NMR Training Course
(Instrument and Experiments)

JEOL Ltd.
Taiwan
陳國豐 Edward Chen

Instrument

Model:

JEOL JNM-ECZL400S

Temperature：

-100 ~ +150℃

Nuclei：

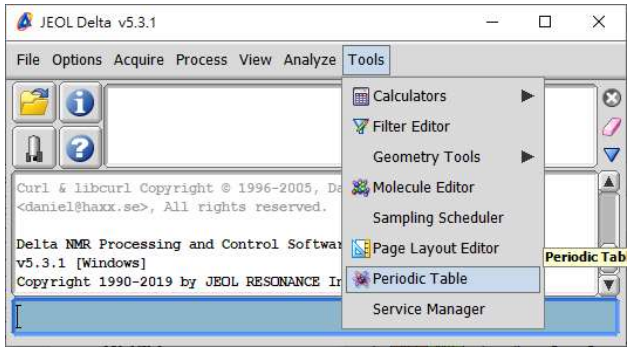
HF: 1H · 19F

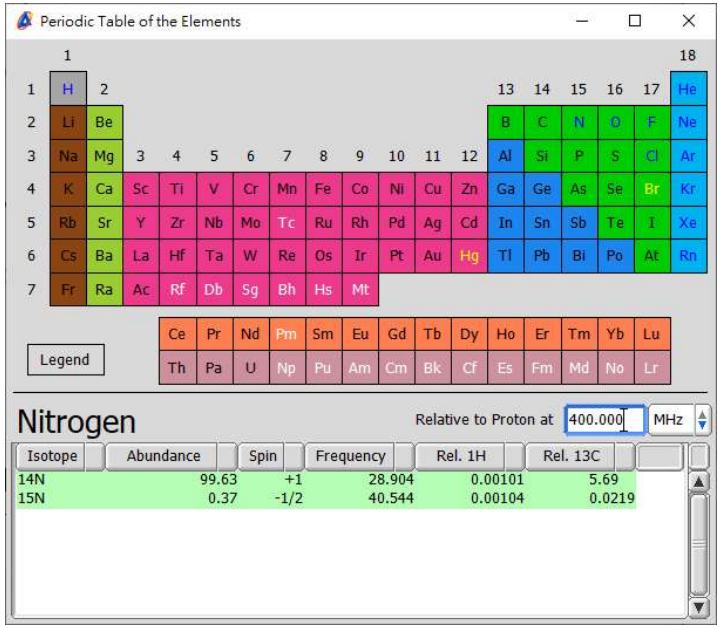
LF: 31P~15N, 39K, 109Ag

(Auto-Tune)

Auto-sampler：

ASC-24







Liquid Probe

(Application V.S. Sensitivity)

Reverse Probe

JEOL: TH5

Bruker: BBFO

Agilent: SW

Good for 13C

13C = 100%

1H = 50%

Inverse Probe

JEOL: H5X

Bruker: BBI

Agilent: ID

Good for 1H & 2D

13C = 50%

1H = 100%

2 in 1 Probe

JEOL: Royal

Bruker: smart (iprobe)

