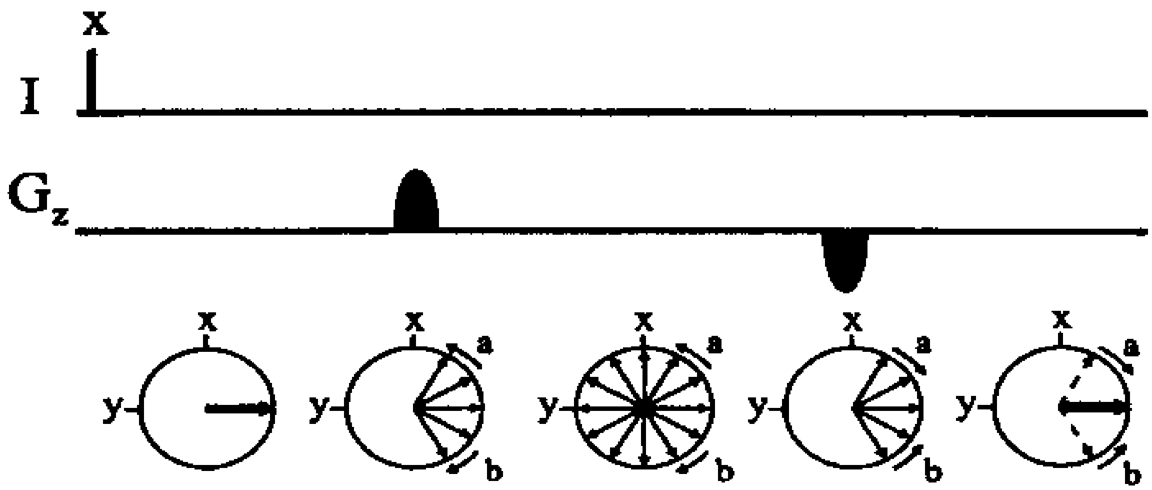
C. **The coherence can be refocused by another gradient pulse (gradient echo).**



Pulsed Field Gradient (PFG)

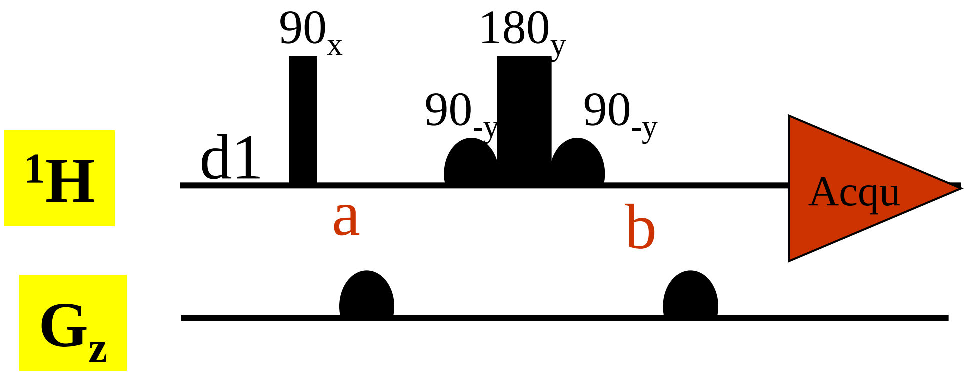


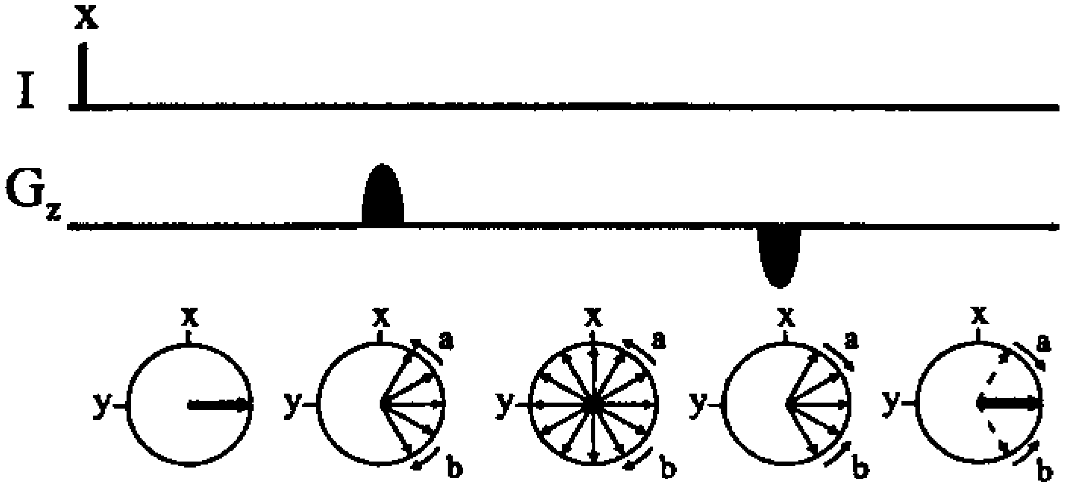
Viewing from the Z-axis:



