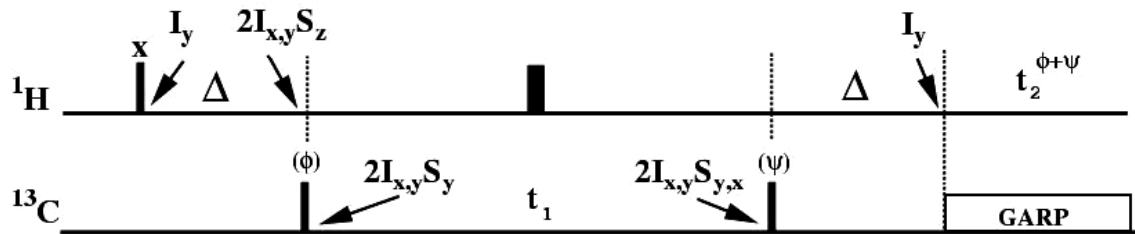


## Basic heteronuclear correlations: HMQC

HMQC: heteronuclear multiple quantum correlation



$$\begin{aligned}\phi &= X - X \\ \psi &= X \quad X - X - X \\ \phi_{\text{rec}} &= X - X - X \quad X\end{aligned}$$

$\delta(^1\text{H})$ : refocused

$\delta(^{13}\text{C})$ : evolution during  $t_1$

$J(^1\text{H}, ^{13}\text{C})$ : active during  $\Delta$

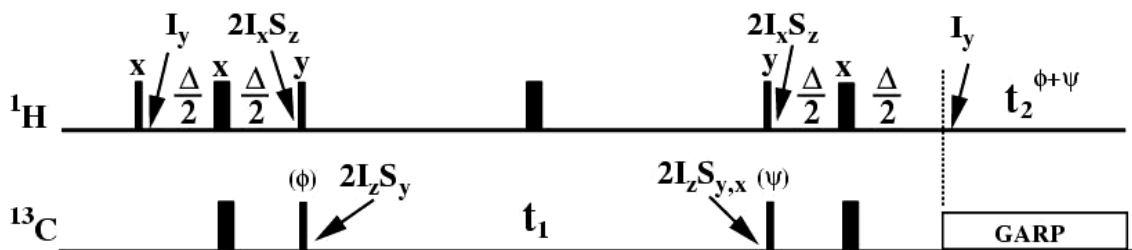
$J(\text{H}, \text{H})$ : active !

$J(\text{C}, \text{C})$ : active !

Relaxation during  $t_1$ : multiple quantum line-narrowing

## Basic heteronuclear correlations: HSQC

HSQC: heteronuclear single quantum correlation



$$\begin{aligned}\phi &= X - X \\ \psi &= X \quad X - X - X \\ \phi_{\text{rec}} &= X - X - X \quad X\end{aligned}$$

$\delta(^1\text{H})$ : refocused

$\delta(^{13}\text{C})$ : evolution during  $t_1$

$J(^1\text{H}, ^{13}\text{C})$ : active during  $\Delta$

$J(\text{H}, \text{H})$ : not active

$J(\text{C}, \text{C})$ : active !

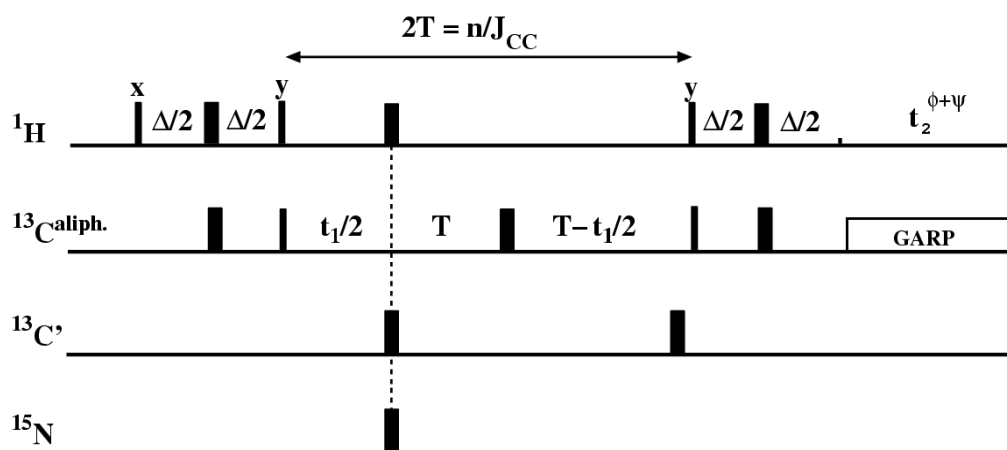
Relaxation during  $t_1$ :  $T_1(^1\text{H})$ ,  $T_2(^{13}\text{C})$

## Constant-time HSQC

→ Set  $2T = n/J_{CC}$  to refocus evolution of homonuclear C,C couplings during  $2T$

→ J-coupling evolution:  $\cos(\pi J_{CC} 2T)^n = -1^n$

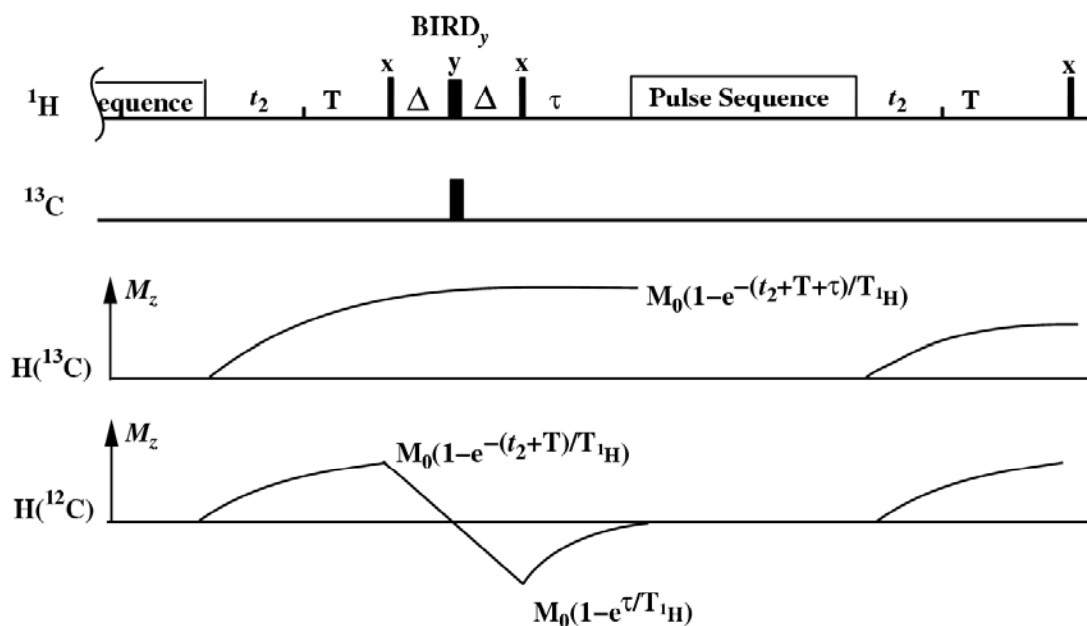
### CT-HSQC



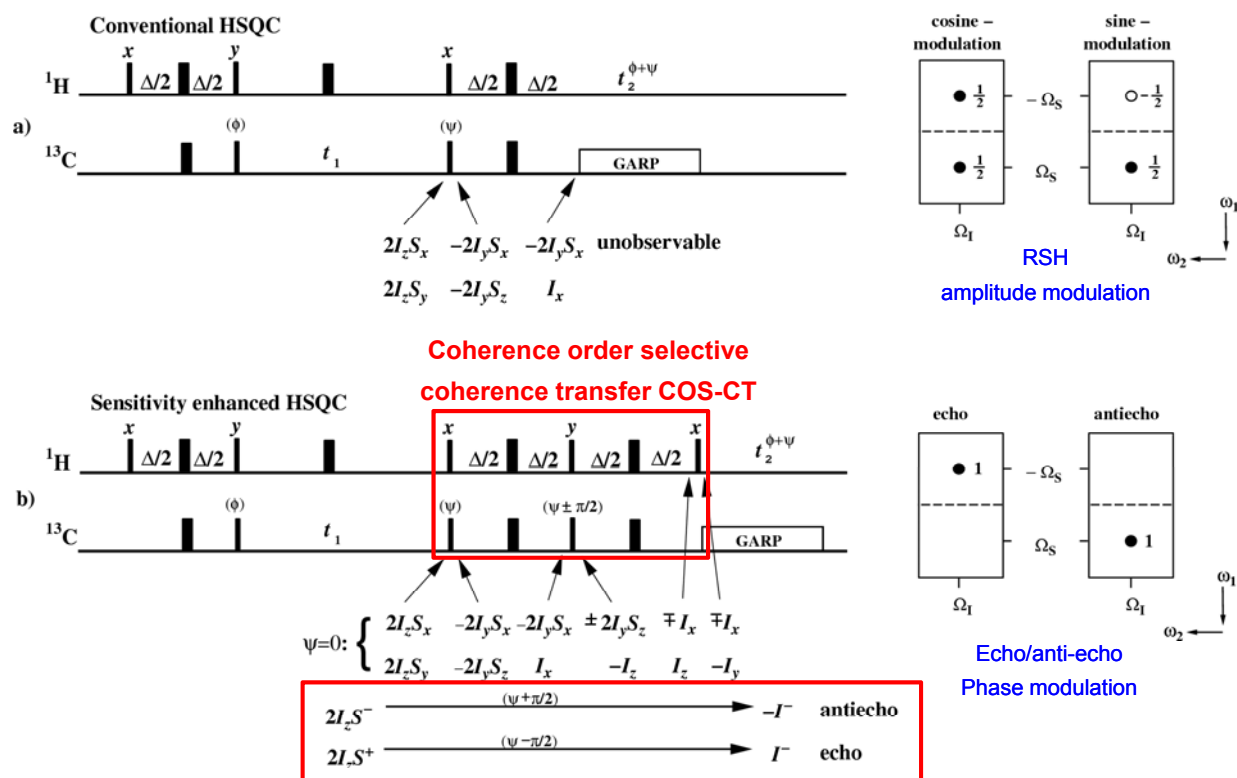
## BIRD filter to suppress $^{12}\text{C}$ magnetization

Excellent  $^{12}\text{C}$  suppression and fast acquisition (small, unlabeled molecules!)