Agilent: One

Good for Both

13C ≈ 100%

1H = 100%

4 Nucleus Probe

JEOL: TFH

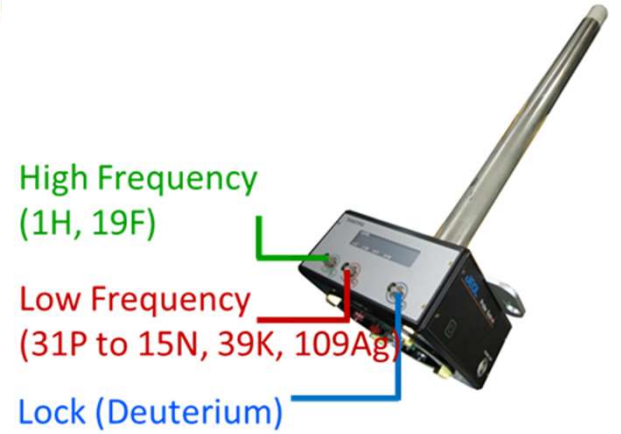
Bruker: QNP

Agilent: 4nu

Low sensitivity

Triple resonance probe

Limited & Low sensitivity & High cost



JEOL & Agilent:	19F at high band
Bruker:	19F at low band

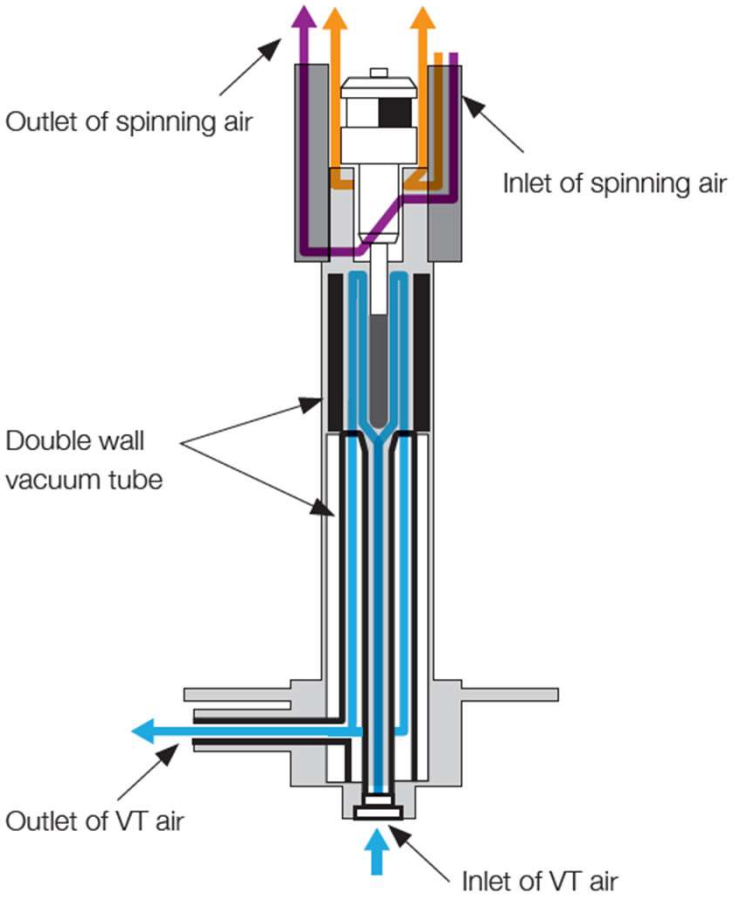
Various temperature

For high- and low-temperature experiments:

- **No nitrogen gas required (carrier gas)**
- **No need to change the rotor material**
- **Normal auto-shimming available**

- **Room temperature to +150 °C (without cryogen)**
- **-100 to +50 °C (with Dewar vessel)**





PCW (Phase Covariance method)

