Training Material Number : UMAL-1507-01 Author : Sun-Phil Han Date : 2022-11-28

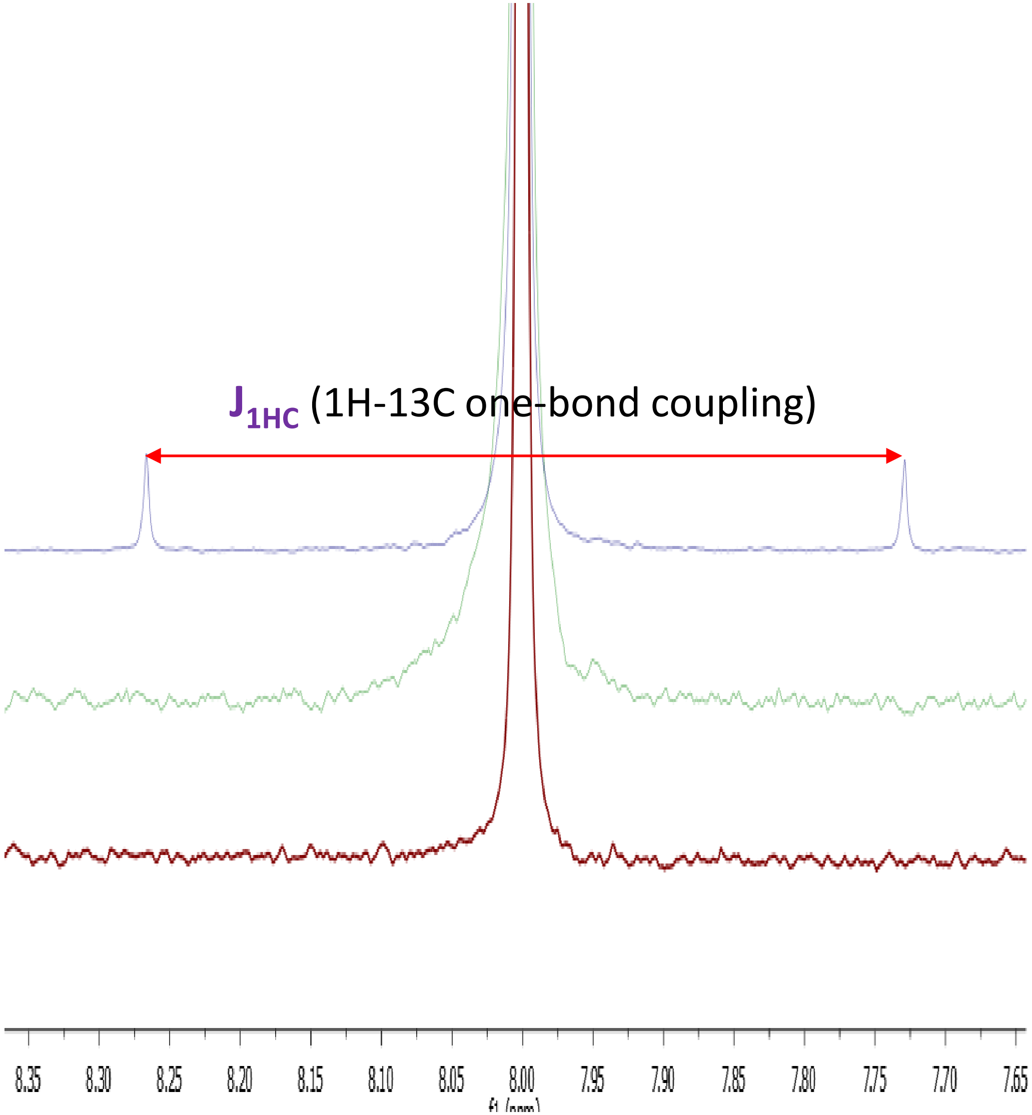
ULSAN NATIONAL INSTITUTE OF SCIENCE AND TECHNOLOGY

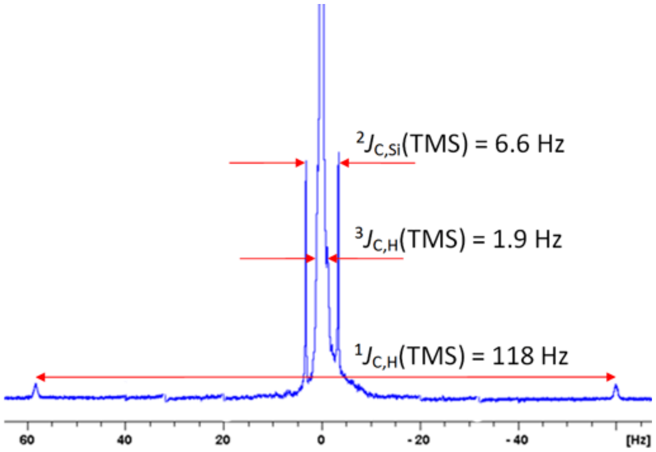
2D NMR Manual

Advanced Operation
Training Course

1. 1H, 13C
Pulse Sequence

1H NMR with C13 decoupling





Normal purpose

PROTON = Non-decoupled 13C

Recommendation

H1_DEC_C13 = Decoupled 13C

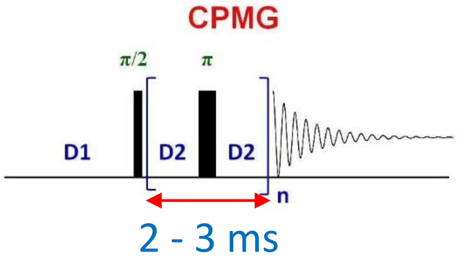
Special purpose

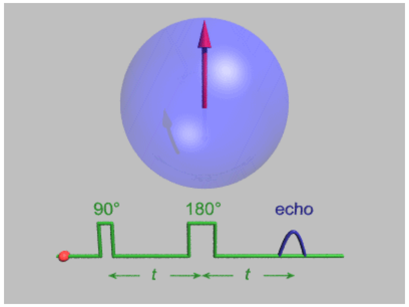
H1_DISPEL_4stage

= Distruction of Interfering Satllites

by Perfect Echo Low-pass filtration

CPMG (Suppress background)





CPMG(Spin echo) Effect

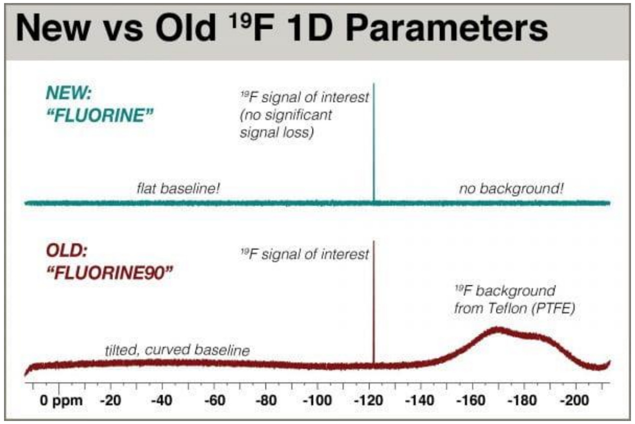
1. Suppress magnetic field inhomogeneous
2. Background signal decay

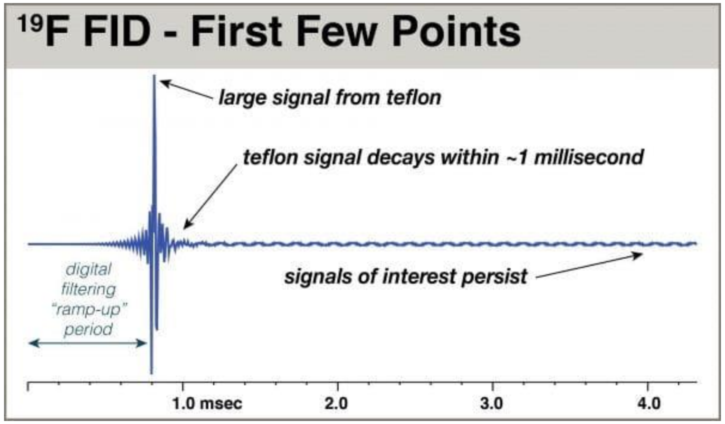
D2 = 2 ~ 3 ms

(Teflon decay time < 1 ms)

Borosilicate
Background: B11, O17, Si29

PTFE (Teflon)
Background: C13, F19

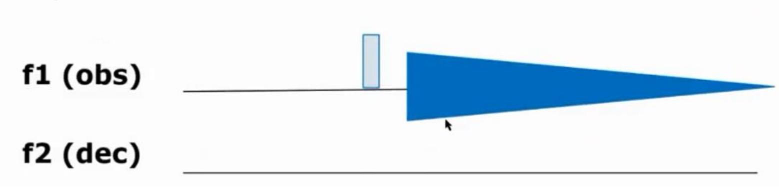




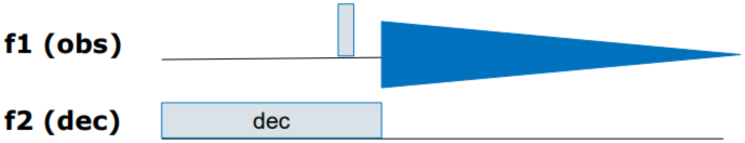
https://voices.uchicago.edu/chemnmr/2020/05/21/background-free-19f-experiments/

C13 with or without 1H Decoupling

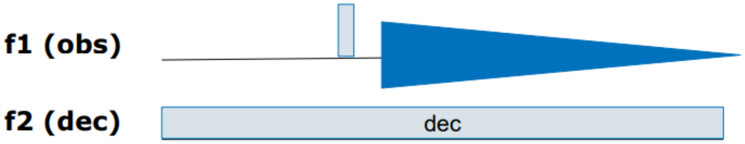
zg (-NOE, -DEC)



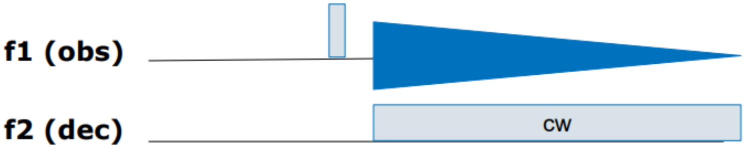
zggd (+NOE, -DEC)



zgdc (+NOE, +DEC)