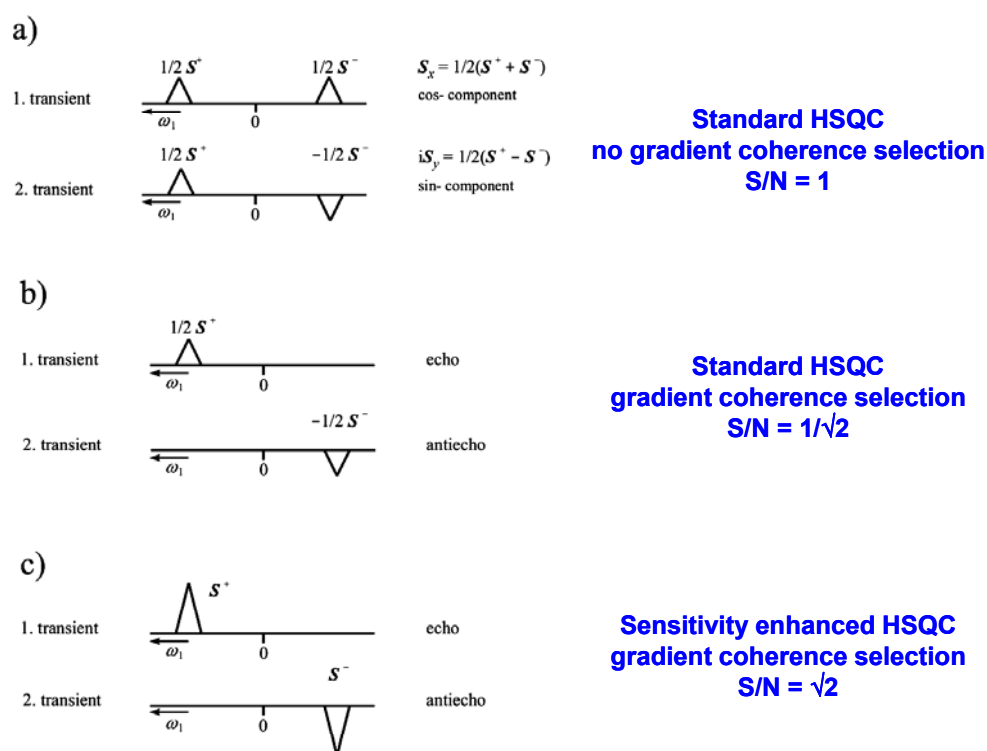
Suppression of non- $^{13}\text{C}$  bound protons with BIRD<sub>y</sub>



## Sensitivity enhancement



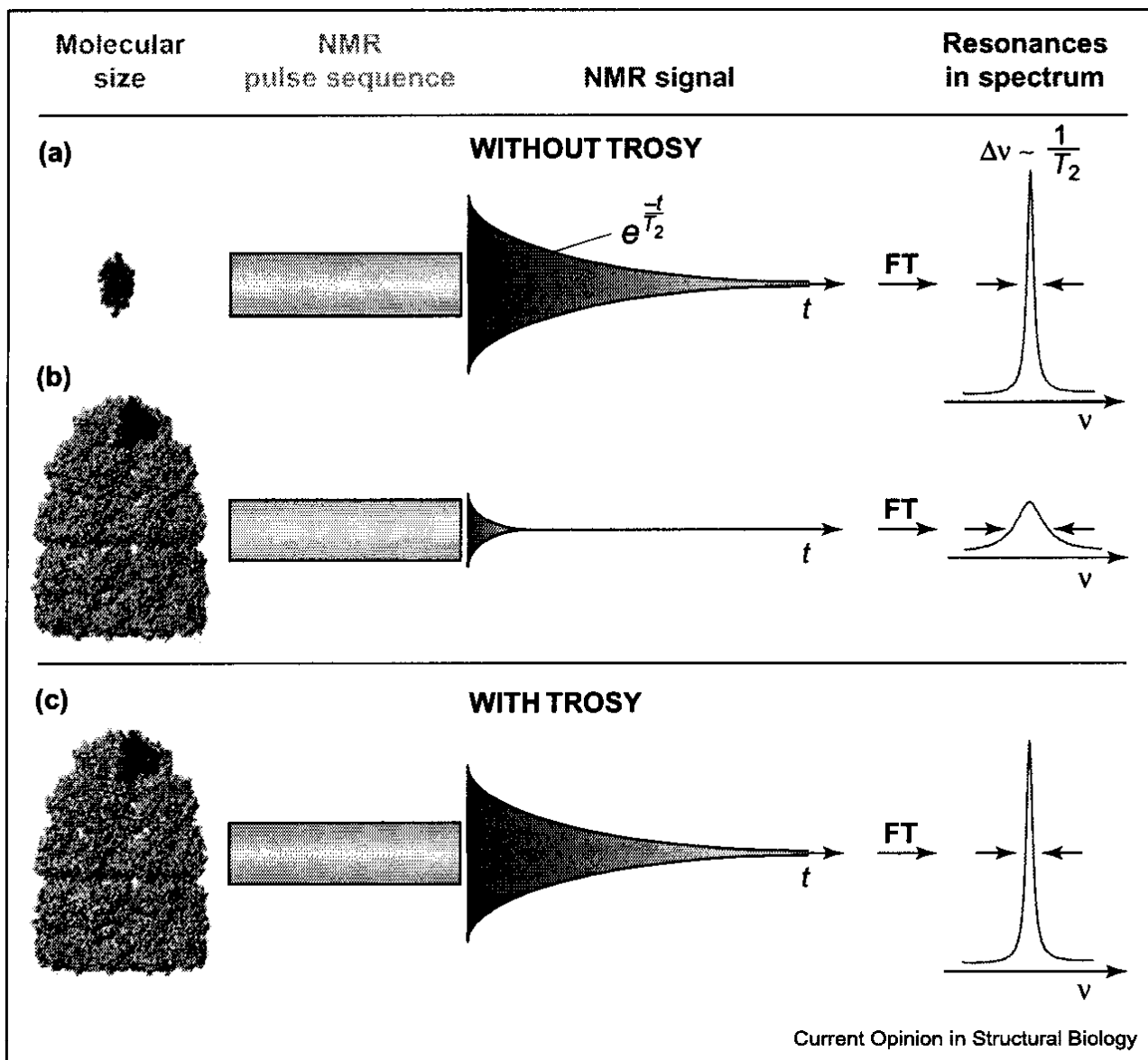
## Sensitivity enhancement / gradient coherence selection



$$S/N =: I/\sqrt{n} \quad (I: \Sigma(\text{intensities}), n: \# \text{ signals})$$

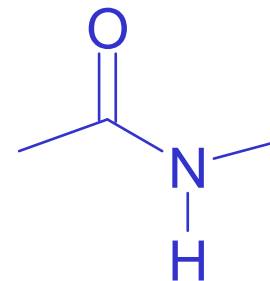
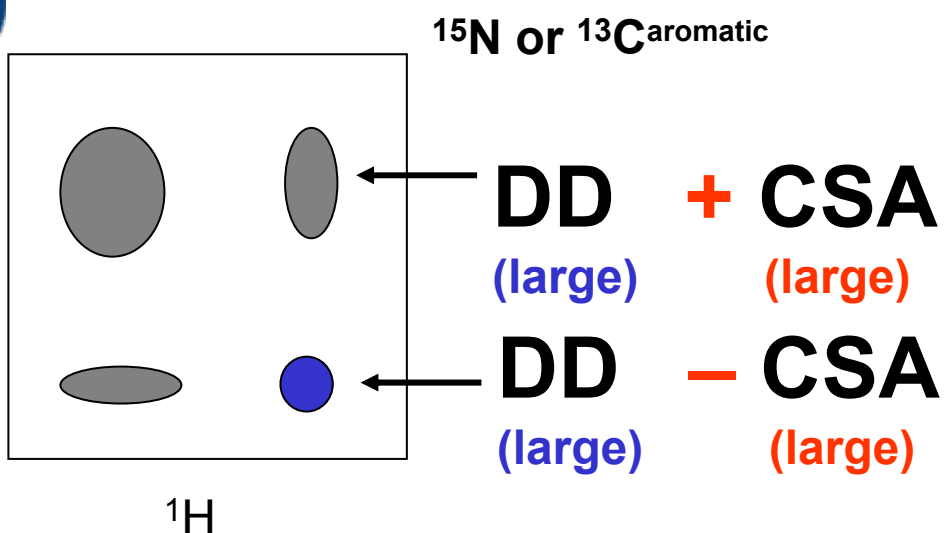