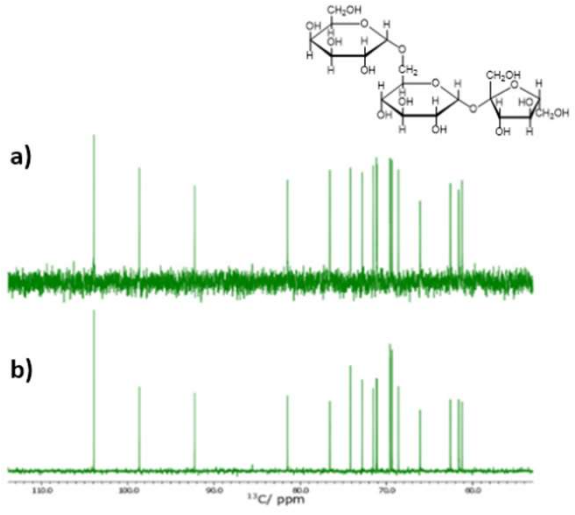


Fig. 1 : ^{13}C NMR spectra of raffinose by a) conventional and b) PCW method

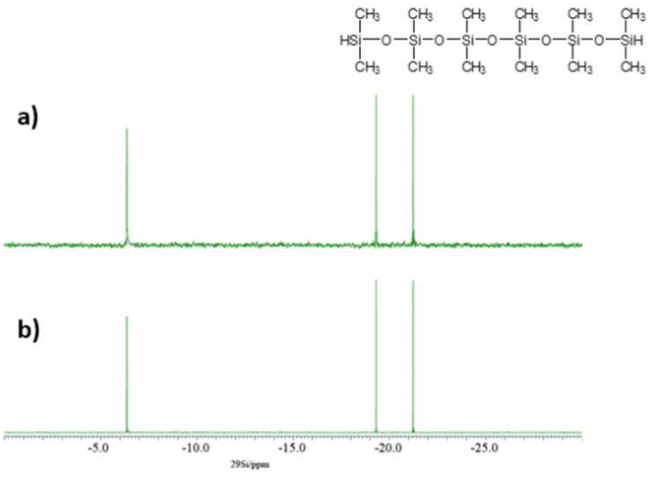
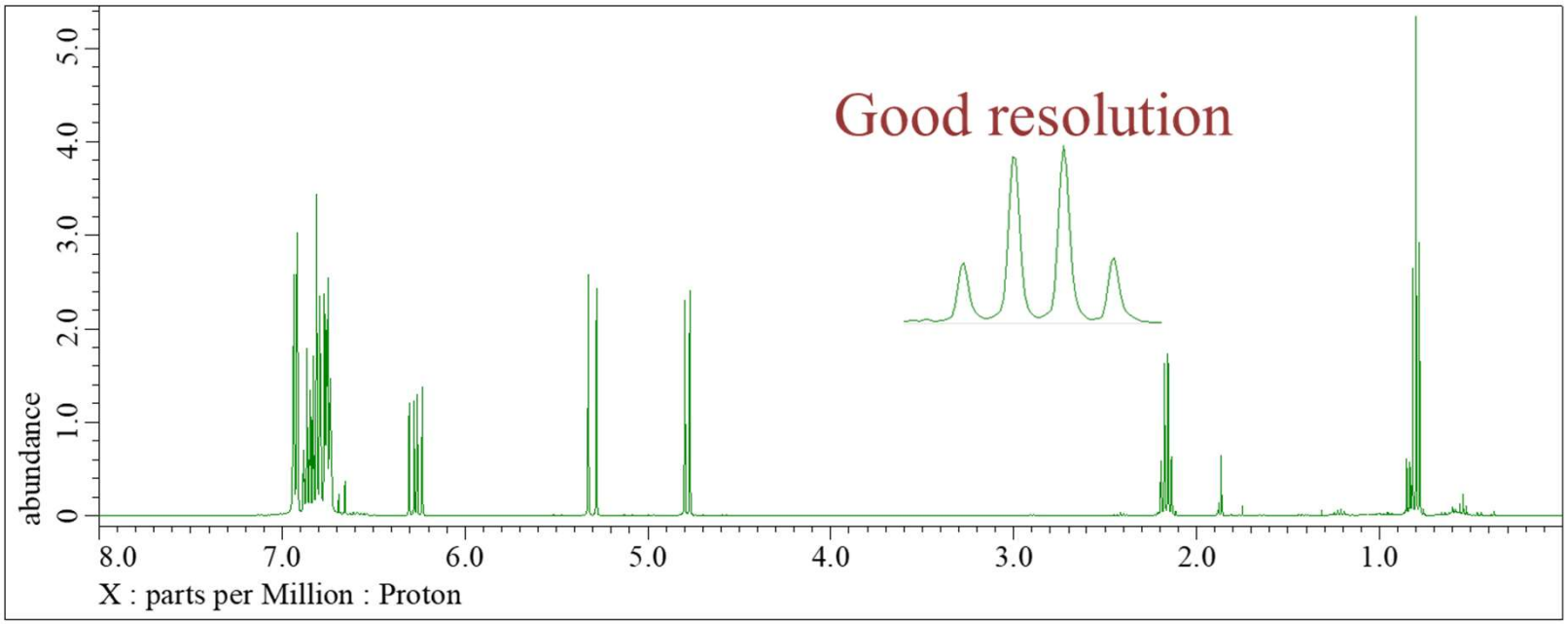