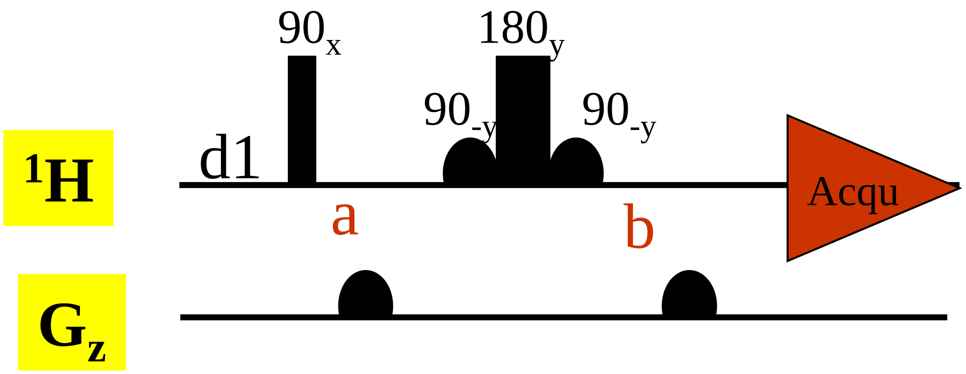
(figure from p106 of Sattler et al. Prog. In Nucl. Mag. Reson. Spect. 34 (1999)

WATERGATE





WATERGATE



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

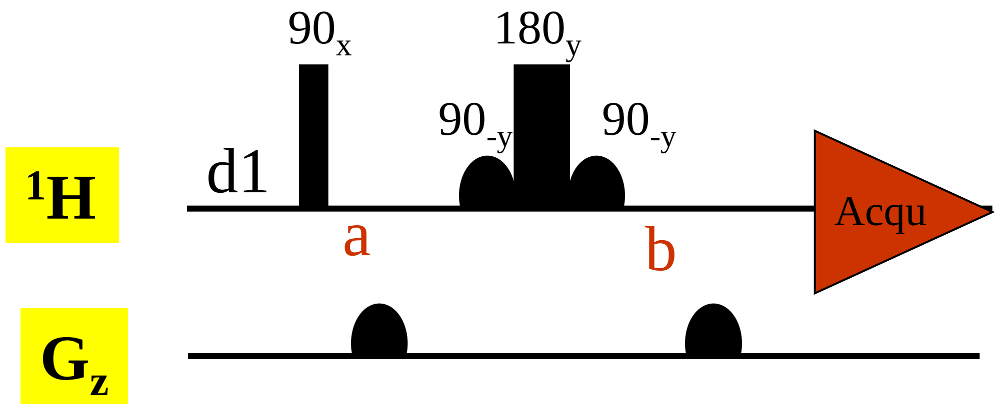
•A destroyed (dephased) magnetization can be refocused by another z-gradient pulse of the same amplitude but of opposite phase. (or use a 180 pulse in between the two identical z-gradient pulse).