# Impact of TROSY on Solution NMR

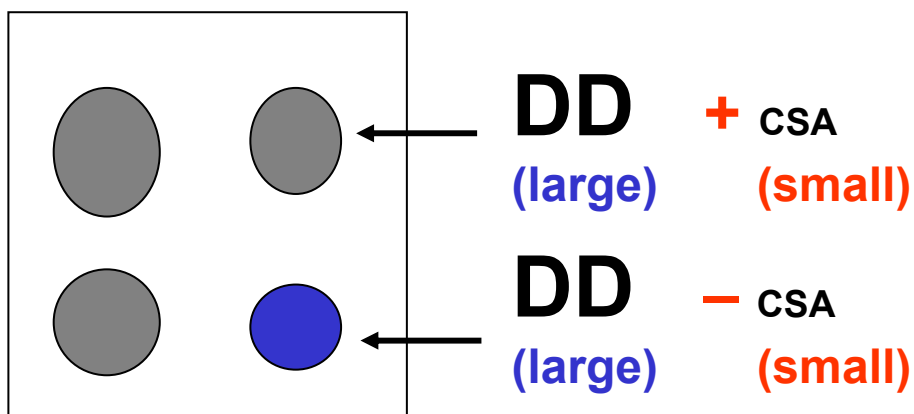




# Interference of Relaxation between DD and CSA Relaxation



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



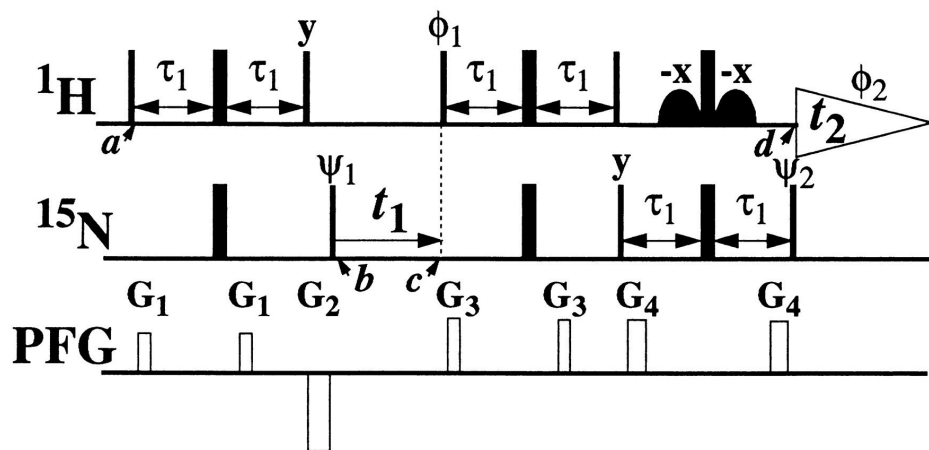
(B) At Low Magnetic Field  
(almost no TROSY line-narrowing effect)

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



# TROSY-HSQC and Conventional HSQC

## TROSY-HSQC



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

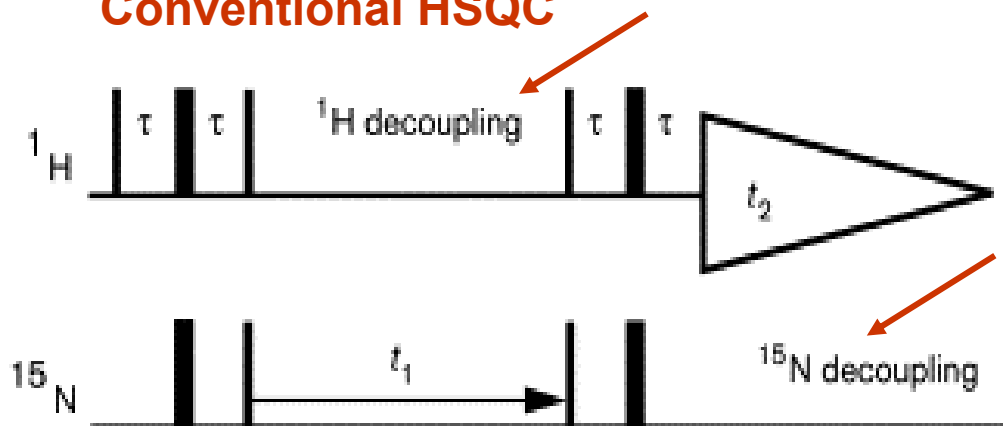
## TROSY-HSQC

(1) No  $^1\text{H}$  decoupling during  $^{15}\text{N}$  evolution.

(2) No  $^{15}\text{N}$  decoupling during  $^1\text{H}$  acquisition.

(3) Use the TROSY-HSQC pulse sequence to selectively observe the most slowly relaxing component.

## Conventional HSQC



Current Opinion in Structural Biology

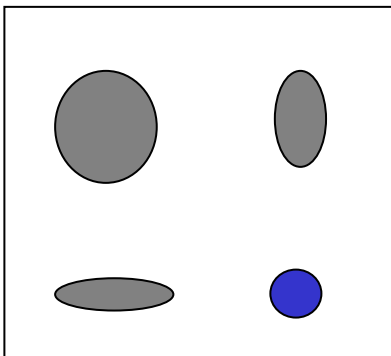
## HSQC

In both  $^1\text{H}$  and  $^{15}\text{N}$  dimensions, both J-split components are mixed by hetero-nuclear decoupling. This collapses each  $^{15}\text{N}$  and  $^1\text{H}$  doublet into a single peak in each dimension.

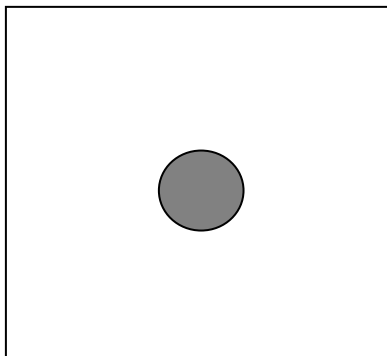


# Transverse Relaxation-Optimized Spectroscopy (TROSY)

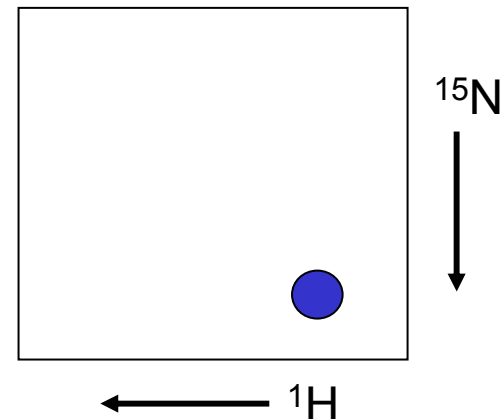
(a). None-decoupled HSQC



(b). Decoupled HSQC



(c). TROSY-HSQC



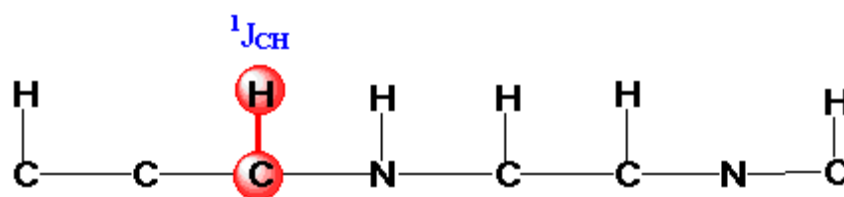
1. **Main relaxation source for  $^1\text{H}$  and  $^{15}\text{N}$ :** dipole-dipole (DD) coupling and, at high magnetic fields, chemical shift anisotropy (CSA).
2. **Different relaxation rates (line width)** for each of the four components of  $^{15}\text{N}$ - $^1\text{H}$  correlation.
3. **The narrowest 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).**

## 2D Experiments

### 2D HSQC

#### DESCRIPTION

The **2D HSQC (Heteronuclear Single-Quantum Correlation) experiment** permits to obtain a 2D heteronuclear chemical shift correlation map between directly-bonded  $^1\text{H}$  and X-heteronuclei (commonly,  $^{13}\text{C}$  and  $^{15}\text{N}$ ). It is widely used because it is based on proton-detection, offering high sensitivity when compared with the conventional carbon-detected [2D HETCOR experiment](#). Similar results are obtained using the [2D HMQC experiment](#).



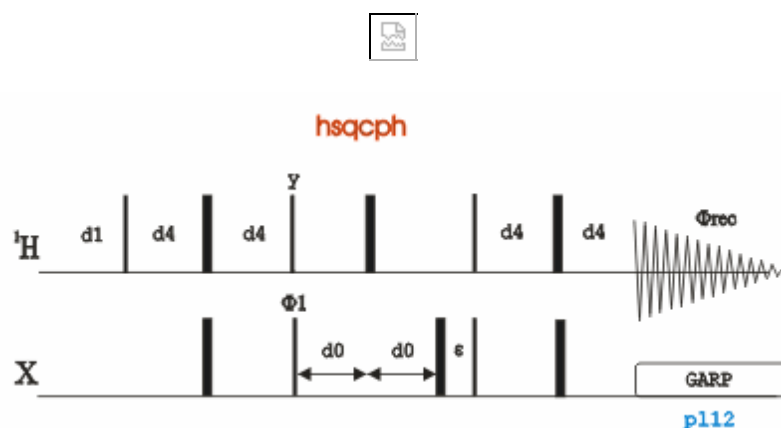
#### REQUIREMENTS

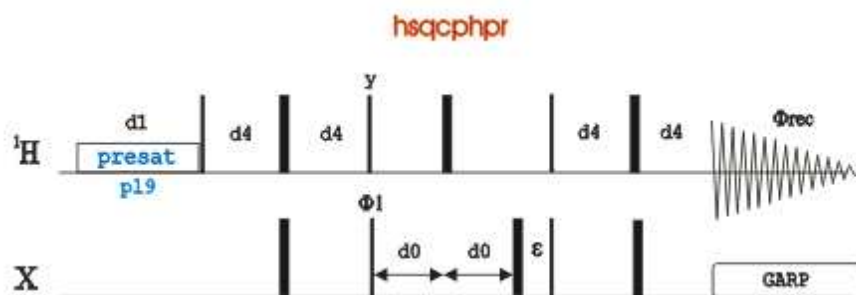
Easy implementation on any AVANCE spectrometer equipped with an inverse probehead

#### VERSIONS

The basic 2D HSQC pulse sequence consists of four independent parts ( [80CPL185](#) and [86JMR546-69](#) ):

1. An **initial INEPT pulse train** transfers polarization from  $^1\text{H}$  to X via  $1J(\text{XH})$  (see [INEPT block](#)).
2. The antiphase  $^{13}\text{C}$  magnetization evolves during the **variable evolution t1 period** under the effect of X chemical shift. Heteronuclear  $^1\text{H}$ -X couplings are refocused by applying a  $180^\circ$   $^1\text{H}$  pulse at the middle of this period (see [1H-decoupled X evolution block](#)).
3. A **retro-INEPT pulse train** converts X magnetization to in-phase  $^1\text{H}$  magnetization (see [reverse-INEPT block](#)).
4. **Proton acquisition** is performed with X decoupling (see [1H-acquisition with X-decoupling](#)).