Fig. 2 : ^{29}Si NMR spectra of 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethylhexasiloxane by a) conventional and b) PCW method

No-D Sample ^{1}H-NMR

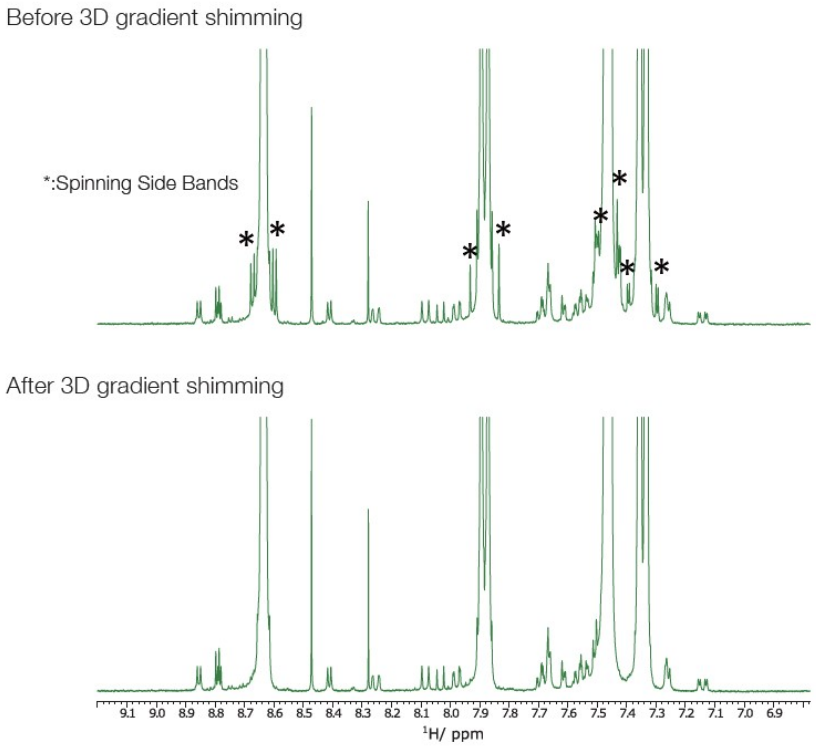
- Auto-measurement (<5min)
- Auto-shimming
- Auto-gain
- Auto-process



Various Shimming Application

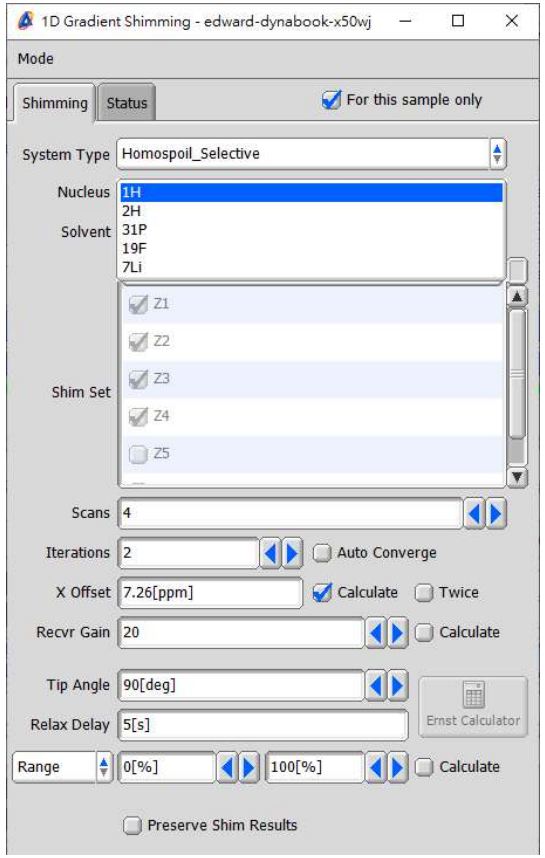
Automatic 3D shimming system (System maintenance is easy)