•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



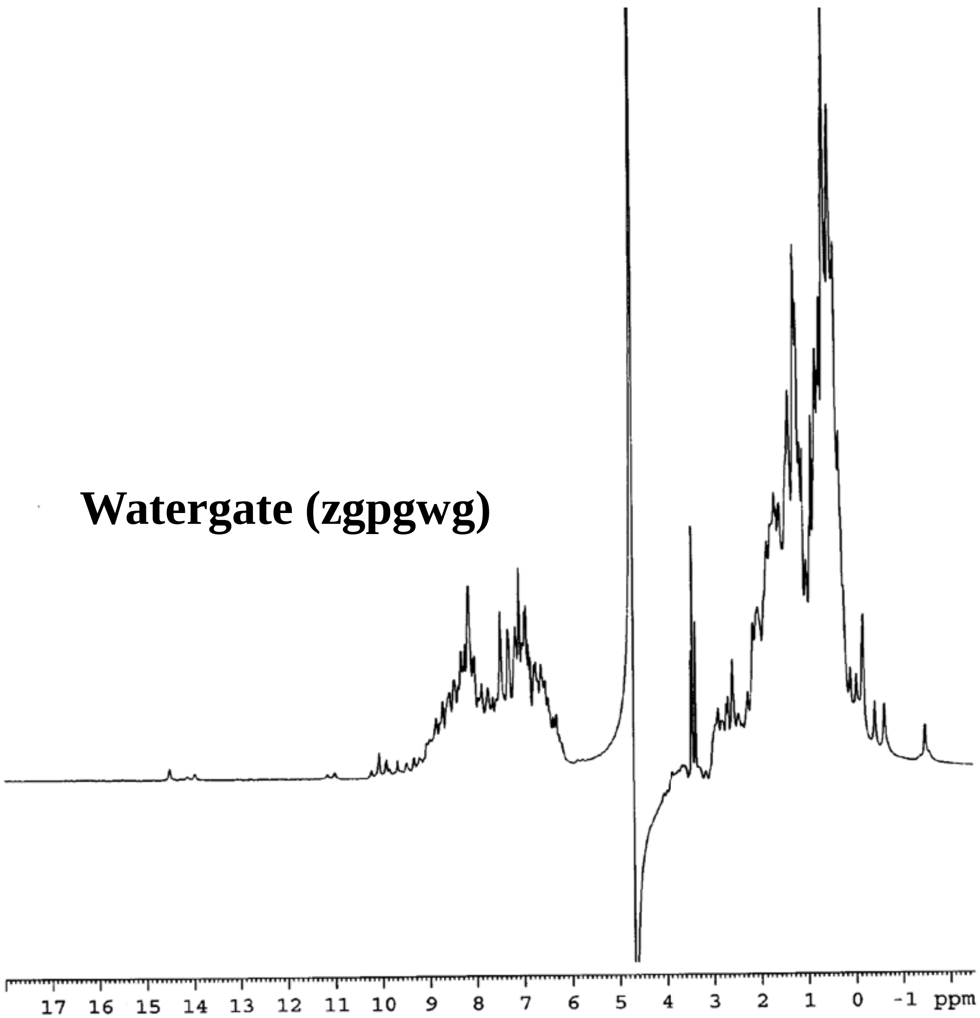
- Parameter adjustment, Pulprog=zgpgwg
- **p11: pulse length for 90 degree shaped pulse**
- **sp1: power level for 90 degree shaped pulse**
- **spnam1: name of shaped pulse**

For example: set spnam1=Sinc1.1000, p11=1 msec,

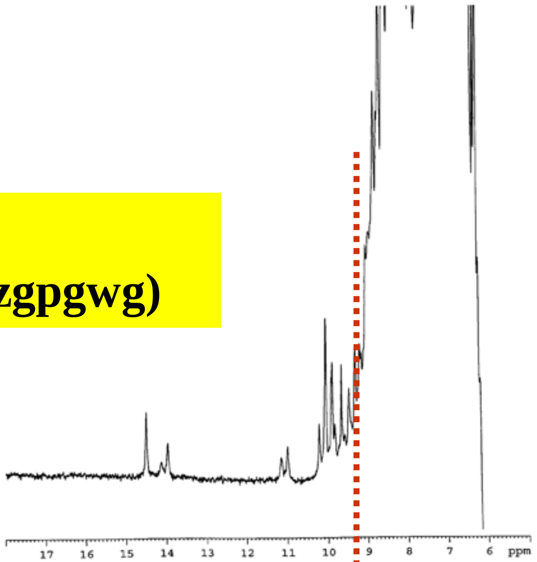
Minimize the water fid by adjusting sp1 in the “gs” utility.

WATERGATE V.S. Presaturation

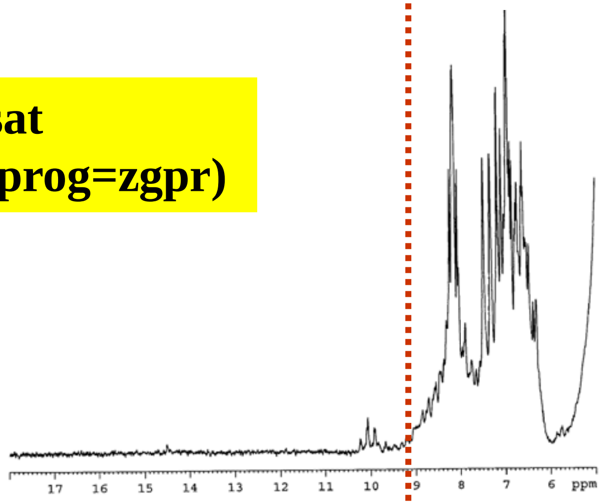
Watergate (zgpgwg)