A number of alternatives have been proposed:

- Use of spin-lock pulses for purging undesired coherences ( [88JMR569-76](#) and [89JMR608-85](#) and [91JMR394-94](#) ).
- Improved sensitivity can be obtained using the PEP methodology ( [91JMR151-93](#) and [93AR1](#) ). A second retro-INEPT pulse train is inserted prior to acquisition in order to select orthogonal components evolving during the t1 period.
- Multiplicity-editing by inserting an heteronuclear spin-echo during the t1 period ( [90JMR589-90](#) and [91JMR665-91](#) ).
- The decoupled HSQC (or Double INEPT) experiment in which the initial INEPT and the last retro-INEPT pulse trains are replaced by refocused INEPT pulse trains in order to achieve in-phase carbon magnetization during the t1 period ( [90JMR304-86](#) and [90JMR488-87](#) ).
- [Constant-Time HSQC experiment](#) to remove <sup>13</sup>C-<sup>13</sup>C scalar coupling during the t1 period.
- Gradient-enhanced versions (see [ge-2D HSQC experiment](#)).
- Removing axial peaks artifacts by modified phase cycling ( [94JMRA246-109](#) and [96JMRB91-110](#) ).
- Semi-selective versions to achieve better resolution in the indirect dimension (see [Semi-selective HSQC experiment](#))

## EXPERIMENTAL DETAILS

The 2D HSQC experiment can be recorded in routine/automation modes. Minor changes are required if a predefined parameter set is available. The interpulse d2 delay is optimized to 1/2\*JCH (3.3-3.8 ms).

More details on practical implementation of ge-2D HSQC experiments on AVANCE spectrometers can be found in the corresponding tutorials:

- [2D HSQC experiment](#) (phase-sensitive)
- [2D HSQC experiment](#) (phase-sensitive with presaturation)

## SPECTRA

The HSQC spectrum correlates chemical shifts of heteronucleus X (F1 dimension) and protons (F2 dimension) via the direct heteronuclear coupling 1J(XH). Carbon decoupling is usually performed during proton acquisition and the corresponding satellites collapse to a single resonance showing all proton-proton couplings.

See spectra

NMRSIM: 2D HSQC

## RELATED TOPICS

Comparison of different 2D inverse correlations have been carried out ( [90JMR304-86](#) and [90JMR488-87](#) ).

Related sequences:

- 2D HSMQC experiment ( [90JMR346-86](#) ).
- 2D Double DEPT sequence ( [89METH134](#) and [91JMR151-93](#) ).
- [2D Inverse experiments](#)
- [2D Inverse gradient-enhanced experiments](#)

More about HSQC ....

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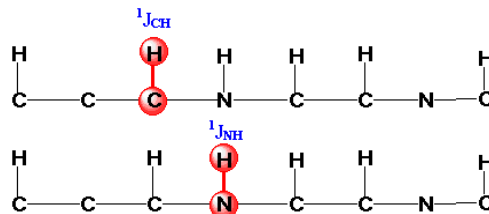
BACK

## 2D Experiments

### ge-2D HSQC

#### DESCRIPTION

The **ge-2D HSQC experiment** is the gradient-enhanced version of the conventional [2D HSQC experiment](#) in which coherence selection is achieved by means of PFG. Thus, clean 2D HSQC spectra can be recorded in a single scan per  $t_1$  increment without need for phase cycle when sample concentration is high. Other advantages are the optimal dynamic range, improved water and artefact suppression, and reduced  $t_1$  noise in the minimally required experiment time. The HSQC experiment allows to trace out directly bonded  $^1\text{H}$ -X pairs via the large  $^1J_{\text{HX}}$  coupling constant



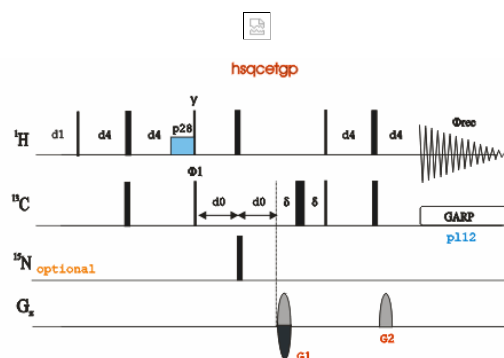
#### REQUIREMENTS

Easy implementation on any AVANCE spectrometer equipped with pulsed field gradients (PFGs) and inverse probehead.

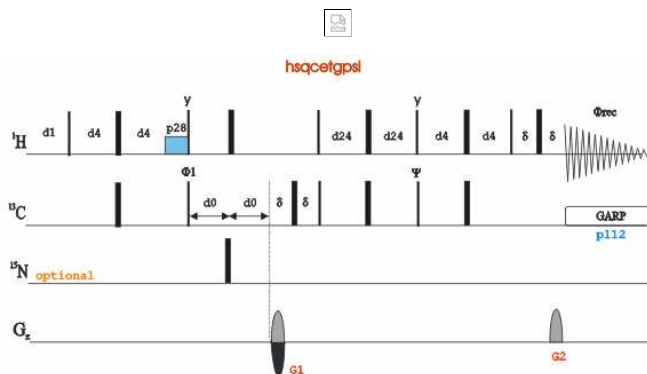
#### VERSIONS

In addition of the [advantages to use PFGs](#), an important aspect when PFG are incorporated in the HSQC pulse sequence, are the *sensitivity* and *resolution* requirements. The effect on the sensitivity of several gradient-based HSQC and other related schemes have been extensively discussed ([95JB11-6](#), [95JMRB235-108](#), [94JMRA70-111](#), [96JMRA64-122](#), [94JB301](#), [93ANG1489](#), and [96JMRA17-1191](#)). It is possible, for instance, to design six different basic versions of the 2D  $^1\text{H}$ -X HSQC experiments using PFGs. The use of each version will depend on the sample under study.

- The dephasing gradient  $G_1$  is applied into the variable  $t_1$  period, obtaining a magnitude mode spectrum ([91JACS9688](#) and [92JMR282-100](#)). This is an excellent option for routine samples in which sensitivity and resolution are not critical. In this case, two gradients with a 4:1 ratio select N-type data (shaded line corresponding to a  $p_1=+1$ ) whereas a 4:-1 ratio would select P-type data (continuous line corresponding to a  $p_1=-1$ ).
- The echo-antiecho version of this experiment uses the same sequence, but the intensity of the refocusing gradient  $G_2$  is inverted on alternated scans to obtain the N- and P-type data separately ([92JMR207-98](#) and [92JMR660-98](#)). After proper processing, this approach allows for obtain phase-sensitive spectra but with sensitivity losses by a factor of square(2) with respect to the classical phase-cycled experiment.



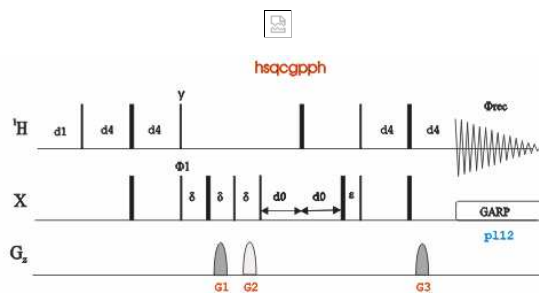
- Use of the PEP methodology ([91JMR429-91](#), [91JMR151-93](#), and [93AR1](#)). This is the best option to record phase-sensitive 2D HSQC spectra with maximum sensitivity ([92JACS10663](#)). The selection procedure use the same principles described for the echo-antiecho approach, but the pulse sequence must be modified adding a second retro-INEPT block in order to select both orthogonal components of the magnetization ( $I_{S_x}$  and  $I_{S_y}$ ) present during  $t_1$ . This basic scheme is widely applied to improve the sensitivity in other related multidimensional experiments ([95JB11-6](#), [95JMRB235-108](#), [94JB301](#), [93ANG1489](#), and [96JMRA171-119](#)).