Poorly adjusted radial shims under sample rotation (top) and corrected with 3D gradient shimming (bottom)

Various nucleus for gradient shimming use (Apply for different application field)



Automation

Automation Experiments

- Common Experiments
  - 1H, 13C, DEPT, COSY, HSQC, HMBC, NOESY, ROESY, TOCSY
- No D Experiments
  - 1H, 13C, COSY, HSQC, HMBC, NOESY, ROESY, TOCSY
- Solvent Suppression
  - pre-saturation, Watergate, Excitation Sculpting, WET
- qNMR
- Fast NMR
  - ASAP, NOAH
- Pureshift
- Various temperature experiment
- 3D shimming

Experiments

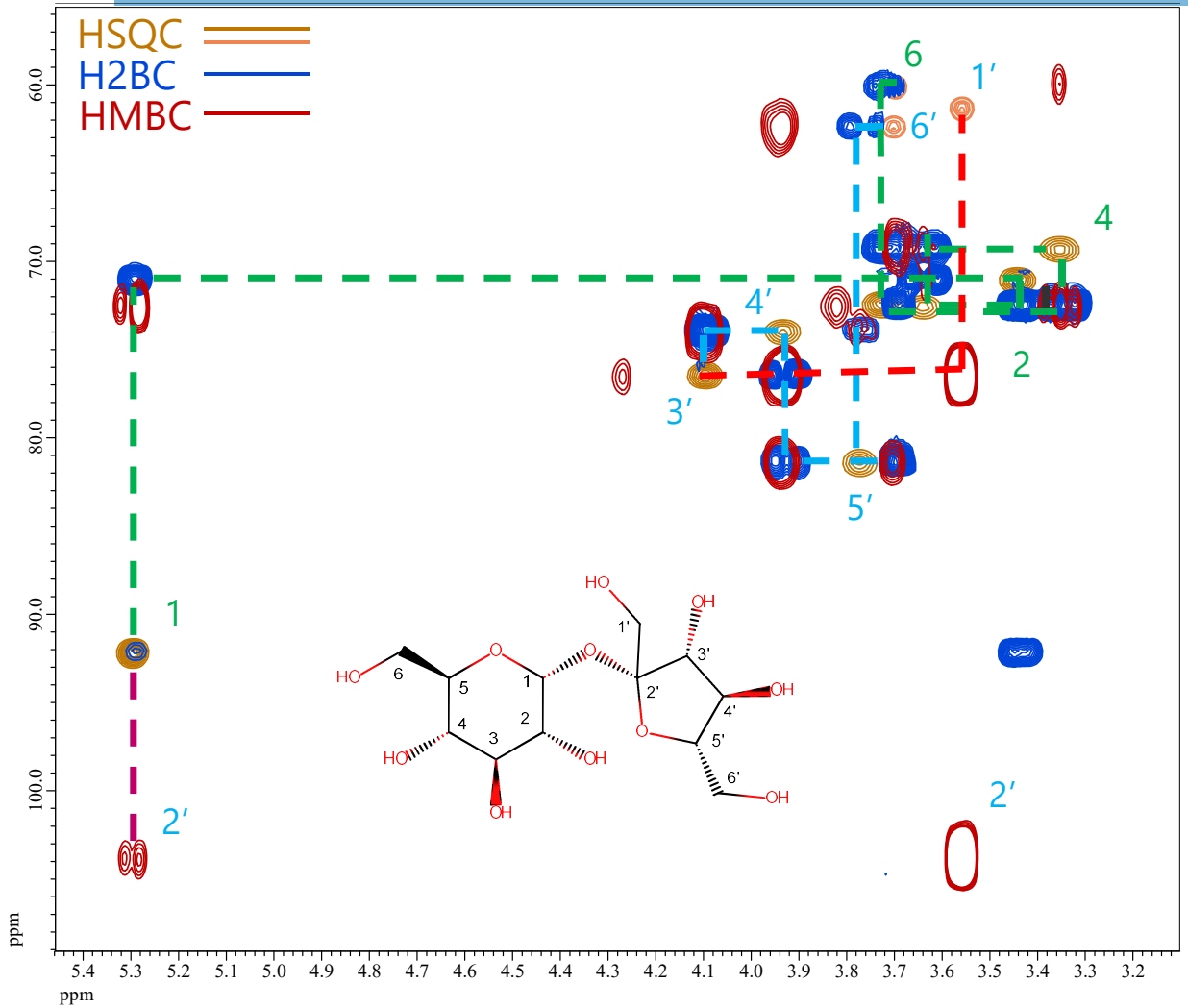