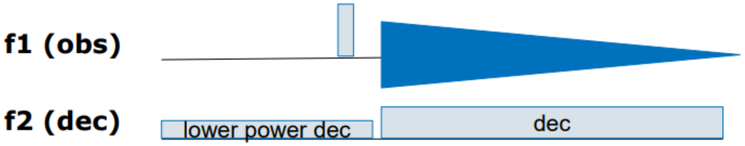


zgcw (-NOE, +DEC_CW)



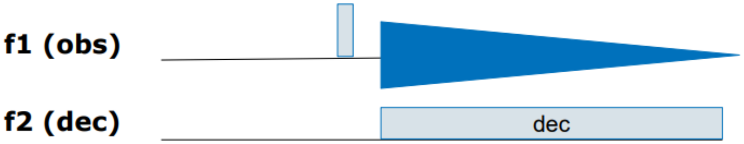
C13CPD

zgpg (+NOE, +DEC_Intensity)



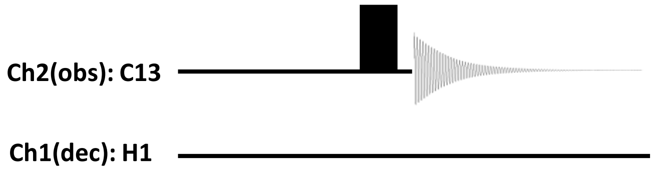
C13IG

zgig (-NOE, +DEC, Integration)



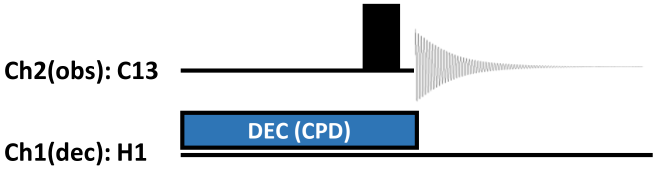
C13 with or without 1H Decoupling

zg (-NOE, -DEC)


Ch2(obs): C13

Ch1(dec): H1

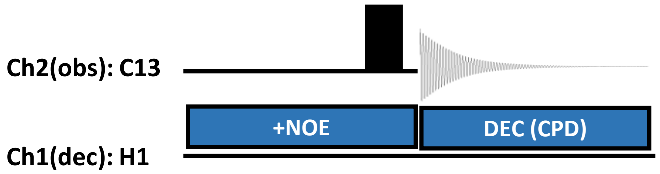
zggd (+NOE, -DEC)


Ch2(obs): C13

DEC (CPD)

Ch1(dec): H1

zgdc (+NOE, +DEC)

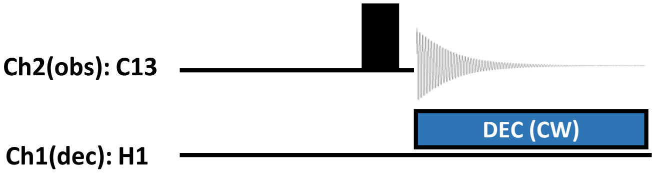

Ch2(obs): C13

+NOE

DEC (CPD)

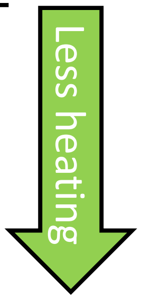
Ch1(dec): H1

zgcw (-NOE, +DEC_CW)


Ch2(obs): C13

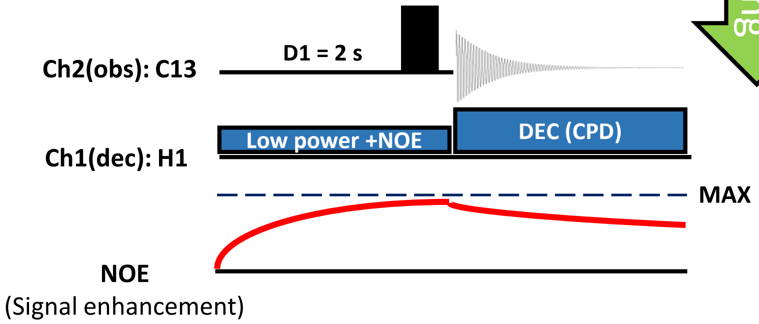
DEC (CW)

Ch1(dec): H1


Less heating

C13CPD (Varian/Agilent = DEC + NOE)

zgpg (+NOE, +DEC_Intensity)


D1 = 2 s

Ch2(obs): C13

Low power +NOE

DEC (CPD)

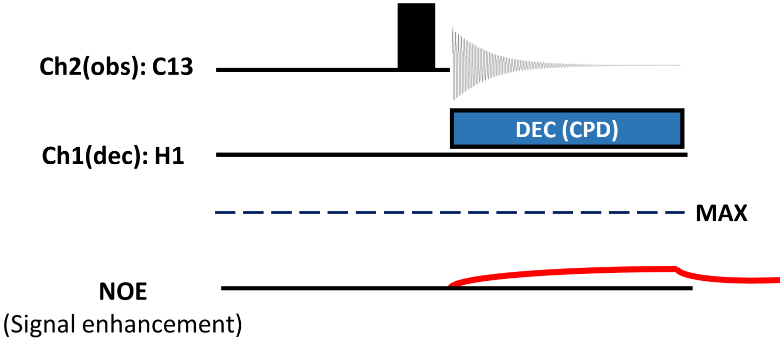
Ch1(dec): H1

MAX

NOE
(Signal enhancement)

C13IG (Varian/Agilent = DEC - NOE)

zgig (-NOE, +DEC, Integration)


Ch2(obs): C13

DEC (CPD)

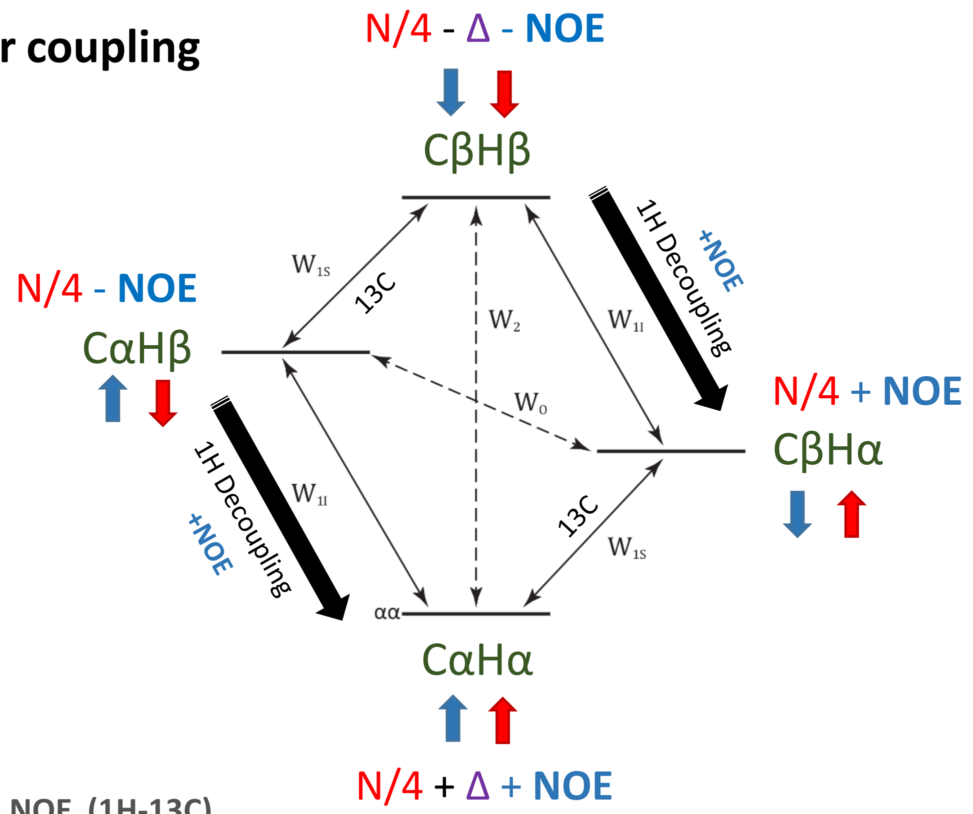
Ch1(dec): H1

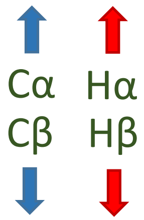
MAX

NOE
(Signal enhancement)

NOE (Nuclear Overhauser Effect)

Dipolar coupling





W (Transition Probability)

W_0 = Zero quantum
W_1 = Single quantum
W_2 = Double quantum

W_{1L} = 1H transition
W_{1S} = 13C transition

N = Total Nucleus
Δ = Population

Hetero NOE (1H-13C)

$$NOE = 1 + \frac{1}{2} * (\frac{r_H}{r_C})$$

$$NOE = 1 + 1.998$$

$NOE = 3$ (Maximum enhancement)

Cross-relaxation rate

$$NOE = 1 + \frac{1}{2} * \frac{W_2 - W_0}{2w_1 + w_2 + w_0} * (\frac{r_H}{r_C})$$