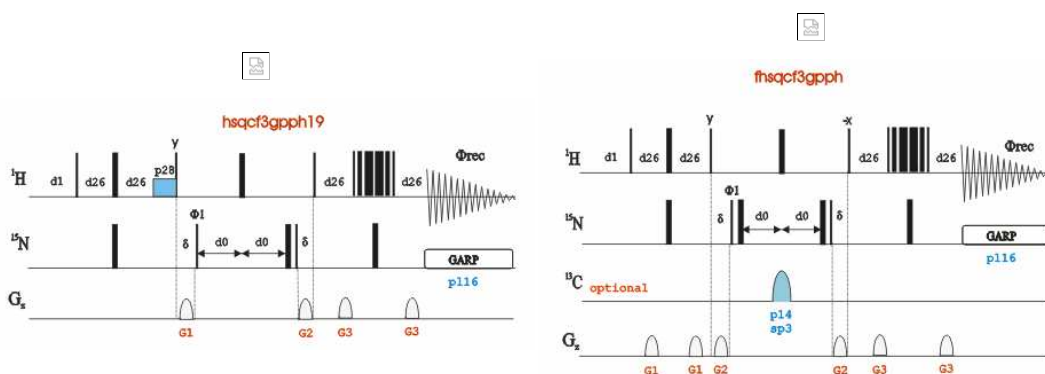
In triple resonance experiments applied to labeled proteins, sensitivity can be further enhanced by simultaneous acquisition ([95JB97](#)). Some examples of modified PEP-HSQC experiments have been described to measure accurate  $^1\text{JNH}$  ([96JMRB245-112](#) and [96JMRB269-112](#)), measure a set of relaxation parameters in  $^{15}\text{N}$ - $^1\text{H}$  spin systems ([96JMRB245-112-111](#) and [96JMRB269-101](#)) or to observe exchange broadened signals ([96JB223](#)).

- A X filter-z (consisting of  $90^\circ(\text{X})$ -PFG- $90^\circ(\text{X})$ ) is applied between the  $t_1$  period and the dephasing  $G_1$  gradient ([93MRC287](#)). A phase-sensitive spectrum is obtained using the classical acquisition and processing procedures (for instance, TPPI), but theoretical sensitivity loss by a factor of two is obtained compared to the phase-cycled experiment. However, this loss can be partially recovered applying the PEP methodology (a second retro-INEPT block shifted  $90^\circ$  in phase) without the need to apply the echo-antiecho approach (

[95JMRB285-108](#) ).

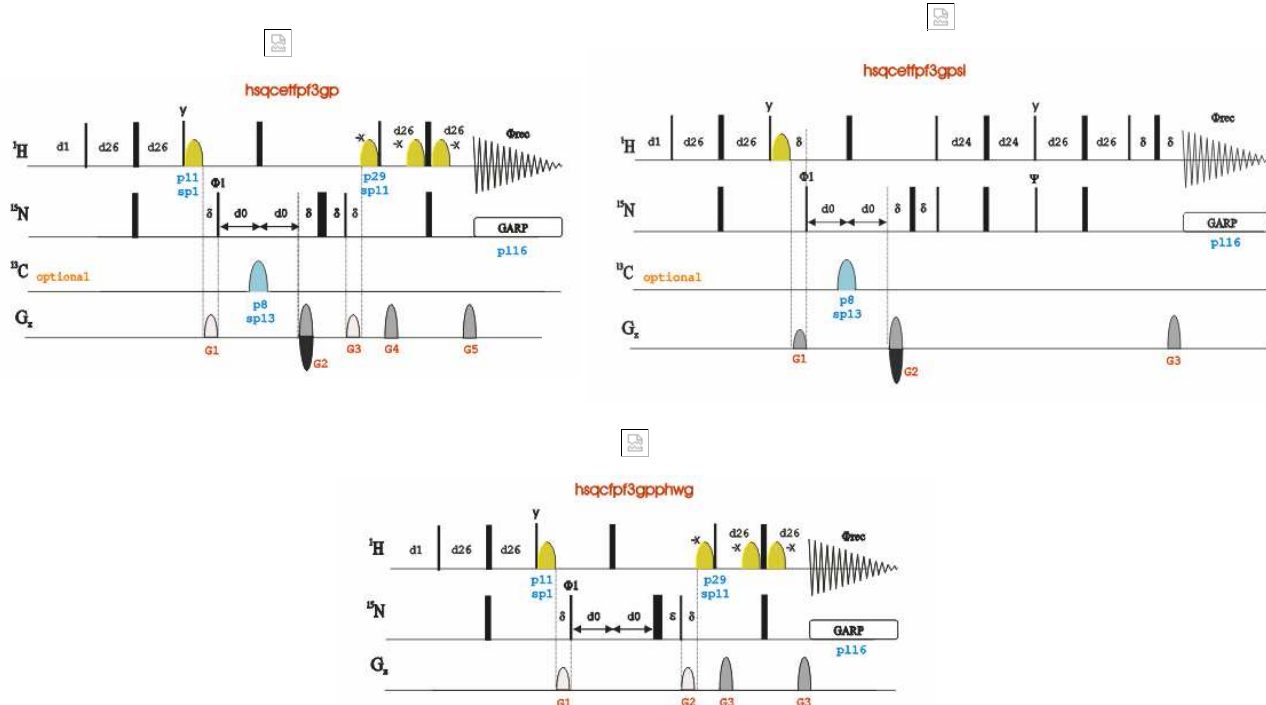


- Use of PFG as purge elements in the original phase cycle sequence. These PFG are placed between the simultaneous  $90^\circ$  pulses of  $^1\text{H}$  and X in order to select the corresponding  $I_z S_z$  magnetization ( [93JMRA113-101](#) and [93JMRB239-102](#) ). Although this is not a pure selection procedure, this approach reduces the number of phase cycle steps and minimizes the suppression artefacts without affecting the overall sensitivity with comparison to the phase cycled analog.
- Much attention has been given to the effect of water suppression on the signal intensity of relatively rapidly exchanging protons, as the NH protons in proteins. As a general approach, the WATERGATE block ( [92JB661](#) and [93JMRA241-102](#) ) is usually applied during the retro-INEPT block of the standard HSQC pulse sequence in order to improve solvent suppression in aqueous samples.



WATERGATE can also be combined with the water flip-back approach ( [93JACS12593](#) ) which ensures that the water magnetization is little perturbed and oriented along the +z axis during most of the experiment, especially just prior to acquisition, to minimize the saturation of water. This is commonly applied to proteins and nucleic acids dissolved in  $\text{H}_2\text{O}$ .

Other related approaches have been proposed to avoid the saturation transfer from water in HSQC experiments ( [93JMRB315-101](#) , [94JMRA178-107](#) , [94JMRB45-105](#) , [95JMRB94-108](#) , and [96JACS5510](#) ).



Other related versions:

- Simultaneous acquisition of  $^1\text{H}$ - $^{13}\text{C}$  and  $^1\text{H}$ - $^{15}\text{N}$  2D HSQC spectra can be obtained using the [Time-Shared method](#)
- Incorporation of an X-filter prior to acquisition to remove ABX strong coupling signals ( [99JMR89-138](#) )
- Improved resolution in the  $F_1$  dimension can be achieved by applying a band-selective carbon pulse (see [Semi-selective HSQC experiment](#)). This approach can be applied in any version of the mentioned HSQC experiment.

## EXPERIMENTAL DETAILS

The ge-2D HSQC experiment is usually recorded in routine and automation modes. Minor changes are required if a predefined parameter set is available. The user must define the strength, the duration, and the shape of the gradients and the recovery delay.

More details on practical implementation of ge-2D HSQC experiments on AVANCE spectrometers can be found in the corresponding tutorials:

- [Tutorials: 2D inverse experiments](#)
- [Tutorials: 2D gradient-based inverse experiments](#)

## SPECTRA

The HSQC spectrum correlates chemical shifts of heteronucleus X ( $F_2$  dimension) and protons ( $F_1$  dimension) via the direct heteronuclear coupling  $^1J(XH)$ . The effective suppression of unwanted  $^1H$ - $^{12}C$  or  $^1H$ - $^{14}N$  magnetization by means of PFGs allows to obtain ultra-clean 2D spectra from which clear analysis can be done.

[See Some Examples](#)

## RELATED TOPICS

Related experiments:

- [2D Inverse experiments](#)
- [2D Inverse gradient-enhanced experiments](#)

[More about 2D HSQC ....](#)

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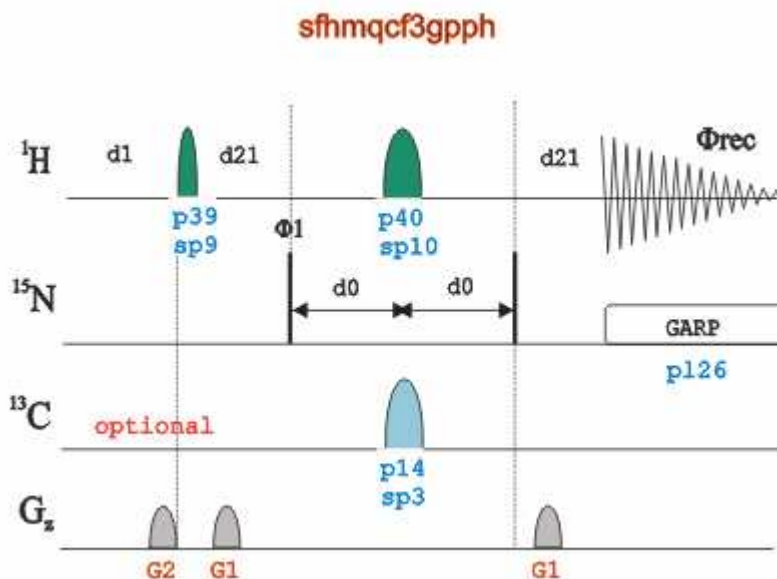
Previous pp:[seqtrhncagp3d](#)Next pp:[shmbcgpdqf](#)

# Pulse Programs

NMRSIM Pulse Diagram

Relevant parameters: [ased](#)More info on [sfhmqcf3gpqh](#)