Common NMR Experiments

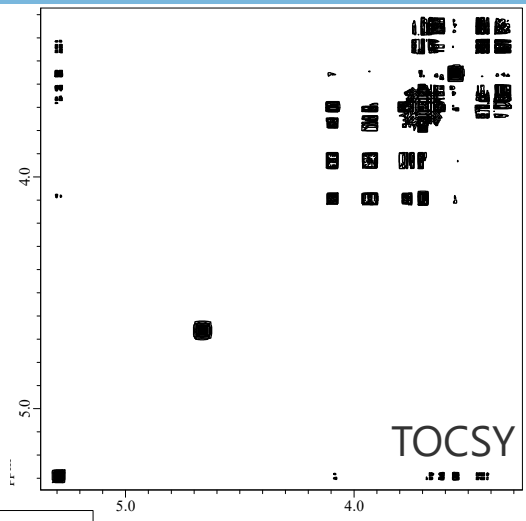
- 1H
  - Relaxation (T1, T2)
  - Homodecouple
  - NOE
- 13C (multinuclear)
  - DEPT
- 1D/2D
  - COSY, TOCSY
  - NOESY, ROESY
  - HMQC, HSQC
  - J-resolve
  - HMBC, H2BC, Cigar, LRHSQMBC
  - HSQC-TOCSY, HSQC-NOESY, Inadequate, Adequate

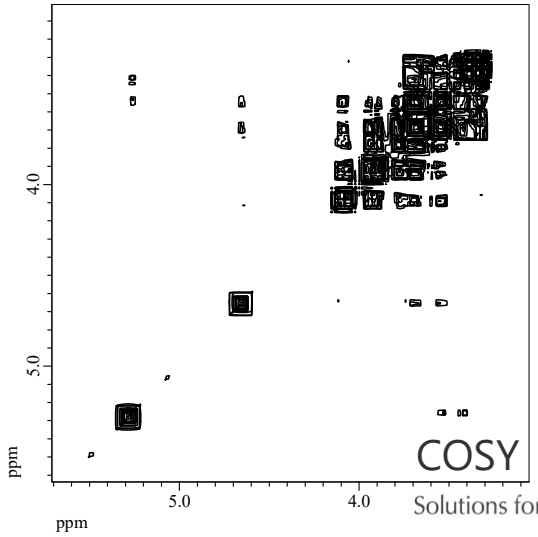
Advanced NMR Experiments and Setting

- No D Experiments
  - 1D, 2D
- DOSY
  - 1D, 2D, 3D
- Fast Experiments
  - NUS, NOAH, ASAP
- Pureshift
  - 1D, 2D
- CRAFT (Process)
- Kinetic

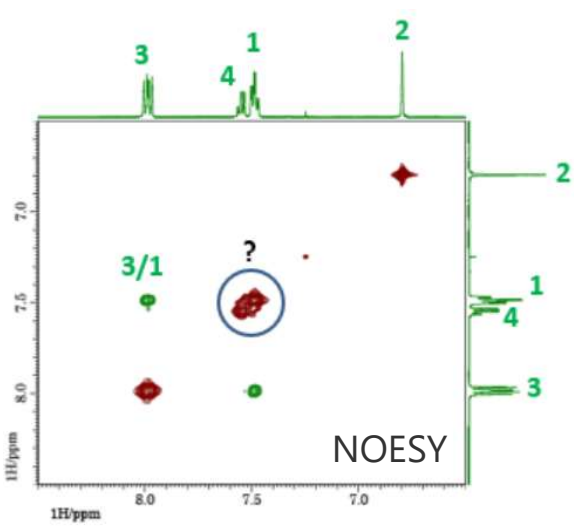
Sensitivity V.S. Resolution Experiments in 2D NMR