Total longitudinal dipolar relaxation rate

2. 2D NMR Pulse Sequence

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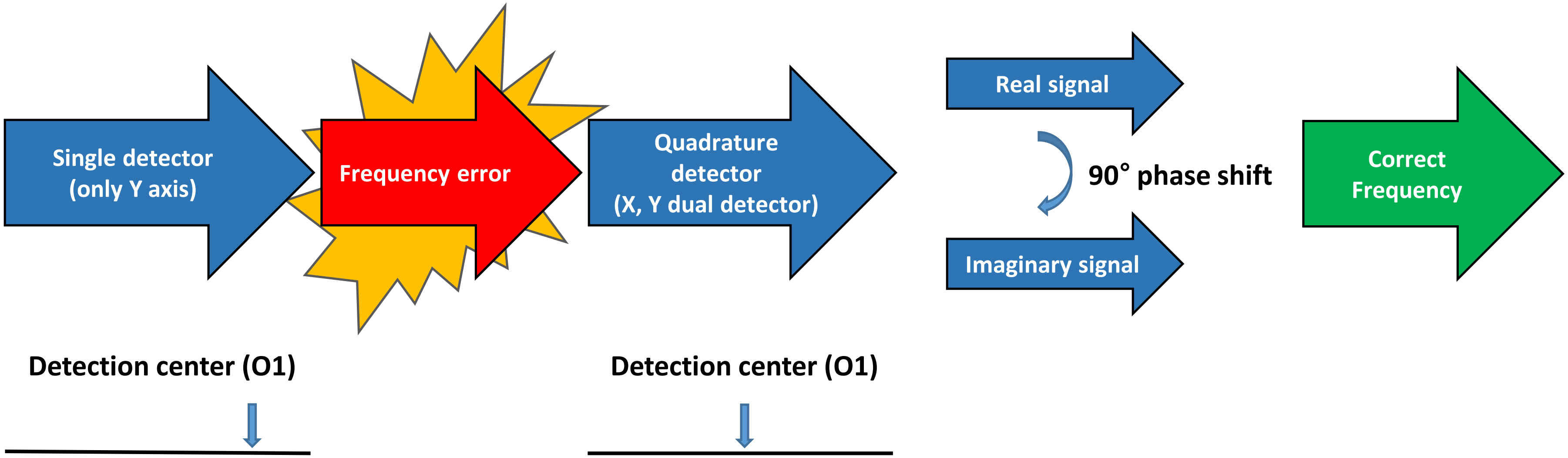
Quadrature Detector



Single detector
(only Y axis)

Frequency error

Quadrature
detector
(X, Y dual detector)

Real signal

90° phase shift

Imaginary signal

Correct
Frequency

Detection center (O1)

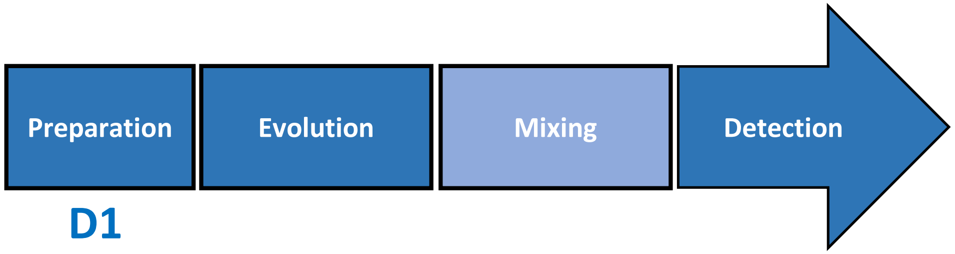
Detection center (O1)

- Spectrum Center가 한쪽에 치우쳐져 있음
- Spectrum Center가 중심에 존재

2D NMR

<Interpretation for 2D NMR pulse sequence>

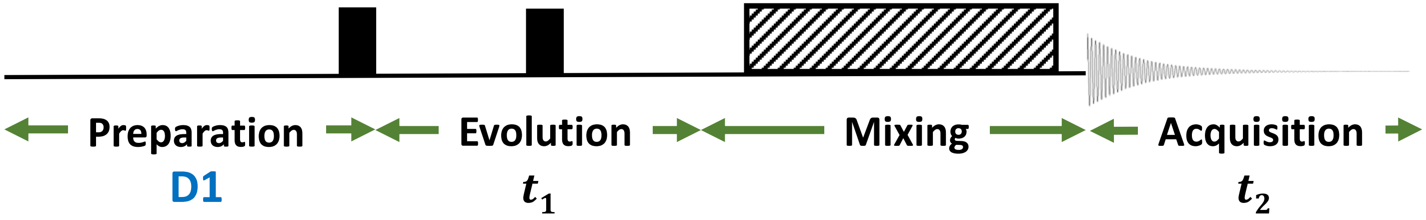
1. Rotating frame
2. Product operator formalism
3. Density matrix (Quantum mechanics)



Preparation
Evolution
Mixing
Detection
D1

- 2D NMR의 pulse sequence를 이해/해석하는 방법에는 상기 3가지 방법이 존재
- 주로 Evolution time or Mixing time 기간 동안 에너지의 전달 및 특정 상호작용의 추출이 이루어짐

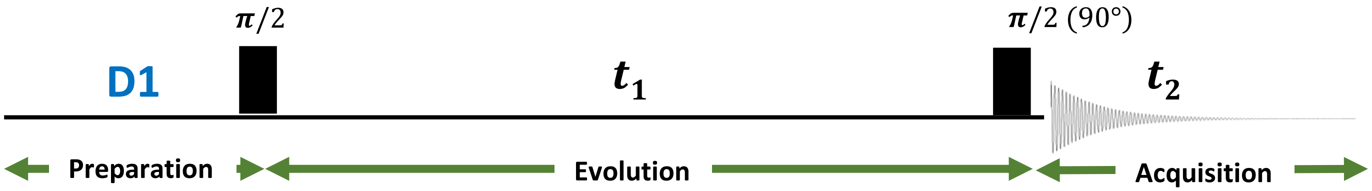
<Pulse sequence of 2D NMR>



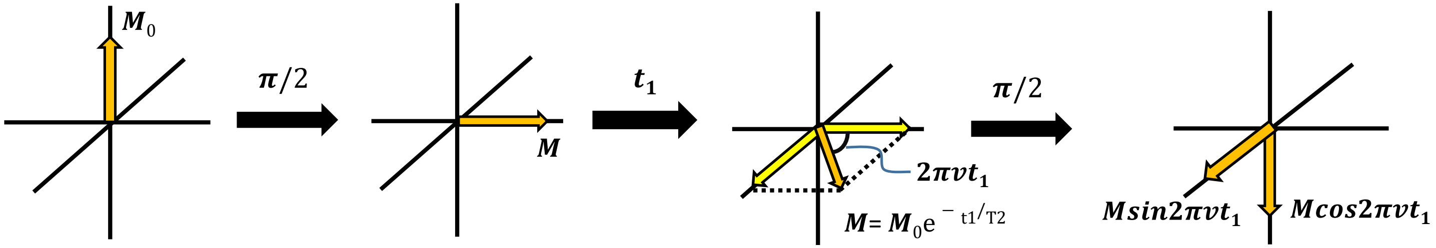
Preparation
D1
Evolution
t_1
Mixing
Acquisition
t_2

COSY (Correlation SpectroscopY)

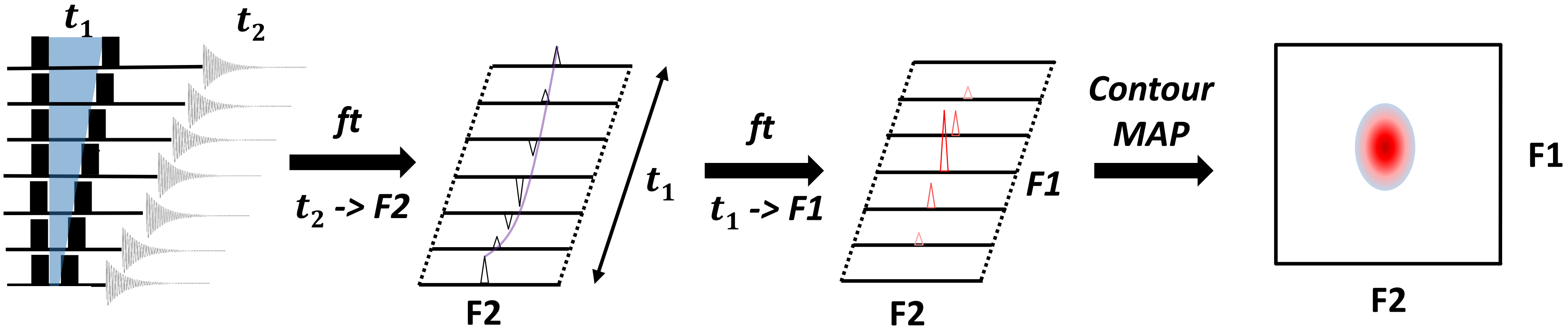
<Pulse sequence of COSY>



<Vector of 2D NMR>

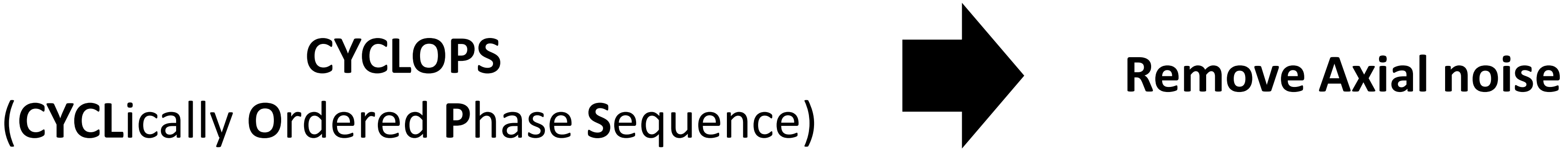


<Example of 2D NMR >



- t_1 을 증가시키며 얻은 여러 개의 Spectrum을 Contour(등고선) MAP으로 표현
- t_1 을 증가를 통해 선택적으로 COSY(J2 or J3) peak (상호작용)만 추출해 낼 수 있음

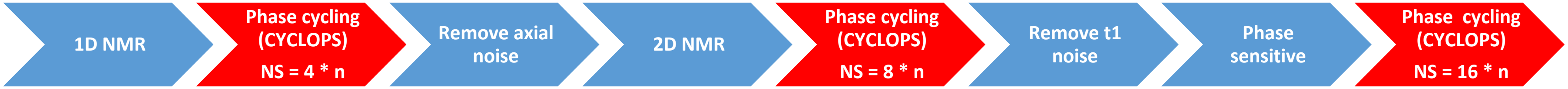
CYCLOPS
(CYCLically Ordered Phase Sequence)


CYCLOPS

(**CYCL**ically **O**rdered **P**hase **S**equence)