## Pulse Diagram



## Pulse Program

```

;sfhmqcf3gpqh
;avance-version (09/10/26)
;SOFAS HMQC
;2D H-1/X correlation via heteronuclear zero and double quantum
; coherence
;phase sensitive
;with decoupling during acquisition
;
;P.Schanda and B. Brutscher, J. Am. Chem. Soc. 127, 8014 (2005)
;
;$CLASS=HighRes
;$DIM=2D
;$TYPE=
;$SUBTYPE=
;$COMMENT=

```

```

prosol relations=<triple>

```

```

#include <Avance.incl>
#include <Grad.incl>
#include <Delay.incl>

```

```

"d11=30m"
"d12=20u"
"d21=1s/(cnst4*2)"

```

```

"in0=inf1"

```

```
"d0=in0/2-p21*4/3.1415"
```

```
"DELTA1=d21-p16-d16-p39*cnst39"
```

```
"DELTA2=p39*cnst39-de-4u"
```

```
"spoff23=bf1*(cnst19/1000000)-o1"
```

```
"spoff24=bf1*(cnst19/1000000)-o1"
```

```
1 ze
```

```
d11 pl26:f3
```

```
2 d1 do:f3
```

```
3 d12 pl3:f3
```

```
50u UNBLKGRAD
```

```
p16:gp2
```

```
d16
```

```
(p39:sp23 ph1):f1
```

```
p16:gp1
```

```
d16
```

```
# ifdef LABEL_CN
```

```
(center (p40:sp24 ph2):f1 (p8:sp13 ph1):f2 (DELTA1 p21 ph3 d0 p21 ph4 DELTA1):f3 )
```

```
# else
```

```
(center (p40:sp24 ph2):f1 (DELTA1 p21 ph3 d0 p21 ph4 DELTA1):f3 )
```

```
# endif /*LABEL_CN*/
```

```
DELTA2
```

```
p16:gp1
```

```
d16 pl26:f3
```

```
4u BLKGRAD
```

```
go=2 ph31 cpd3:f3
```

```
d1 do:f3 mc #0 to 2
```

```
F1PH(calph(ph3, +90), caldel(d0, +in0))
```

```
exit
```

```
ph1=0
```

```
ph2=0
```

```
ph3=0 2
```

```
ph4=0 0 2 2
```

```
ph31=0 2 2 0
```

```
;pl3 : f3 channel - power level for pulse (default)
```

```
;pl26: f3 channel - power level for CPD/BB decoupling (low power)
```

```
;sp13: f2 channel - shaped pulse 180 degree (adiabatic)
```

```
;sp23: f1 channel - shaped pulse 120 degree
```

```
; (Pc9_4_120.1000 or Q5.1000)
```

```
;sp24: f1 channel - shaped pulse 180 degree (Rsnob.1000)
```

```
;p8 : f2 channel - 180 degree shaped pulse for inversion (adiabatic)
```

```
;p16: homospoil/gradient pulse [1 msec]
```

```
;p21: f3 channel - 90 degree high power pulse
```

```
;p39: f1 channel - 120 degree shaped pulse for excitation
```

```
; Pc9_4_120.1000 (120o) (3.0ms at 600.13 MHz)
```

```
; (or Q5.1000 (90o) (2.0ms at 600.13 MHz) )
```

```
;p40: f1 channel - 180 degree shaped pulse for refocussing
```

```
; Rsnob.1000 (1.0ms at 600.13 MHz)
```

```
;d0 : incremented delay (2D) = in0/2-p21*4/3.1415
;d1 : relaxation delay
;d11: delay for disk I/O [30 msec]
;d12: delay for power switching [20 usec]
;d16: delay for homospoil/gradient recovery
;d21 : 1/(2J)NH
;cnst4: = J(NH)
;cnst19: H(N) chemical shift (offset, in ppm)
;cnst39: compensation of chemical shift evolution during p39
; Pc9_4_120.1000: 0.529
; Q5.1000: -0.07
;inf1: 1/SW(N) = 2 * DW(N)
;in0: 1/ SW(N) = 2 * DW(N)
;nd0: 1
;NS: 2 * n
;DS: 16
;aq: <= 50 msec
;td1: number of experiments
;FnMODE: States-TPPI, TPPI, States or QSEC
;cpd3: decoupling according to sequence defined by cpdprg3: garp4.p62
;pcpd3: f3 channel - 90 degree pulse for decoupling sequence
; use pulse of >= 350 usec

;use gradient ratio: gp 1 : gp 2
; 11 : 7

;for z-only gradients:
;gpz1: 11%
;gpz2: 7%

;use gradient files:
;gpnam1: SMSQ10.100
;gpnam2: SMSQ10.100

;preprocessor-flags-start
;LABEL_CN: for C-13 and N-15 labeled samples start experiment with
; option -DLABEL_CN (eda: ZGOPTNS)
;preprocessor-flags-end

;Processing

;PHC0(F1): 90
;PHC1(F1): -180
;FCOR(F1): 1

;$Id: sfhmqcf3gpqh,v 1.8.2.2 2009/12/14 12:35:13 ber Exp $
```

---

NMRGuide 4.3 - TOPSPIN 3.0

Written by [Teodor Parella](#)

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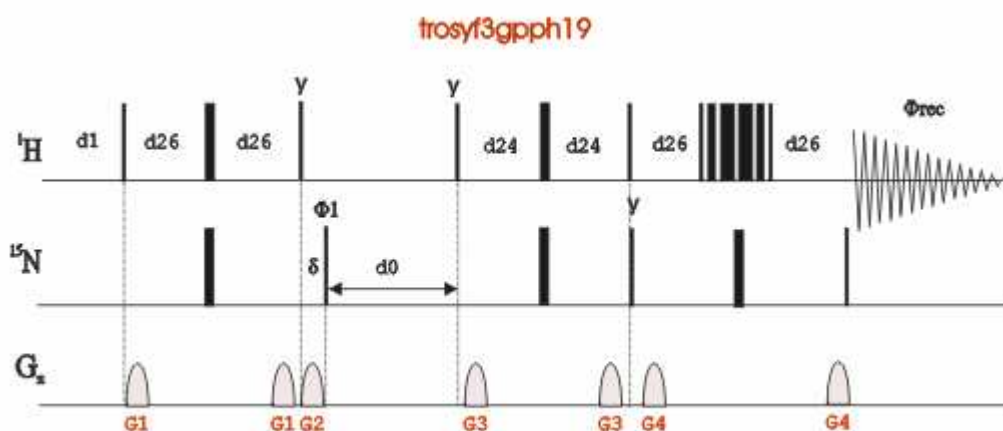
Previous pp:[trosyf3gpdpbwg](#)Next pp:[trosyf3gpphi19](#)

# Pulse Programs

NMRSIM Pulse Diagram

Relevant parameters: [ased](#)More info on [trosyf3gpphi19](#)

## Pulse Diagram



## Pulse Program

```

;trosyf3gpphi19
;avance-version (09/04/17)
;2D H-1/X correlation via TROSY
;phase sensitive
;using f3 - channel
;water suppression using 3-9-19 pulse sequence with gradients
;(use parameterset TROSYF3GPPH19)
;
;K. Pervushin, R. Riek, G. Wider & K. Wuethrich, Proc. Natl. Acad.
; Sci. USA, 12366-12371 (1997)
;M. Piotto, V. Saudek & V. Sklenar, J. Biomol. NMR 2, 661 - 666 (1992)
;V. Sklenar, M. Piotto, R. Leppik & V. Saudek, J. Magn. Reson.,
; Series A 102, 241 -245 (1993)
;
;$CLASS=HighRes
;$DIM=2D
;$TYPE=
;$SUBTYPE=
;$COMMENT=

```

```

#include <Avance.incl>
#include <Grad.incl>
#include <Delay.incl>

```

```

"p2=p1*2"
"p22=p21*2"
"d12=20u"
"d26=1s/(cnst4*4)"

```

```

"d0=3u"

```

```

"in0=inf1"

```

```

"DELTA=d19-p22/2"
"DELTA1=d26-p16-d16-p27*2.385-d19*5+p22/2"
"DELTA2=d26-p16-d16-p27*2.154-p0*0.231-d19*5+p22/2-8u-p21"
"DELTA3=d26-p16-4u"

1 ze
2 d1
3 d12 p11:f1
50u UNBLKGRAD
(p1 ph1)
4u
p16:gp1
DELTA3
(center (p2 ph1) (p22 ph6):f3 )
4u
p16:gp1
DELTA3
(p1 ph2)
3u
p16:gp2
d16
(p21 ph3):f3
d0
(p1 ph5)
4u
p16:gp3
DELTA3
(center (p2 ph1) (p22 ph1):f3 )
4u
p16:gp3
DELTA3
(center (p1 ph1) (p21 ph4):f3 )
DELTA1
p16:gp4
d16 p118:f1
p27*0.231 ph7
d19*2
p27*0.692 ph7
d19*2
p27*1.462 ph7
DELTA
(p22 ph1):f3
DELTA
p27*1.462 ph8
d19*2
p27*0.692 ph8
d19*2
p0*0.231 ph8
4u
p16:gp4
d16
4u BLKGRAD
DELTA2
(p21 ph9):f3
go=2 ph31
d1 mc #0 to 2 F1PH(calph(ph3, +90) & calph(ph6, +90), caldel(d0, +in0))
exit

ph1=0
ph2=1

```

```

ph3=1 3 2 0
ph4=1
ph5=1 1 1 1 3 3 3 3
ph6=0
ph7=0
ph8=2
ph9=0 0 0 0 2 2 2 2
ph31=0 2 3 1 0 2 1 3