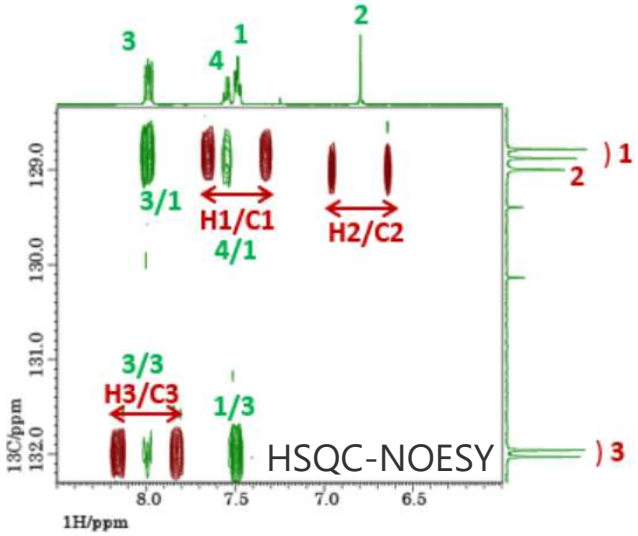


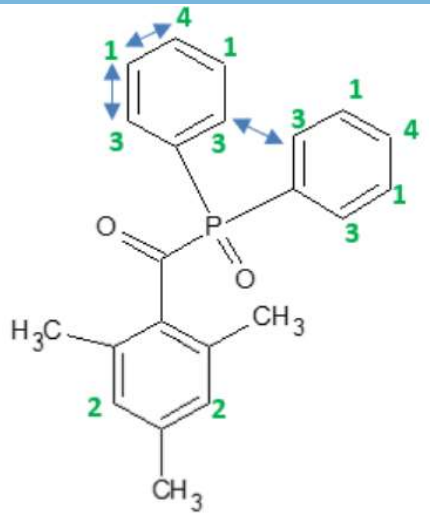


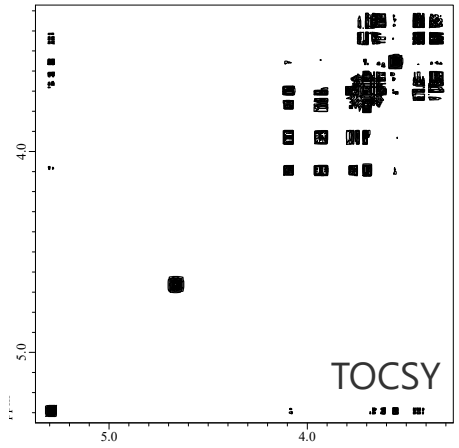


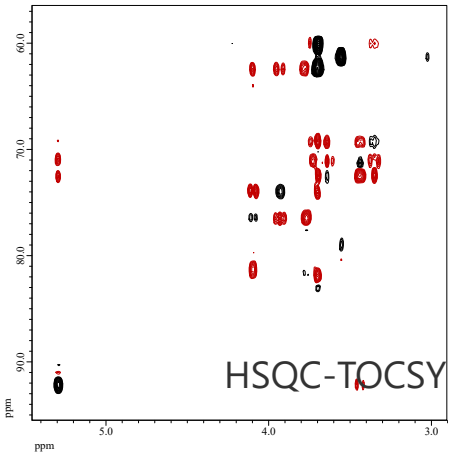
Sensitivity V.S. Resolution Experiments in 2D NMR