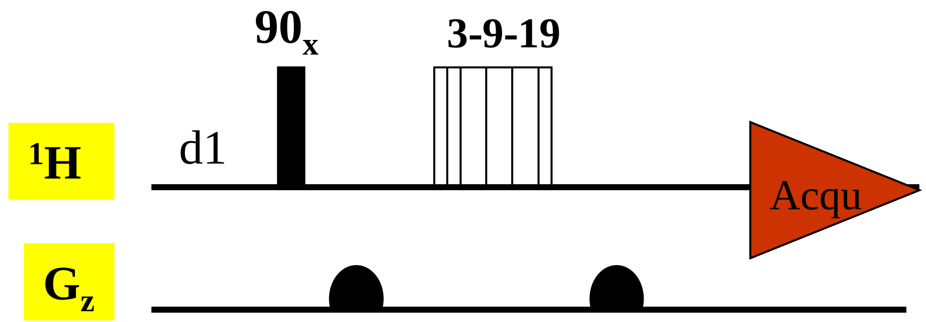
Watergate (pulprog=zgpgwg)



Presat (pulprog=zgpr)

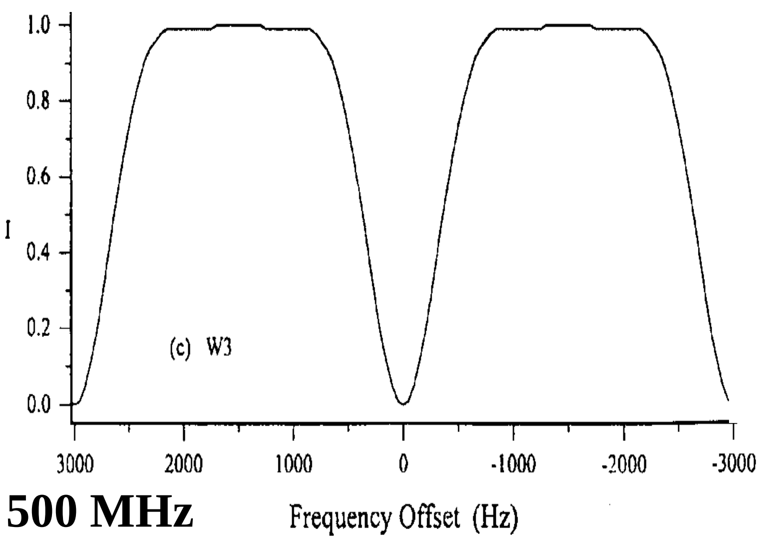


3-9-19 WATERGATE



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".)**



500 MHz

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

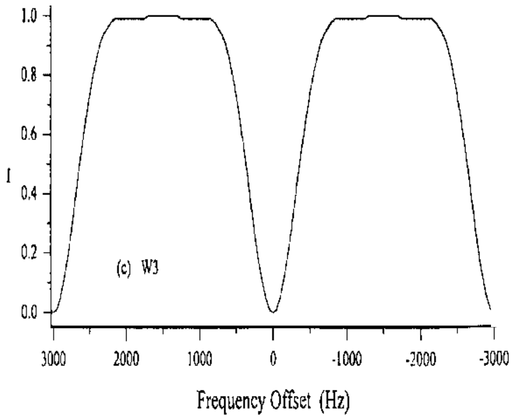
Delay $\tau = 1/(4\ \Delta\upsilon_{max})$, where $2\ \Delta\upsilon_{max}$= distance of next null (Hz).
(The delay τ is field-dependent !!)

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

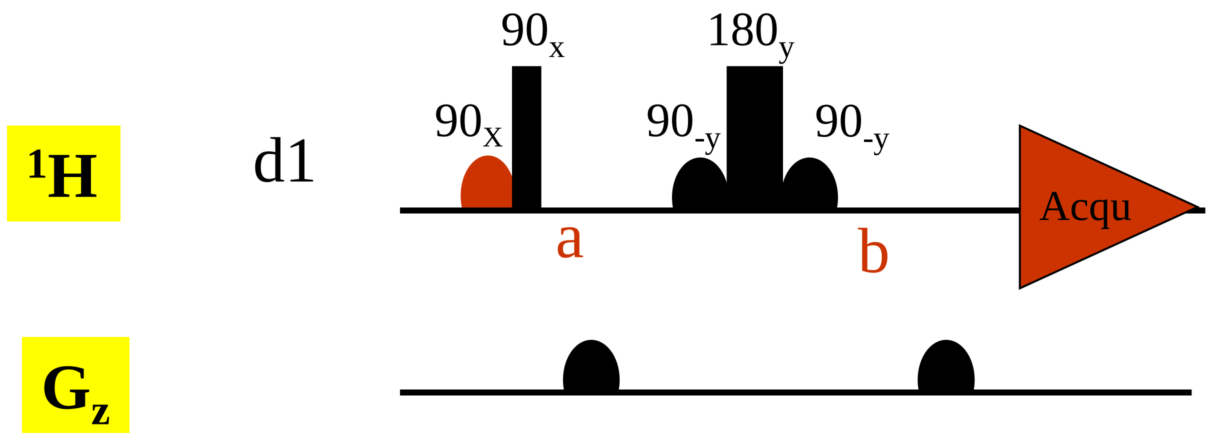
τ=1/[4*(8.2-4.75)*600.13]=121 usec @600 MHz machine
τ=1/[4*(8.2-4.75)*500.13]=145 usec @500 MHz machine