```

```

;p11 : f1 channel - power level for pulse (default)
;p13 : f3 channel - power level for pulse (default)
;p118: f1 channel - power level for 3-9-19-pulse (watergate)
;p0 : f1 channel - 90 degree pulse at p118
; use for fine adjustment
;p1 : f1 channel - 90 degree high power pulse
;p2 : f1 channel - 180 degree high power pulse
;p16: homospoil/gradient pulse
;p21: f3 channel - 90 degree high power pulse
;p22: f3 channel - 180 degree high power pulse
;p27: f1 channel - 90 degree pulse at p118
;d0 : incremented delay (2D) [3 usec]
;d1 : relaxation delay; 1-5 * T1
;d12: delay for power switching [20 usec]
;d16: delay for homospoil/gradient recovery
;d19: delay for binomial water suppression
; d19 = (1/(2*d)), d = distance of next null (in Hz)
;d26 : 1/(4J)YH
;cnst4: = J(YH)
;inf1: 1/SW(X) = 2 * DW(X)
;in0: 1/(1 * SW(X)) = 2 * DW(X)
;nd0: 1
;NS: 8 * n
;DS: 16
;td1: number of experiments
;FnMODE: States-TPPI (or TPPI)

```

```

;use gradient ratio: gp 1 : gp 2 : gp 3 : gp 4
; 24 : -60 : 40 : 57.6

```

```

;for z-only gradients:
;gpz1: 24%
;gpz2: -60%
;gpz3: 40%
;gpz4: 57.6%

```

```

;use gradient files:
;gpnam1: SMSQ10.100
;gpnam2: SMSQ10.100
;gpnam3: SMSQ10.100
;gpnam4: SMSQ10.100

```

```

;$Id: trosyf3gp19,v 1.6 2009/07/02 16:40:47 ber Exp $

```

Next pp:[trosyf3gpdpw](#)

More info on **trosvetf3gpsi.2**



prosol relations=<triple>

```
#include <Avance.incl>
#include <Grad.incl>
#include <Delay.incl>

define list<gradient> EA2 = { 0.8750 1.0000 }
define list<gradient> EA4 = { 1.0000 0.6667 }
define list<gradient> EA6 = { 0.6595 1.0000 }

"p2=p1*2"
"p22=p21*2"
"d11=30m"
"d26=1s/(cnst4*4)"

"in0=inf1/2"

"d0=6u"

"DELTA1=d26-p16-d16"
"DELTA2=d25-p16-d16"
"DELTA3=d26-p11-p16-d16-8u"

# ifdef LABEL_CN
"DELTA=d0*2+p8-p21*4/3.1416+8u"
# else
"DELTA=d0*2-p21*4/3.1416+6u"
# endif /*LABEL_CN*/

1 ze
2 d11
3 d1 p11:f1
50u UNBLKGRAD

(p1 ph3)
p16:gp1
d16
DELTA1
(center (p2 ph2) (p22 ph1):f3 )
DELTA1
p16:gp1
d16
(p1 ph2)

p16:gp2*EA2
d16

(p21 ph5):f3
DELTA
(p22 ph1):f3

# ifdef LABEL_CN
d0 gron0
2u groff
(p8:sp13 ph1):f2
d0 gron0*-1
2u groff
# else
d0 gron0
d0 gron0*-1
```

```
2u groff
# endif /*LABEL_CN*/
```

```
4u
p16:gp2*-1*EA2
d16
```

```
(p1 ph6)
p16:gp3
d16
DELTA2
(center (p2 ph2) (p22 ph2):f3 )
DELTA2
p16:gp3
d16
(p1 ph1)
```

```
p16:gp4*EA4
d16
```

```
(p21 ph7):f3
p16:gp5
d16
DELTA3 p10:f1
(p11:sp1 ph4:r):f1
4u
4u p11:f1
(center (p2 ph2) (p22 ph8):f3 )
4u p10:f1
(p11:sp1 ph4:r):f1
4u
DELTA3
p16:gp5
d16 p11:f1
(p21 ph9:r):f3
```

```
p16:gp6*EA6
d16
4u BLKGRAD
```

```
go=2 ph31
d11 mc #0 to 2
F1EA(calgrad(EA2) & calgrad(EA4) & calgrad(EA6) & calph(ph6, +180) & calph(ph7, +180),
caldel(d0, +in0) & calph(ph5, +180) & calph(ph31, +180))
exit
```

```
ph1=0
ph2=1
ph3=2
ph4=3
ph5=0 2
ph6=3
ph7=0 0 2 2
ph8=1 1 3 3
ph9=3 3 1 1
ph31=0 2 2 0
```

```
;p10 : 0W
;p11 : f1 channel - power level for pulse (default)
;p13 : f3 channel - power level for pulse (default)
;sp1: f1 channel - shaped pulse 90 degree (H2O on resonance)
```

```
;sp13: f2 channel - shaped pulse 180 degree (adiabatic)
;p1 : f1 channel - 90 degree high power pulse
;p2 : f1 channel - 180 degree high power pulse
;p8 : f2 channel - 180 degree shaped pulse for inversion (adiabatic)
;p11: f1 channel - 90 degree shaped pulse [1 msec]
;p16: homospoil/gradient pulse [1 msec]
;p21: f3 channel - 90 degree high power pulse
;p22: f3 channel - 180 degree high power pulse
;d0 : incremented delay (2D) [6 usec]
;d1 : relaxation delay; 1-5 * T1
;d11: delay for disk I/O [30 msec]
;d16: delay for homospoil/gradient recovery
;d25 : 1/(4J')NH,
; compensation delay for suppression of other Trosy peaks
;d26 : 1/(4J)NH
;cnst4: = J(NH)
;inf1: 1/SW(N) = 2 * DW(N)
;in0: 1/(2 * SW(N)) = DW(N)
;nd0: 2
;NS: 4 * n
;DS: 16
;td1: number of experiments
;FnMODE: echo-antiecho
```

```
;use gradient ratio: gp 0 : gp 1 : gp 2 : gp 3 : gp 4 : gp 5 : gp 6
; 3 : 3 : 80 : 5 : 30 : 7 :30.13
```

```
;for z-only gradients:
;gpz0: 3%
;gpz1: 3%
;gpz2: 80%
;gpz3: 5%
;gpz4: 30%
;gpz5: 7%
```

---

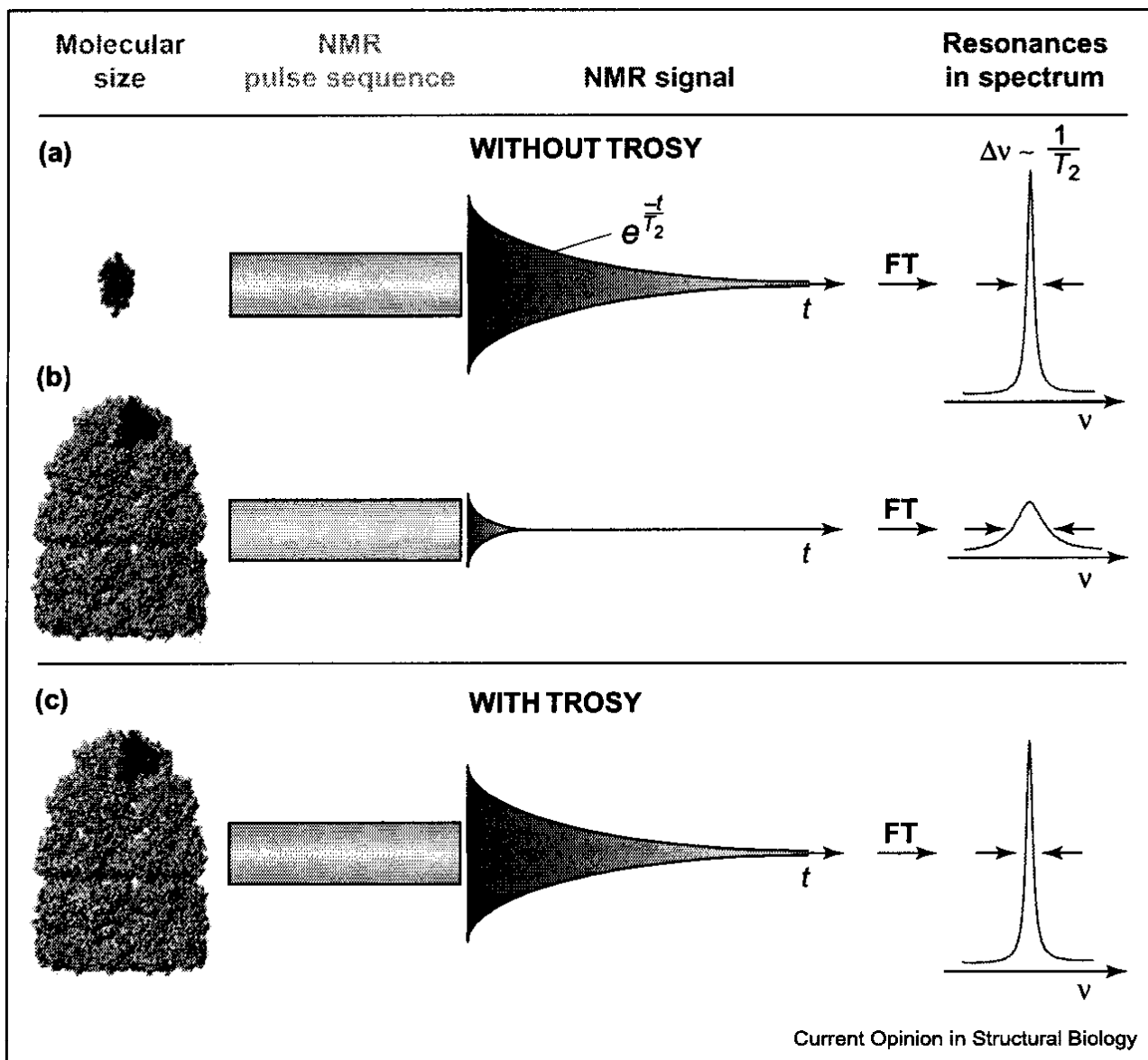
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Written by [Teodor Parella](#)