Remove Axial noise

<Number of scans for 2D NMR>

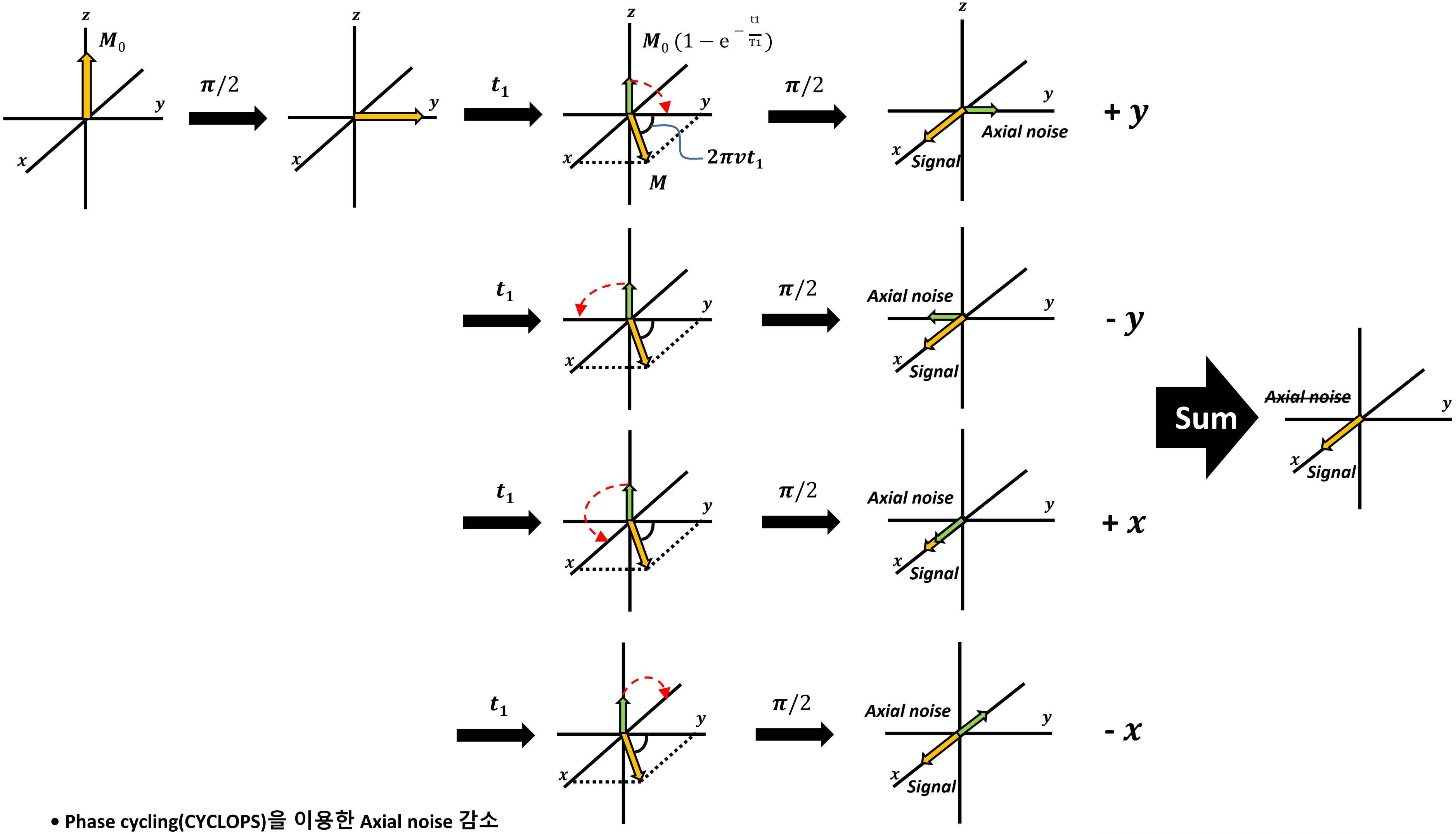


<At best condition>

- **Phase cycling(CYCLOPS)을 이용하여 Axial noise를 제거할 수 있음**
- **Pulse sequence에 따라 다르지만 최소 NS=4 or NS=8의 스캔 수가 필요함**

CYCLOPS
(CYCLically Ordered Phase Sequence)

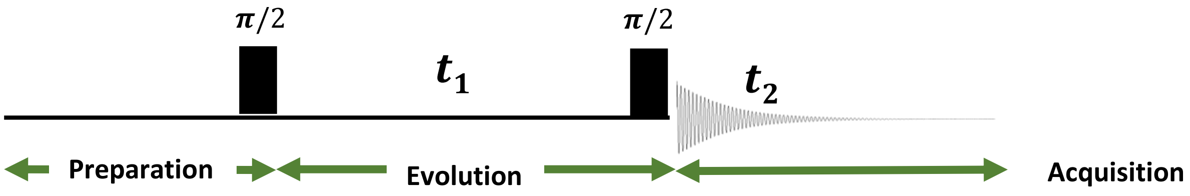
<Phase cycling for Quadrature detector>



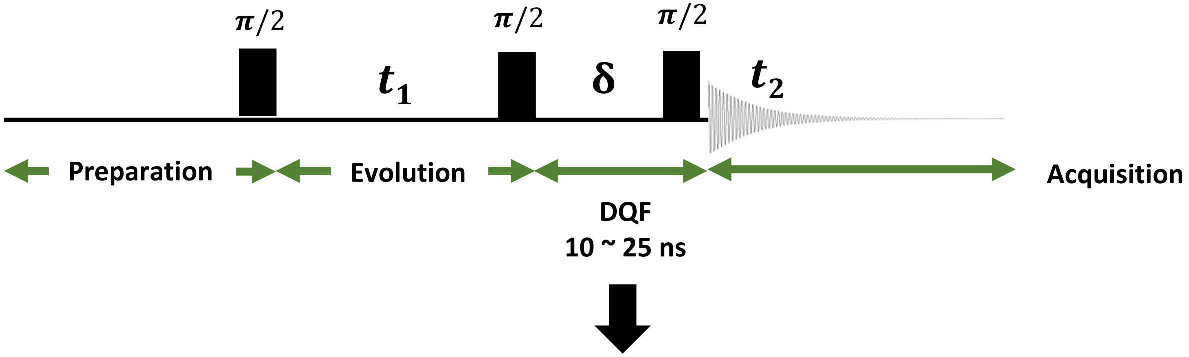
- Phase cycling(CYCLOPS)을 이용한 Axial noise 감소

COSY (Correlation SpectroscopY)

<COSY>



<DQF(Double Quantum Filtered)-COSY>



1) Suppress Singlet
2) Diagonal dispersion
But, Low S/N ratio
DQF-COSY = Phase sensitive mode
COSY = Magnitude mode

- **DQF-COSY의 경우 Phase mode이므로 COSY(Magnitude mode)에 비해 상대적으로 1/2의 S/N ratio를 가짐 (4배의 NS 필요)**

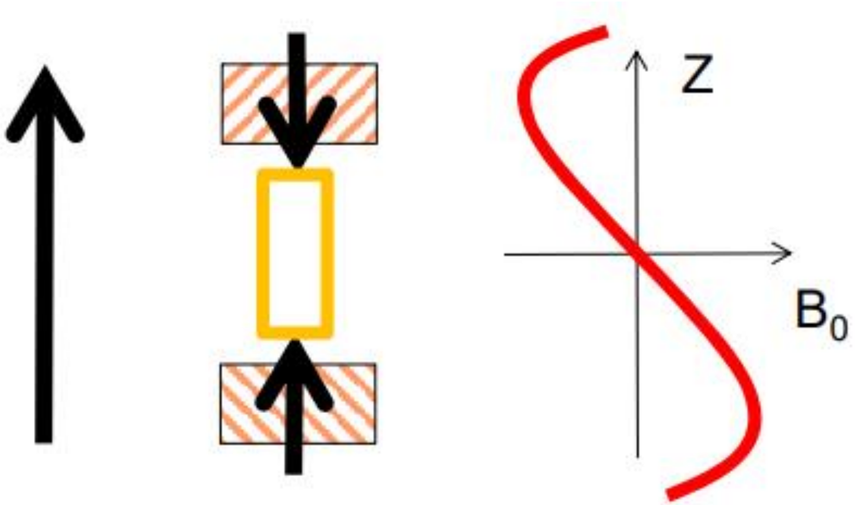
3. Gradient

Pulsed Field Gradients (PFG)

What is Gradient?

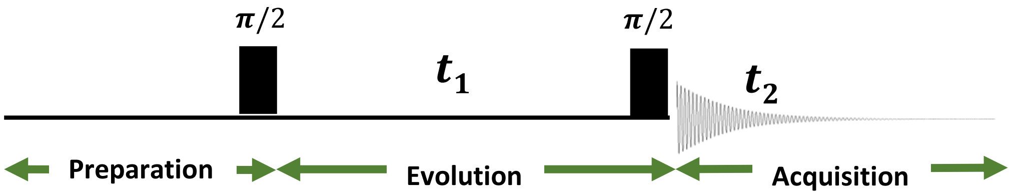
Use Gradient coil -> Make field gradient
-> Force Dephasing / Refocusing for Nuclear spin
-> Save time, Correct good data

Gradient coil을 이용하여 Magnetic field gradient 형성할 수 있음, 이를 이용 강제적으로 Nuclear spin의 Dephasing / Refocusing을 할 수 있음 -> 실험시간 단축, 정확한 데이터 획득 가능



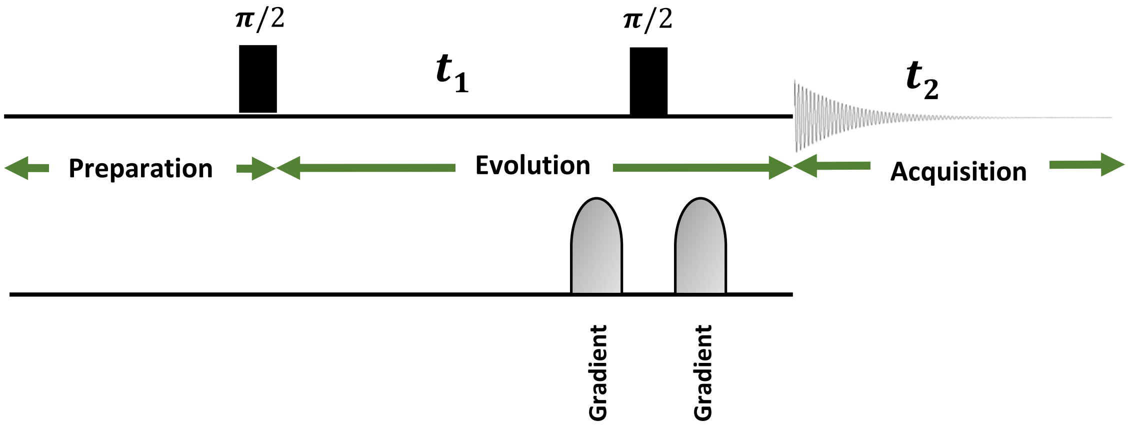
Gradient vs Phase cycling

<COSY>



Phase cycling
(NS = 4 or 8)

<gCOSY, Gradient COSY>



Save Time!!