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.

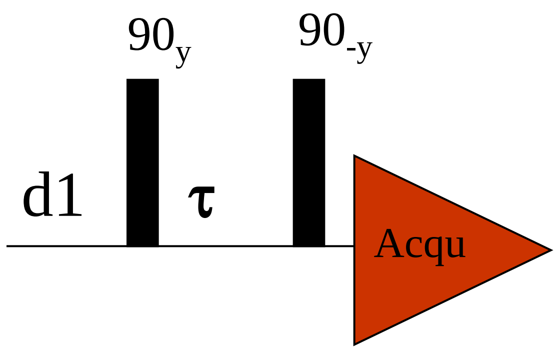
Pulse Sequence for Observing Fast-Exchanging Protons

N-H + $^{*}H_2O$ ⇄ N-*H + *H-O-H

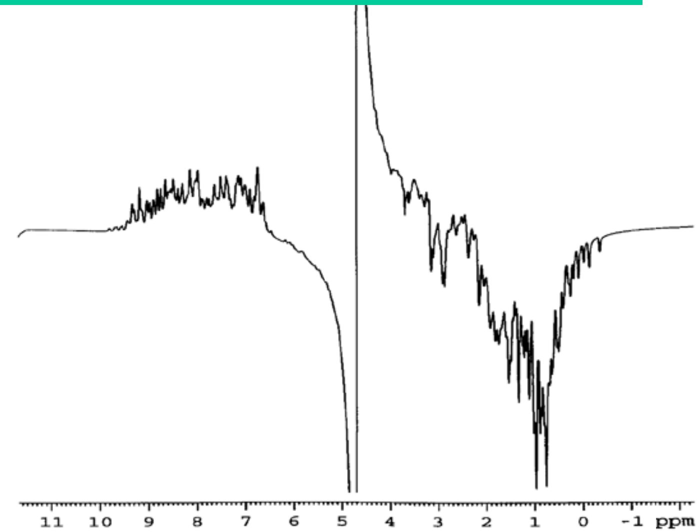
Imino protons in DNA, hydroxyl protons (-OH), Histidine side chain protons in proteins are usually in a fast exchange process with water.

- **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(**$\Omega\tau$**)**
- **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,**