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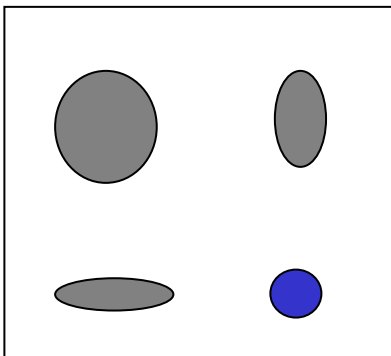
# Impact of TROSY on Solution NMR



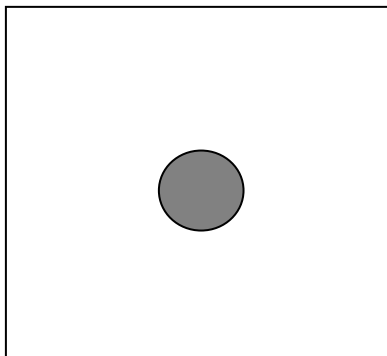


# Transverse Relaxation-Optimized Spectroscopy (TROSY)

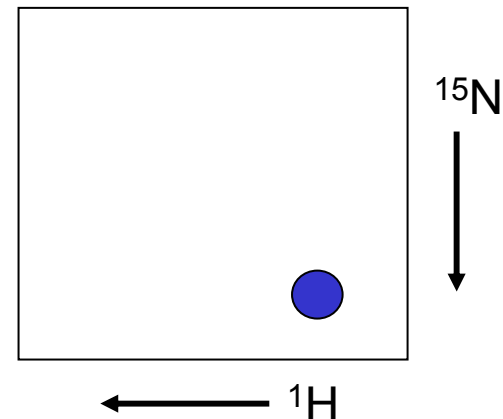
(a). None-decoupled HSQC



(b). Decoupled HSQC



(c). TROSY-HSQC

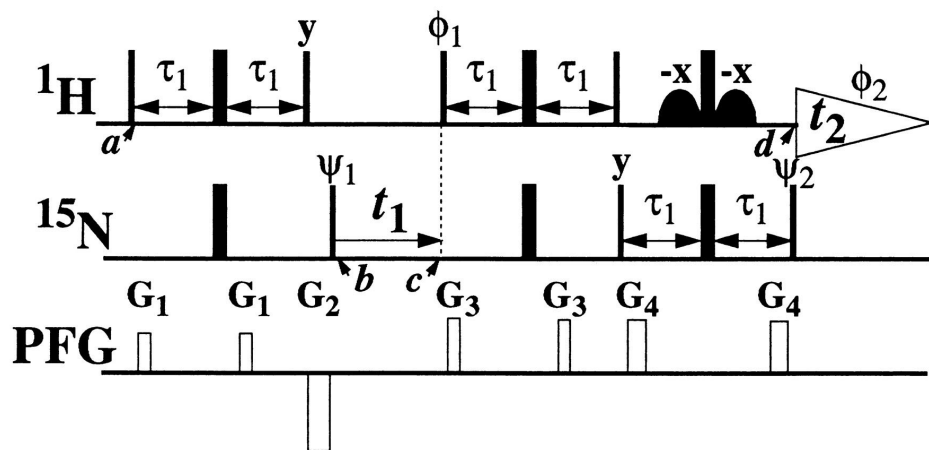


1. **Main relaxation source for  $^1\text{H}$  and  $^{15}\text{N}$ :** dipole-dipole (DD) coupling and, at high magnetic fields, chemical shift anisotropy (CSA).
2. **Different relaxation rates (line width)** for each of the four components of  $^{15}\text{N}$ - $^1\text{H}$  correlation.
3. **The narrowest 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).**



# TROSY-HSQC and Conventional HSQC

## TROSY-HSQC



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

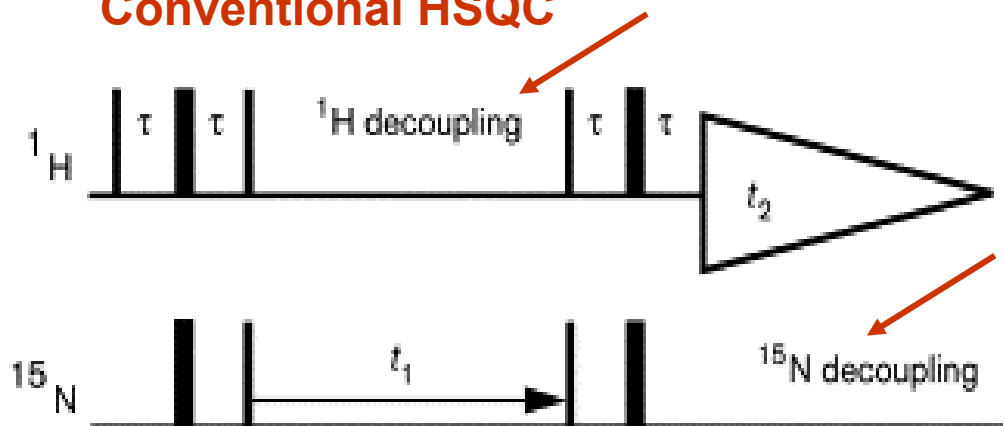
## TROSY-HSQC

(1) No  $^1\text{H}$  decoupling during  $^{15}\text{N}$  evolution.

(2) No  $^{15}\text{N}$  decoupling during  $^1\text{H}$  acquisition.

(3) Use the TROSY-HSQC pulse sequence to selectively observe the most slowly relaxing component.

## Conventional HSQC



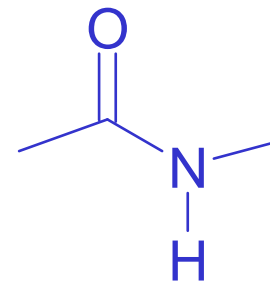
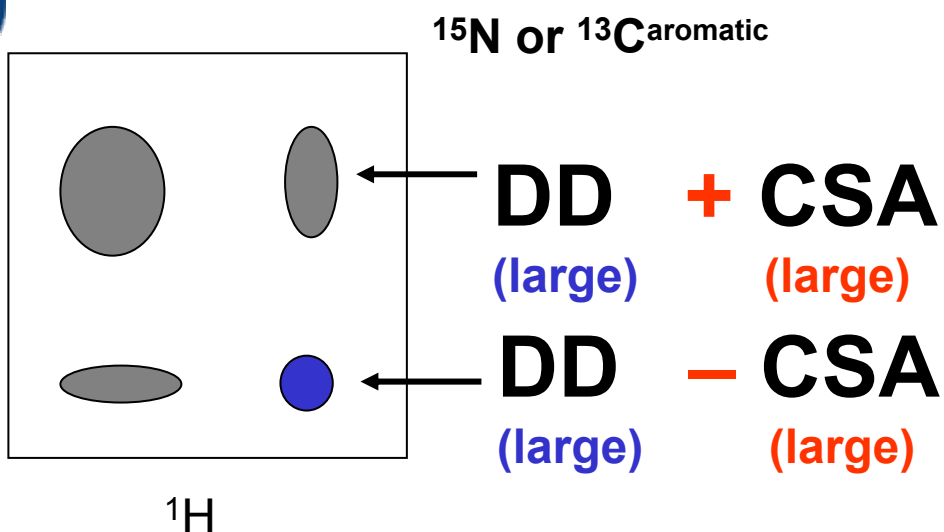
Current Opinion in Structural Biology

## HSQC

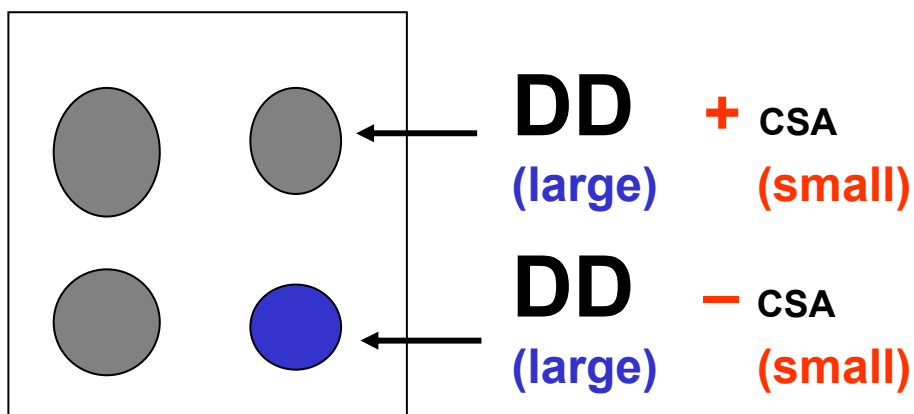
In both  $^1\text{H}$  and  $^{15}\text{N}$  dimensions, both J-split components are mixed by hetero-nuclear decoupling. This collapses each  $^{15}\text{N}$  and  $^1\text{H}$  doublet into a single peak in each dimension.



# Interference of Relaxation 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,  $\text{CSA} \propto B_0^2$ , therefore at high magnetic fields, CSA relaxation can be comparable to DD relaxation, and the interference effect on relaxation can be observed.