Non phase cycling
(NS = 1, Possible)

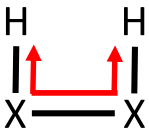
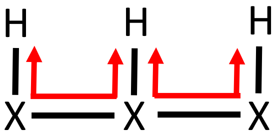
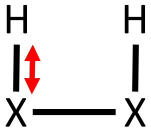
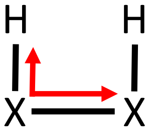
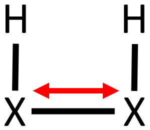
- gCOSY(Gradient COSY)의 경우 Phase cycling을 이용하지 않으므로 NS = 1 로도 분석 가능
  + 보다 S/N ratio가 좋은 데이터 획득 가능

4. Correlation in 2D

Correlation in 2D NMR

Bonding

Scalar coupling (J Coupling)

	J_{2HH} ~ J_{3HH}	1H-1H COSY
	J_{2HH} ~ J_{5HH}	1H-1H TOCSY
	J_{1HX}	1H-X HSQC 1H-X HMQC X->1H HETCOR
	J_{2HX} ~ J_{3HX}	1H-X HMBC
	J_{1XX}	X-X COSY X-X INADEQUATE H-X-X-H AQDEQUATE

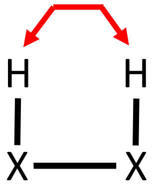
Space

Dipolar coupling

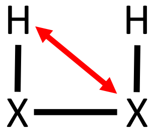
NOESY = Small, Large molecules (along T1)
ROESY = Middle molecules (along T2)

Small < 700 Da < Middle < 1.2 kDa < Large

Distance = 3 ~ 5 Å



1H-1H NOESY
1H-1H ROESY



2D HOESY

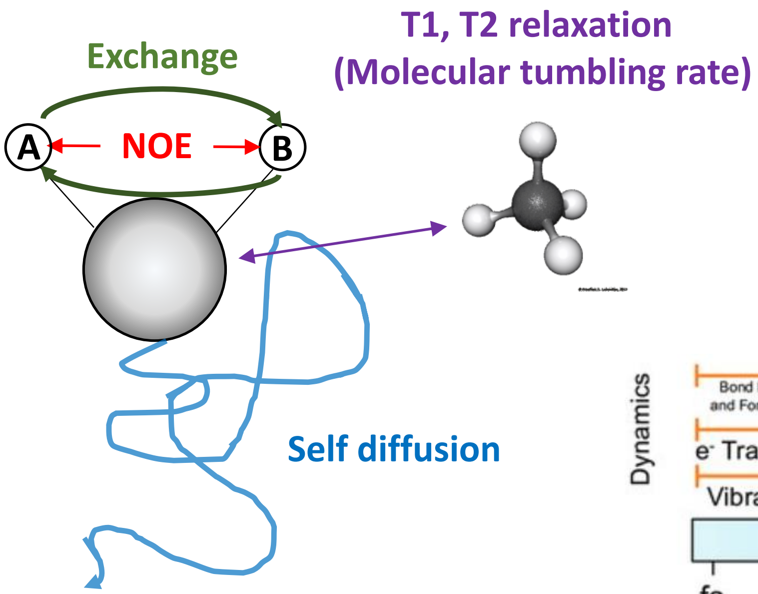
Motion

Self-Diffusion	DOSY (Diffusion)
Tumbling	T1 (Inversion recovery) T2 (CPMG)

- 2D NMR은 Scalar coupling, Dipolar coupling, Motion 을 이용한 상호작용으로 나뉘어짐

Protein dynamics elucidated by NMR technique, 2013

Correlation in 2D NMR



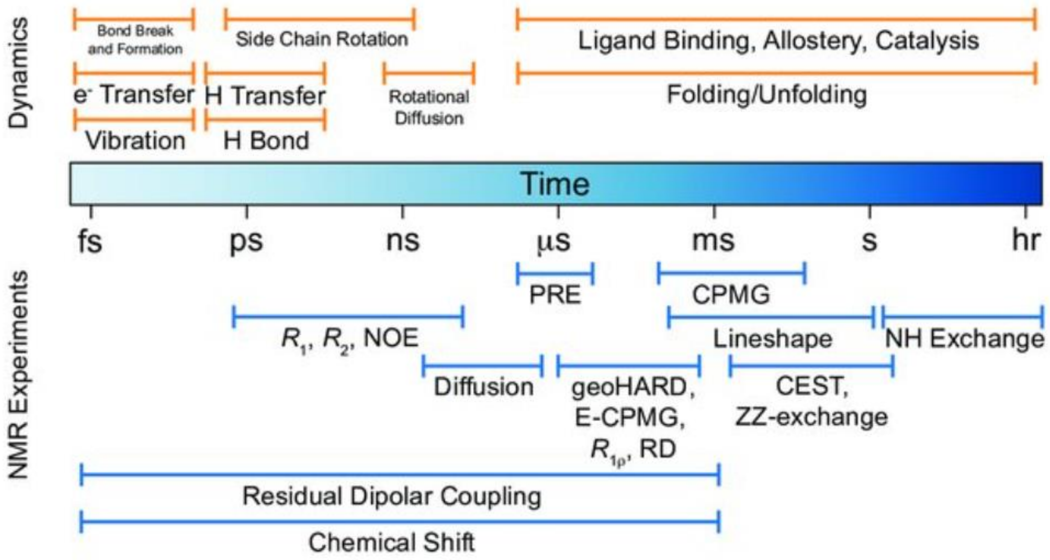
Exchange

A ← NOE → B

T1, T2 relaxation
(Molecular tumbling rate)

Self diffusion

Exchange (EXSY)
NOE (NOESY/ROESY)
Diffusion (DOSY)
T1, T2 (Inversion recovery, CPMG)



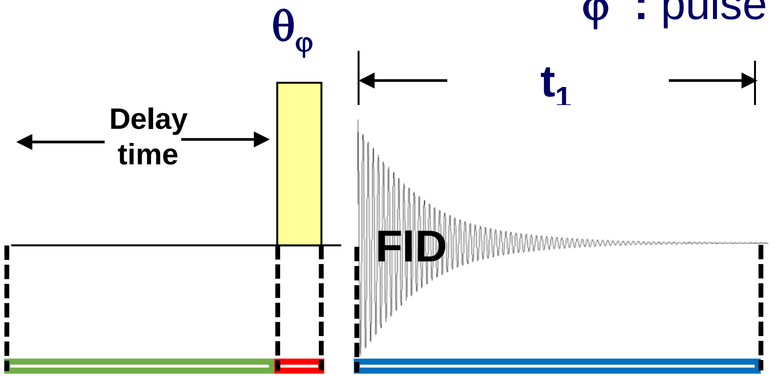
Protein dynamics elucidated by NMR technique, 2013

5. Parameter Calibration

One pulse

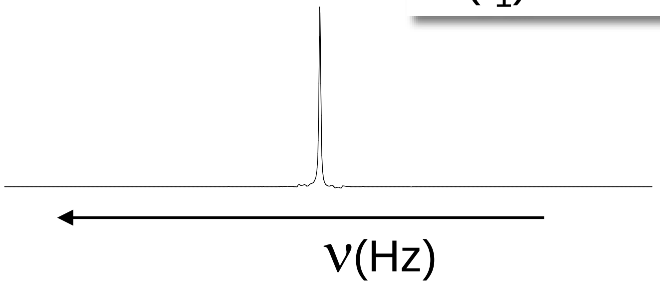
Basic 1D NMR

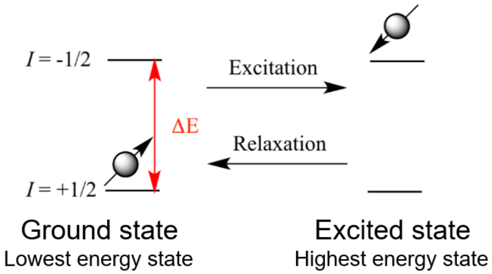
θ : pulse width(μs)
φ : pulse phase



Fourier Transformation

$$G(t_1) \xrightarrow{FT} g(f_1)$$





NMR

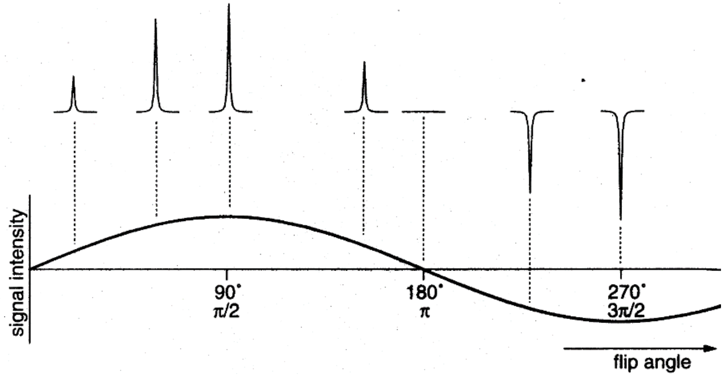
Parameter

- 🟩 **D1 (Relaxation delay time)**
- 🟥 **P1 (Pulse width)**
  PLW1 (Power level)
- 🟦 **AQ (Acquisition time)**

Parameter calibration

1. Find **P1**

1) Type edc – create experiment room
2) Change PULPROG zg30 to **zg**
3) Set D1=10, P1=3, DS=0, NS=1
4) Type rga; zg
5) ProcPars -> Change pHmod no to pk
6) Type efp
7) Correct phase and save
8) Set P1=20 (About 180 °) or P1=40 (About 360 °)
9) Type efp -> Check peak phase (negative or positive)
10) Change P1 (negative -> decrease P1, positive -> increase P1) and find symmetry peak intensity (Exact 180 °)
11) Set P1/2 = 90 degree pulse (=Highest intensity)