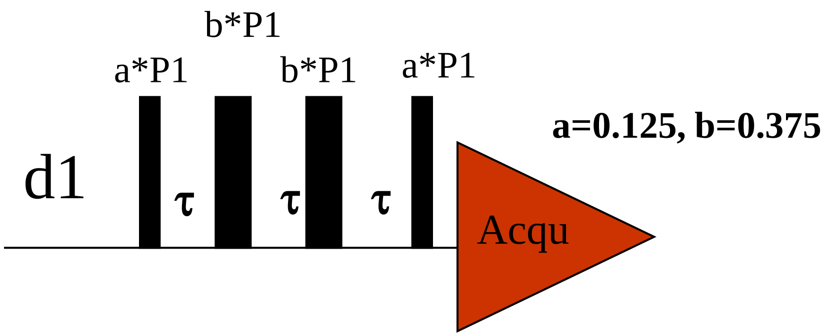
  τ**=1/[4*(14-4.75)*600.13]=45 usec**

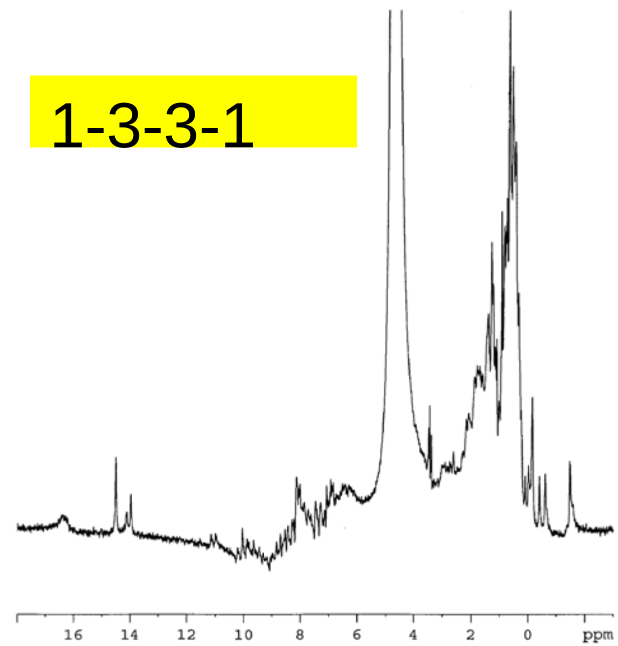
Parameter adjustment: Pulprog=p11

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





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

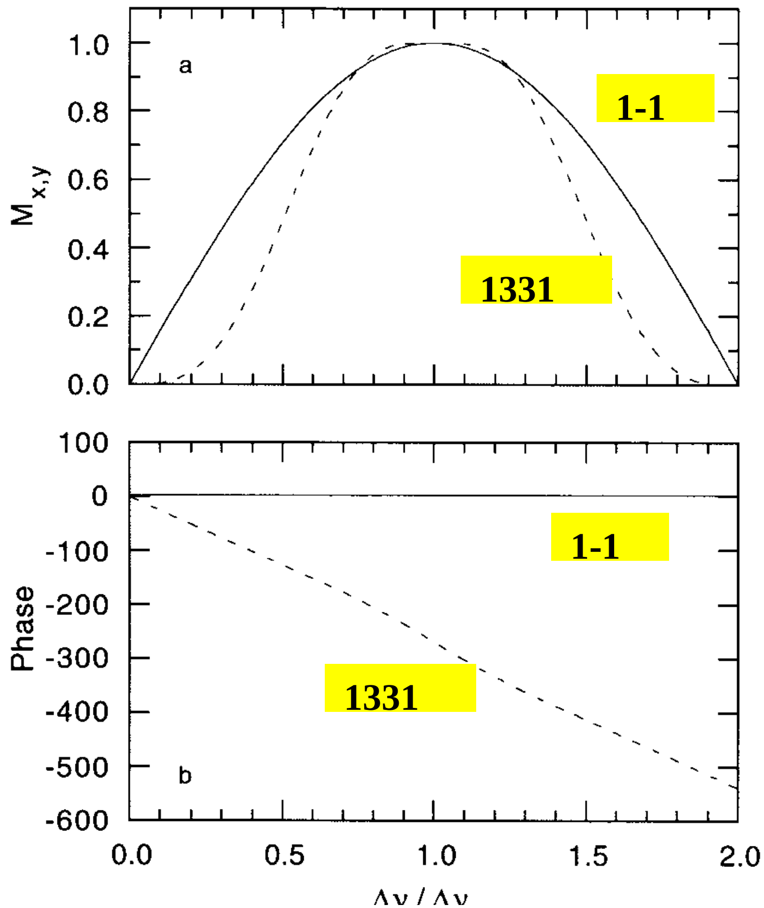
•Delay τ = 1/(2$\Delta\upsilon_{max}$)=1/d,
$\Delta\upsilon_{max}$=distance of maximal intensity
d=distance of next null

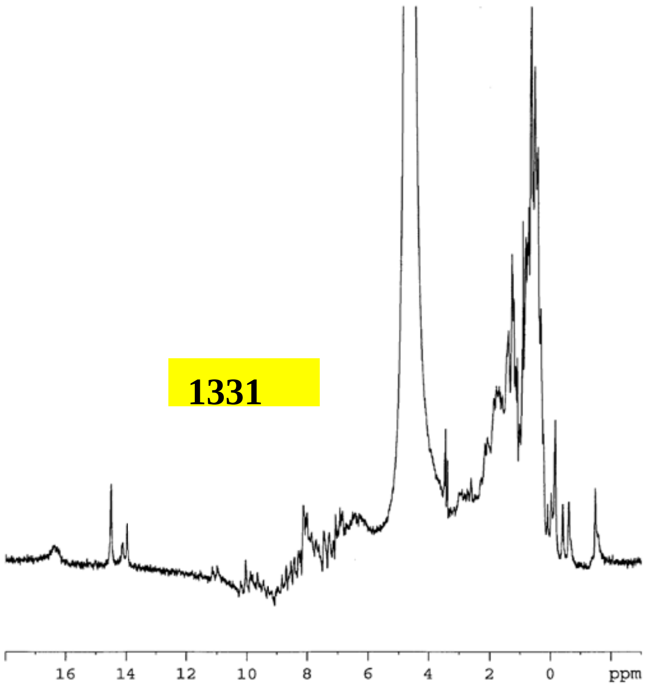
•For example: To observe a peak at 14 ppm at 600 MHz,
τ=1/[2*(14-4.75)*600.13]=90 usec

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.