1-1. Find **P1 (with pulsecal)**

1) Type edc – create experiment room
2) Progress Lock, ATM, (Non-spin), Shim, Prosol, RGA
3) Type ***pulsecal*** -> Find **P1**

Parameter calibration

2. Find T1

1) Type wrpa (ex. exp# 900)
2) Change PULPROG **t1ir**
3) Click AcquPars and change 1D to 2D
4) Set F1 domain TD=10 and set vdlist (ex, T1=20s)
5) Type ased
6) Set DS=0, NS= 1 or 2
7) Type rga; zg
8) Type xf2; abs2
9) Type rser 1 and correct phase and then **save 2d**
10) Type xf2; abs2
11) Click Analyse -> Dynamics -> T1/T2
12) Click Spectrum and put 1 at Slice number
13) Click Peak/Range and click manual integration
14) Export Regions o relaxation module and .ret
15) Click Relaxation and click fitting

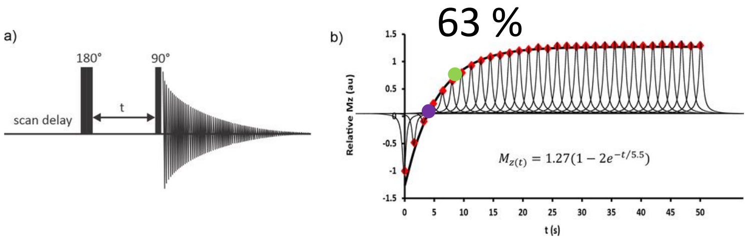
Or

1) Run 'T1 short experiment' in Automation(Icon NMR)!!

2-1. Find D7null(T1)

1) Type wrpa (ex. Exp# 901)
2) Change PULPROG **t1ir1d**
3) Type ased
4) Set D7null=0.002(or 2m)
5) Type zg and efp
6) Check negative peak.
7) Find D7null value
   (zero intensity)

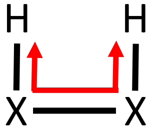
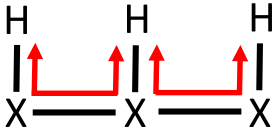
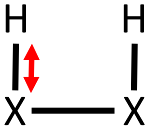
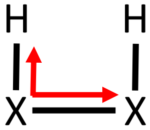
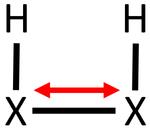
T1 * ln(2) = **D7null**
T1 = **D7null** * 1.443



Key parameter in 2D NMR

Bonding

Scalar coupling (J Coupling)

	J_{2HH} ~ J_{3HH}	**1H-1H COSY**
	J_{2HH} ~ J_{5HH}	**1H-1H TOCSY**
	J_{1HX}	**1H-X HSQC** **1H-X HMQC** **X-1H HETCOR**
	J_{2HX} ~ J_{3HX}	**1H-X HMBC**
	J_{1XX}	**X-X COSY** **X-X INADEQUATE** **H-X-X-H** **AQDEQUATE**

Hz	J_{HH}	J_{HC}	J_{CC}
J_1		125 ~ 250	35-80
J_2	-20 ~ 0	-10 ~ 12	<20
J_3	0 ~ 16	1 ~ 10	0 ~ 7
J_{3+n}	0 ~ 5	<1	<1

Key parameter = D9 (Mixing time, Spin lock)
Common = 20 ~ 30 ms, 120 ms (long range)

Key parameter = J_{1HX}
Common = 145 Hz
Aliphatic, CHn = 125 – 135 Hz
Aliphatic, CHnX = 135 – 155 Hz
Alkene = 155 – 170 Hz
Alkyne 240 – 250 Hz
Aromatic 155 – 165 Hz

Key parameter = J_{2HX} or J_{3HX} (cnst13)

Key parameter = J_{1XX} (1 bond coupling constant)

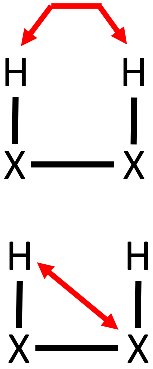
- 대부분의 2D 실험은 (감도가 좋은) 1H를 측정하여 X핵종과의 상호작용을 분석함 (HETCOR의 경우 X핵종을 측정하므로 감도가 낮음)

Key parameter in 2D NMR

Space

Dipolar coupling

Distance = 3 ~ 5 Å



1H-1H NOESY

1H-1H ROESY

1H-X HOESY

Small < 700 Da < Middle < 1.2 kDa < Large

Key parameter = D8 (Mixing time)
Common = 500 ms

Small: 500 ms ~ 1000 ms
Middle: 100 ~ 500 ms
Large: 50 ~ 200 ms

D8 = T1 * 2/3

Small

Positive (Diagonal 1D, EXSY, Indirect NOE)
Negative (NOE)

Positive (Diagonal 1D, EXSY or TOCSY, Indirect NOE)
Negative (ROE)

Large

Positive (Diagonal 1D, EXSY, Indirect NOE)
Positive (NOE)

Positive (Diagonal 1D, EXSY or TOCSY, Indirect NOE)
Negative (ROE)

https://nmr.chem.columbia.edu/sites/default/files/content/NOESY%20and%20ROESY%20experiments.pdf

6. Quick guide (Icon NMR)

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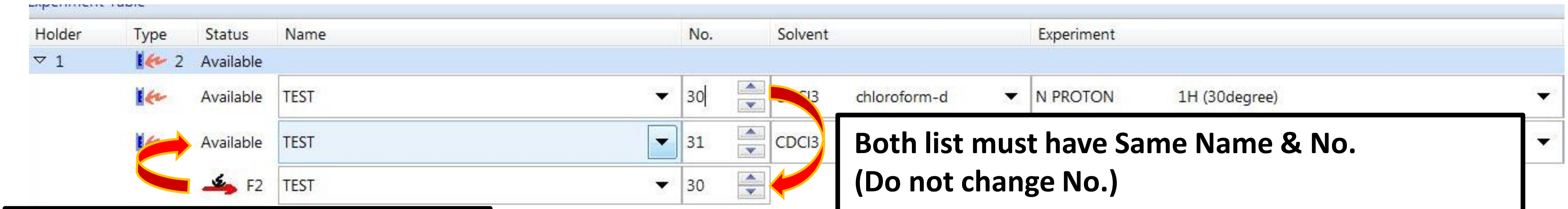
How to run 2D NMR (Quick)

Directly Summit
0. Open Holder list (Double click holder number or click Add)
1. Type sample name, Select NMR solvent
2. Choice 2D NMR in Experiment list (COSY, NOESYPHSW, HSQCEDETGPSISP, etc..)
3. We must have 1H NMR DATA!
4. Change NS or directly run (check exp. time)



Holder	Type	Status	Name	No.	Solvent	Experiment
1	2	Available				
		Available	TEST	30	Cl3 chloroform-d	N PROTON 1H (30degree)
		Available	TEST	31	CDCl3	
		F2	TEST	30		

1D 1H data will automatically use to select 2D NMR spectrum width (Ignore Poor S/N ratio peak)

Both list must have Same Name & No. (Do not change No.)

PROTON = F2 F2

If you have old 1H 1D NMR data, you could use it.

- **It is not optimal parameter, but It should show middle quality data.**

How to run 2D NMR (Optimized)

1. Find **P1 (with pulsecal)**
    1) Type edc – create experiment room
    2) Progress Lock, ATM, (Non-spin), Shim, Prosol, RGA
    3) Type ***pulsecal*** -> Find **P1**

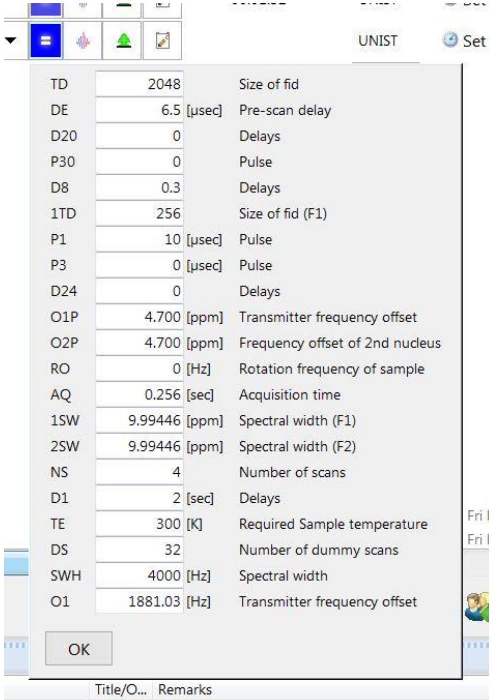
2. Find **T1**

N 1H_T1_relaxation_5min 1H_T1_Short

1) Just Change **P1**
2) Run T1 relaxation_5min 1H_T1_Short
3) Analyse > Dynamics > T1/T2

Relaxation delay in 2D NMR

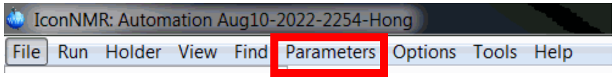
D1 = T1 * 1.3 (Maximum S/N ratio)
= T1 * 5 (Quantitative 2D NMR)



TD	2048		Size of fid
DE	6.5	[μsec]	Pre-scan delay
D20	0		Delays
P30	0		Pulse
D8	0.3		Delays
1TD	256		Size of fid (F1)
P1	10	[μsec]	Pulse
P3	0	[μsec]	Pulse
D24	0		Delays
O1P	4.700	[ppm]	Transmitter frequency offset
O2P	4.700	[ppm]	Frequency offset of 2nd nucleus
RO	0	[Hz]	Rotation frequency of sample
AQ	0.256	[sec]	Acquisition time
1SW	9.99446	[ppm]	Spectral width (F1)
2SW	9.99446	[ppm]	Spectral width (F2)
NS	4		Number of scans
D1	2	[sec]	Delays
TE	300	[K]	Required Sample temperature
DS	32		Number of dummy scans
SWH	4000	[Hz]	Spectral width
O1	1881.03	[Hz]	Transmitter frequency offset

3. Open Holder list (Double click holder number or click AdD)
4. Type sample name, Select NMR solvent
5. Choice 2D NMR in Experiment list (COSY, NOESYPHSW, HSQCEDETGPSISP, etc..)
6. We must have 1H NMR DATA!
7. Set **D1**, **P1,** 8. Set TD2(**TD**) = 1024 or 2048 (1H Dimension, F2)
9. Set TD1(**1TD**) = 128 ~ 512 (t1 increment, F1)
10. Set NS = 4 or 8 (Sequence by sequence) -> gCOSY(Gradient COSY) could set NS =1 (Non-phase cycling)
11. Set **Key parameter**

(Parameters > Edit all acquisition parameter > Click pulse icon > Change **key parameter** > Return to icon-nmr)



7. Processing

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How to process 2D NMR in Mnova

Phase mode

NOESY, HSQC, etc

Hotkey (단축키) w

Sine bell = 90
or Sine Square = 90
(for F1,F2)

Hotkey (단축키) shift + p

Phase correction
(for F1,F2)

Magnitude mode

COSY, HMBC, etc

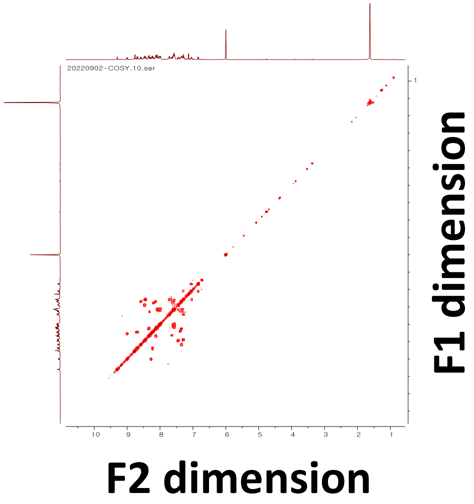
Sine bell = 0
or Sine Square = 0
(for F1,F2)

Hotkey (단축키) b

Baseline correction
(for F1, F2)

Ex) Polynomial (order 2~3)
Bernstein polynomial (order 2~3)

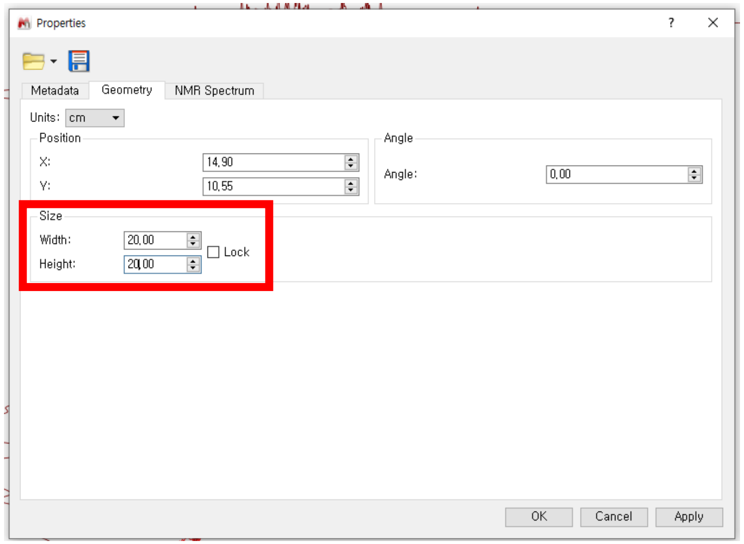
- **Zero Filling and LP (only F1)**
- **Processing -> Reduce t1 noise (case by case)**
- **Processing -> COSY like (ex. COSY, NOESY)**



https://nmr.chem.ucsb.edu/software/2D_processing_Mnova_v4.pdf

How to process 2D NMR in Mnova

1) Make Square



Units: cm

Position

X: 14,90

Y: 10,55

Angle

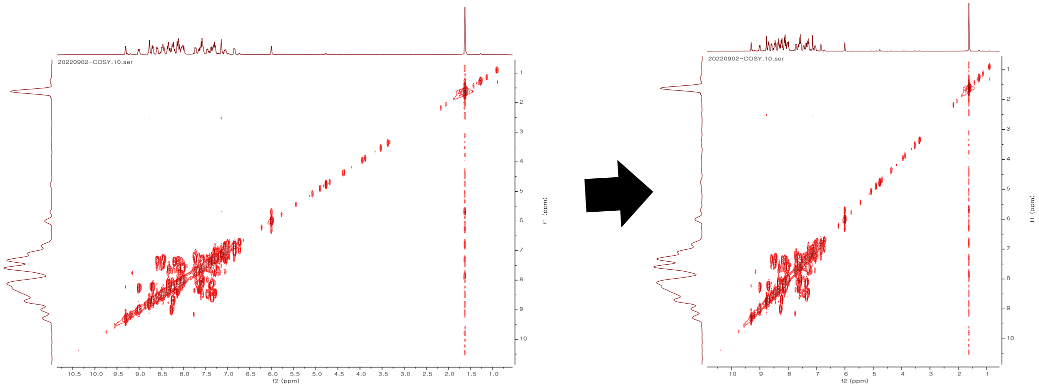
Angle: 0,00

Size

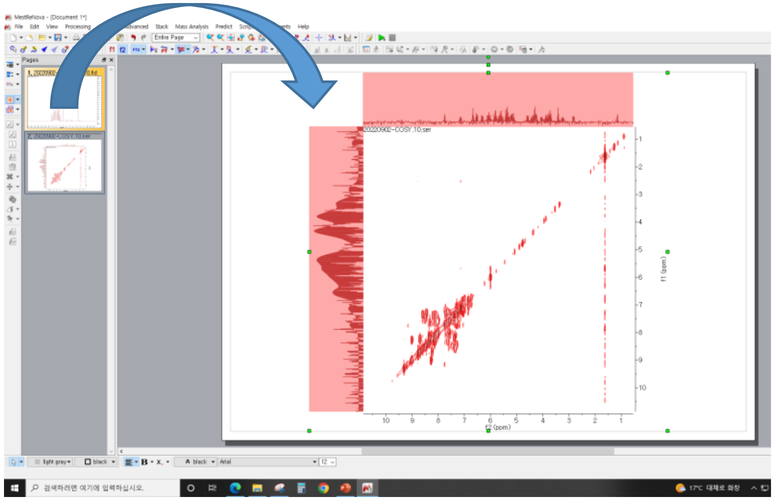
Width: 20,00

Height: 20,00

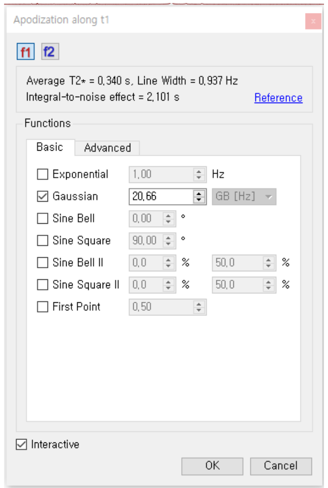
☐ Lock



2) Add 1D



3) Apodization (Hotkey = w)



Average T2* = 0,340 s, Line Width = 0,937 Hz

Integral-to-noise effect = 2,101 s

Functions

☐ Exponential 1,00 Hz

☑ Gaussian 20,66 GB [Hz]

☐ Sine Bell 0,00 °

☐ Sine Square 90,00 °

☐ Sine Bell II 0,0 % 50,0 %

☐ Sine Square II 0,0 % 50,0 %

☐ First Point 0,50

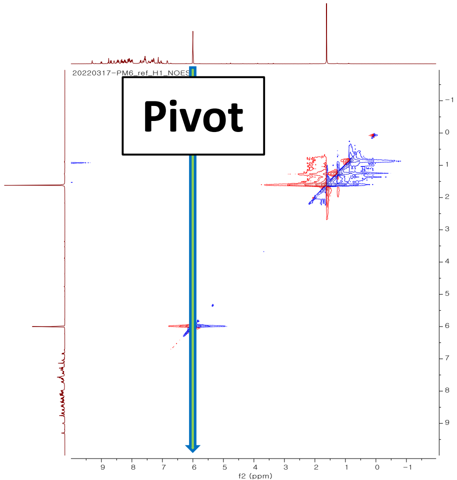
☑ Interactive

Phase mode
Sine Bell or Sine Square
= 90 (for F1, F2)

Magnitude mode
Sine Bell or Sine Square
= 90 (for F1, F2)

How to process 2D NMR in Mnova

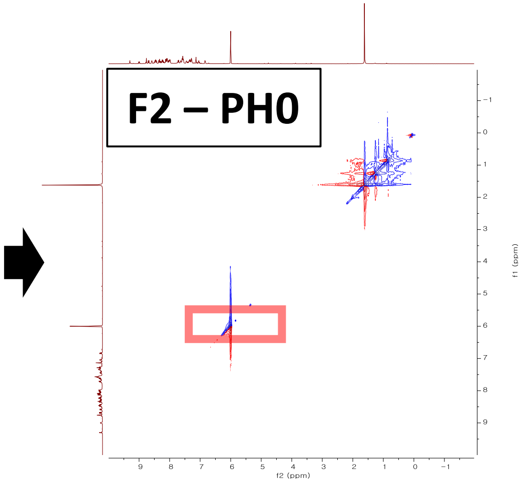
4) Phase correction (Hotkey = Shift + P)



Click F2

Set Pivot (Left or right end)

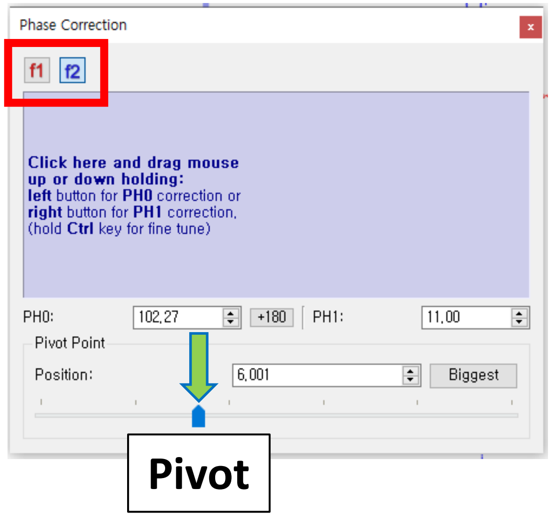
원하는 peak들 중 끝부분에 피벗을 옮김



Correct Phase to use PH0(0 order) / PH1(1 order)