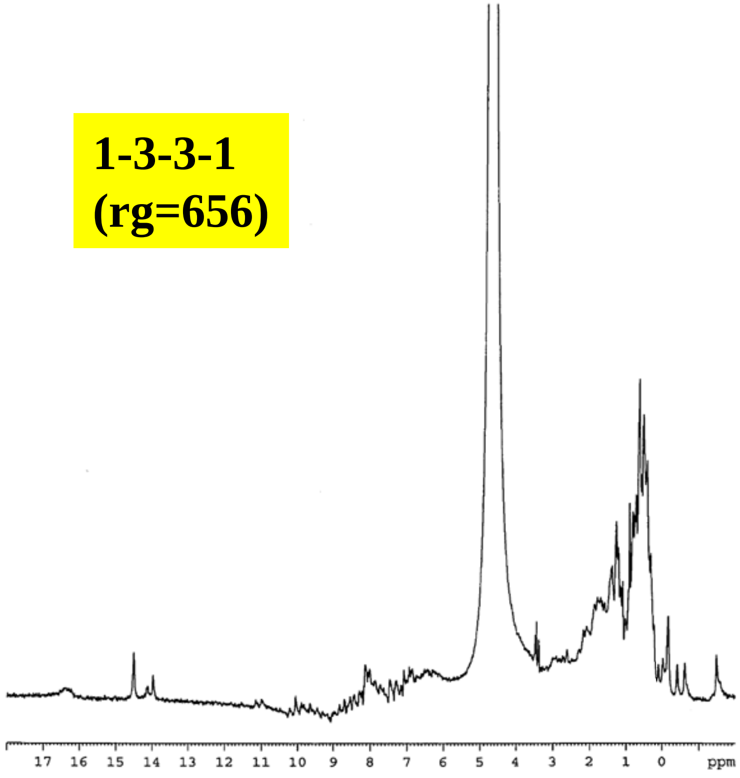
John Cavanagh et al., page 154, "Protein NMR Spectroscopy: Principles and Practice", Academic Press (1995)

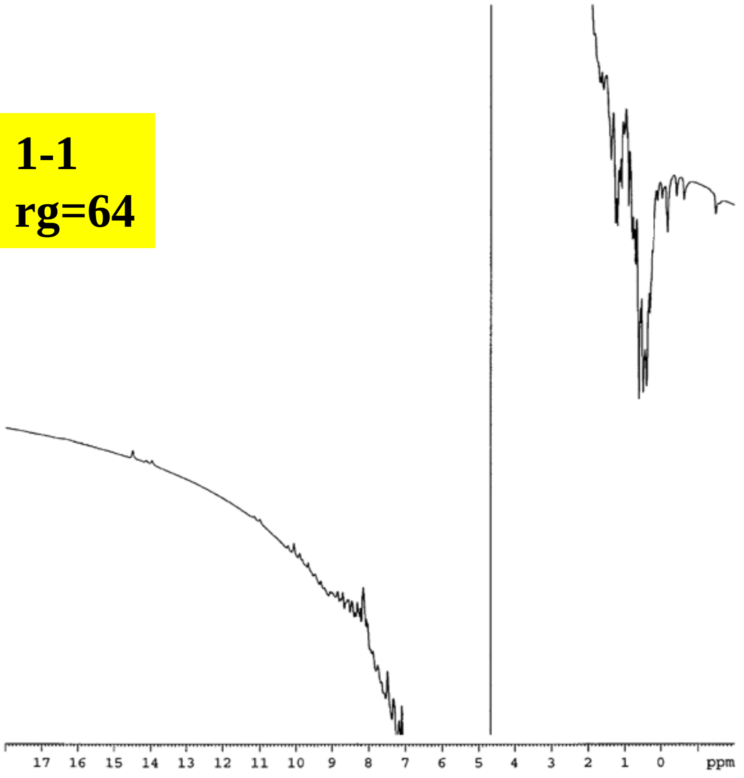
Jump-Return 1-1 and Binominal 1-3-3-1

Both are for observing fast exchanging protons.