PH0 -> Change Same amount, Correct phase on pivot position
(Pivot 위치의 peak에 대한 Phase correction)

PH1 -> Near Pivot: Small correct, Far Pivot: Large correct,
Correct phase other side on pivot position
(Pivot 위치의 반대편 끝 peak에 대한 Phase correction)



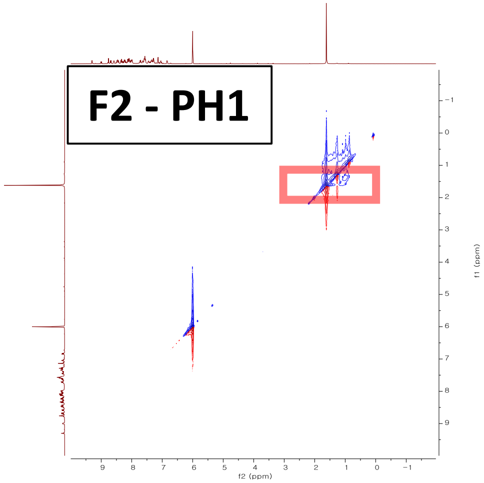
Click here and drag mouse up or down holding:
left button for **PH0** correction or
right button for **PH1** correction.
(hold **Ctrl** key for fine tune)

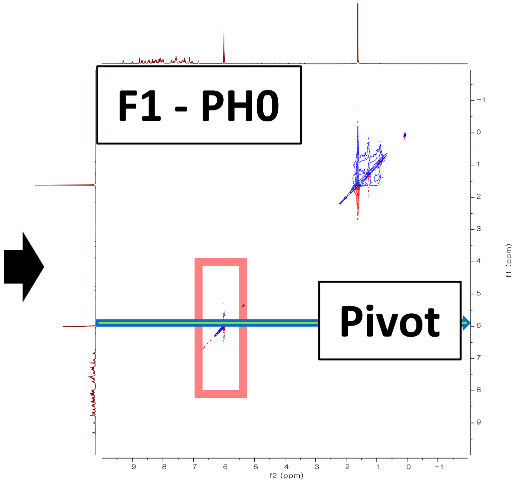
PH0: 102,27

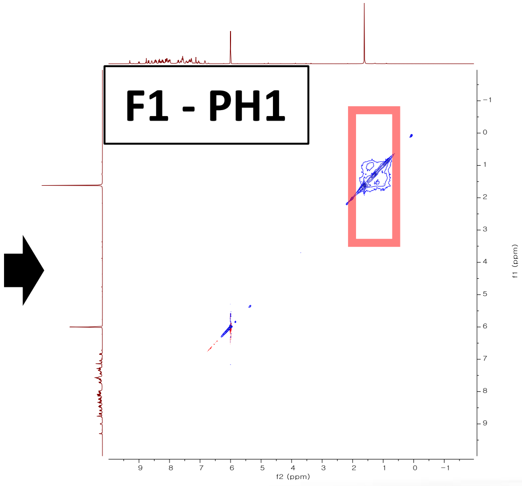
PH1: 11,00

Pivot Point

Position: 6,001

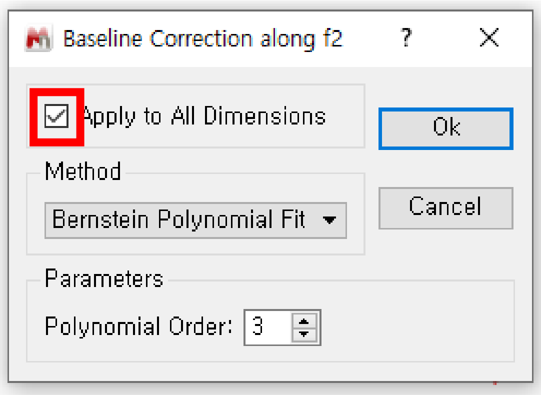






How to process 2D NMR in Mnova

5) Baseline correction (Hotkey = b)



Baseline Correction along f2

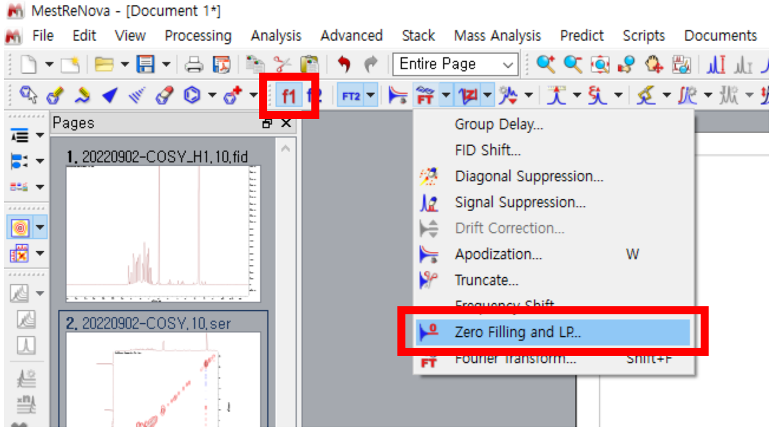
☑ Apply to All Dimensions

Method: Bernstein Polynomial Fit

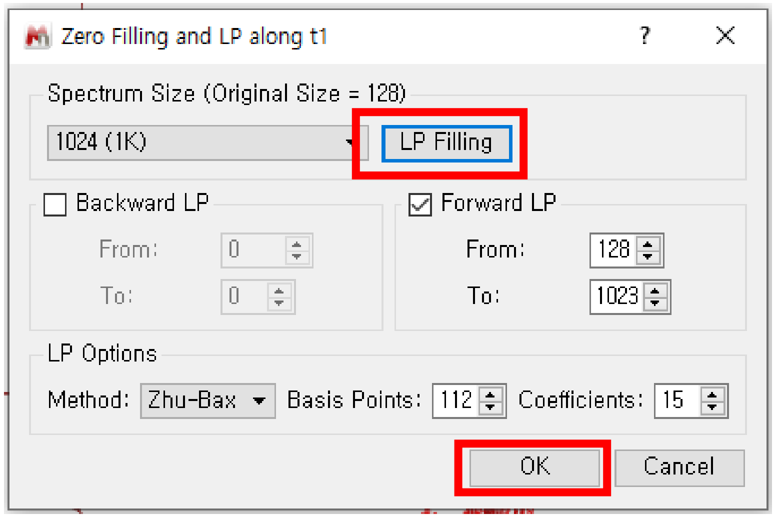
Parameters

Polynomial Order: 3

6) Zero Filling and LP (F1)



MestReNova - [Document 1*]



Zero Filling and LP along t1

Spectrum Size (Original Size = 128): 1024 (1K)

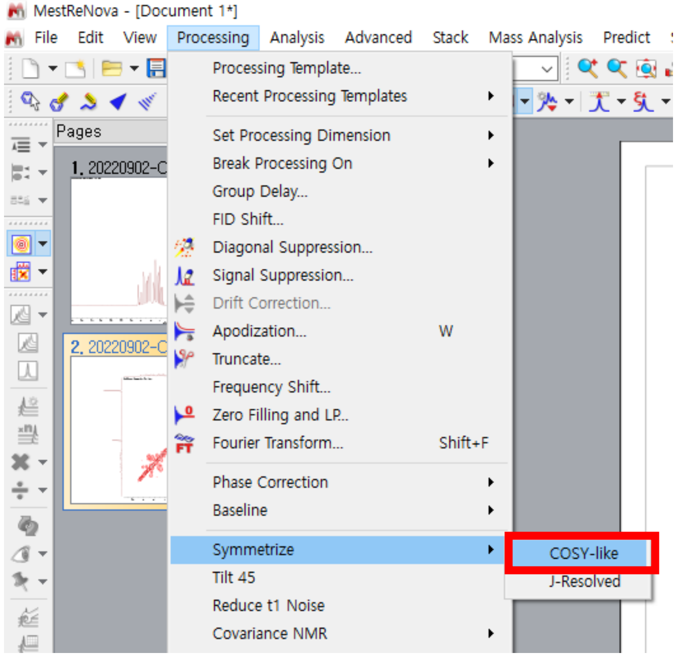
☐ Backward LP — From: 0 To: 0

☑ Forward LP — From: 128 To: 1023

LP Options

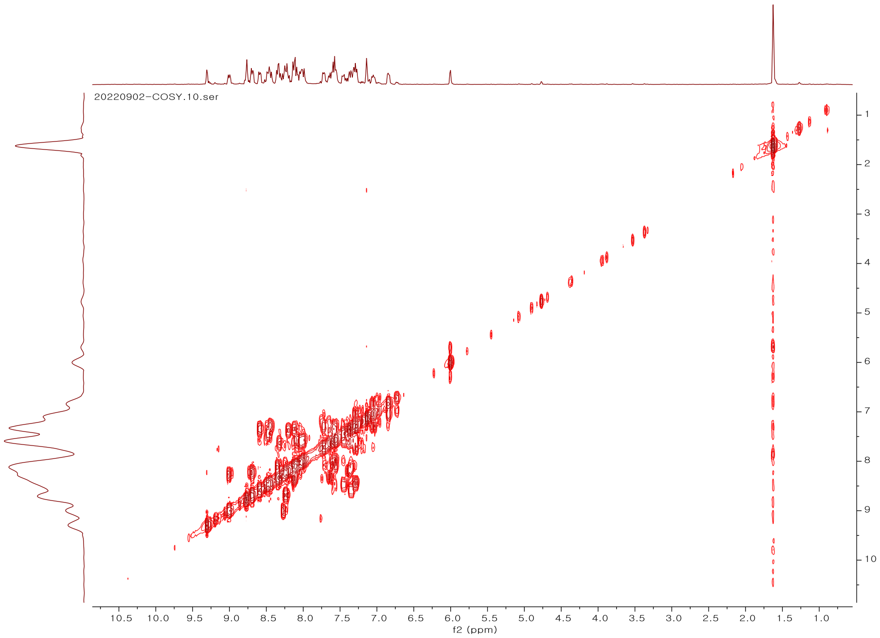
Method: Zhu-Bax Basis Points: 112 Coefficients: 15

7) COSY-like (Make Symmetry)



MestReNova - [Document 1*]

Processing result



Processed