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

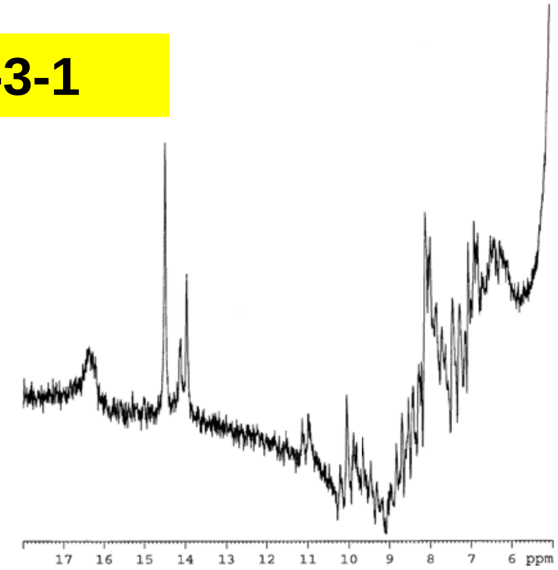
- **1-1: low receiver gain, the dispersive tail of water interferes with the signals of interest.**



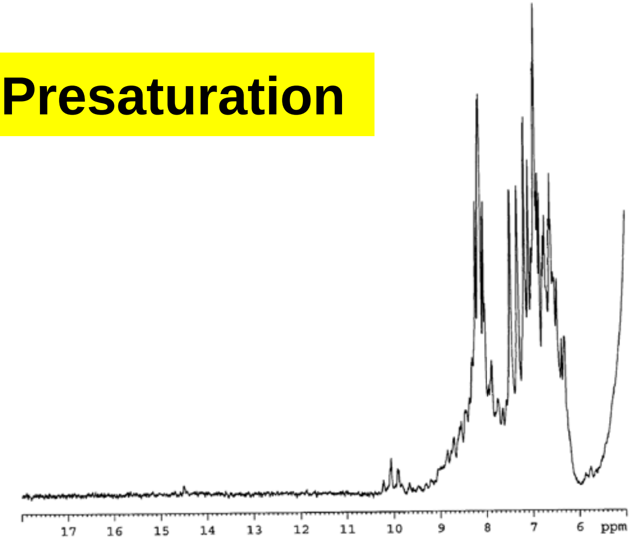


What are you trying to detect ?

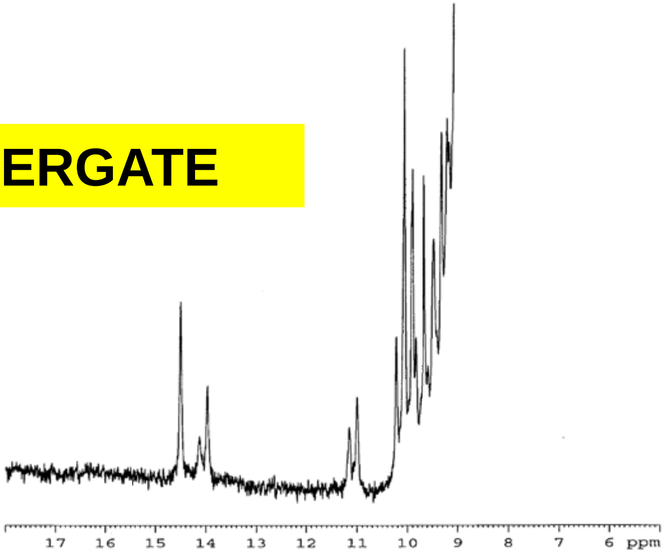
1-3-3-1



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

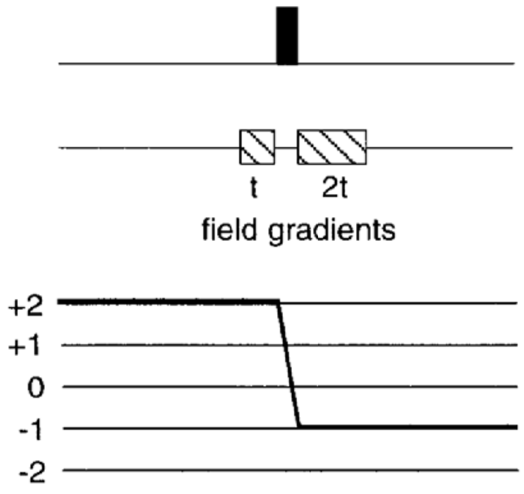


Figure from John Cavanagh et al.,"Protein NMR Spectroscopy: Principles and Practice", Academic Press (1995)

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.