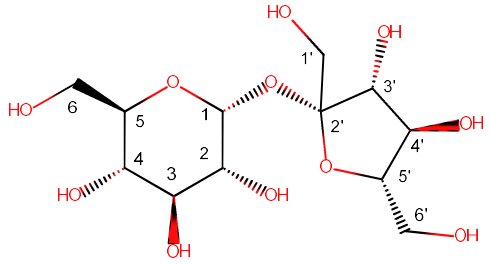




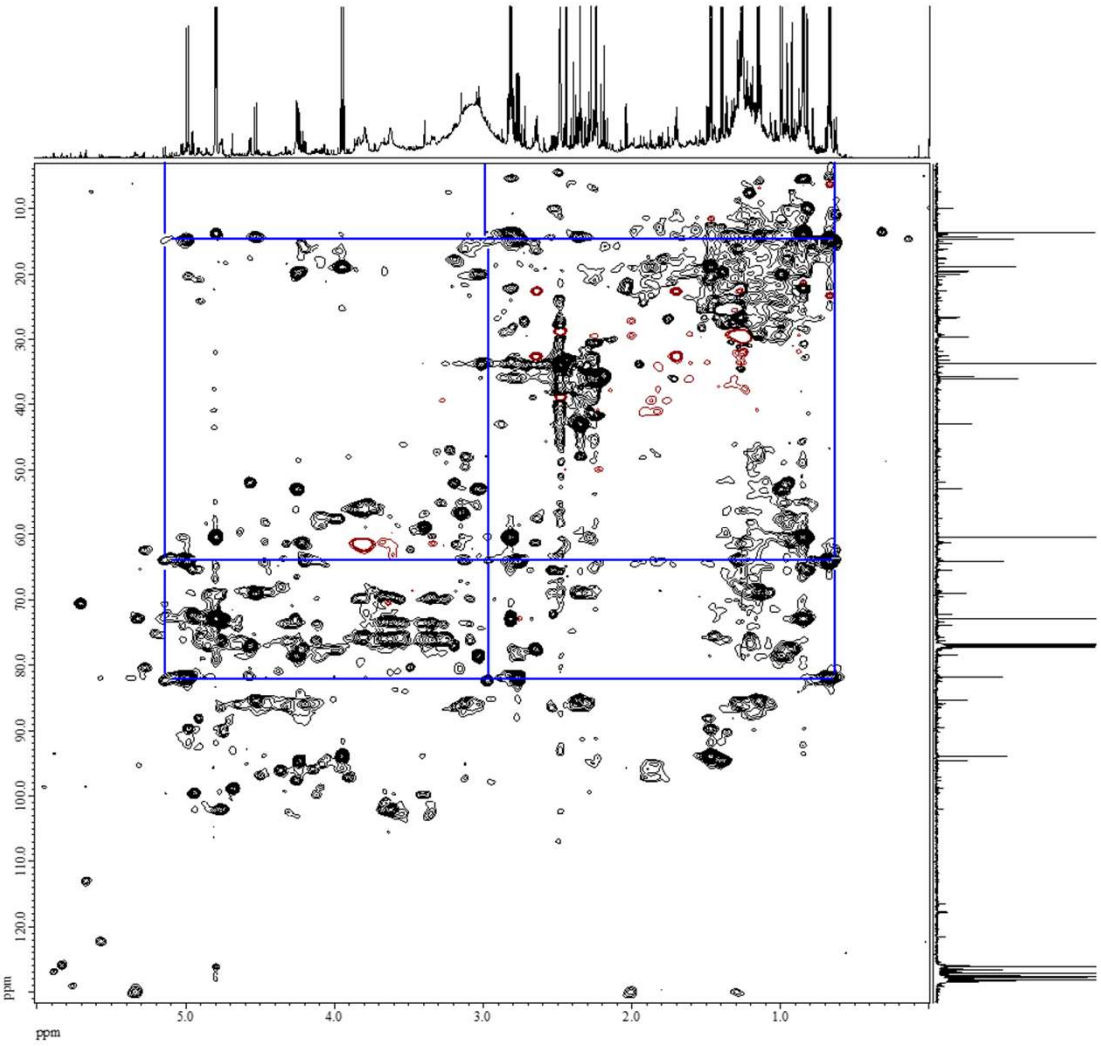


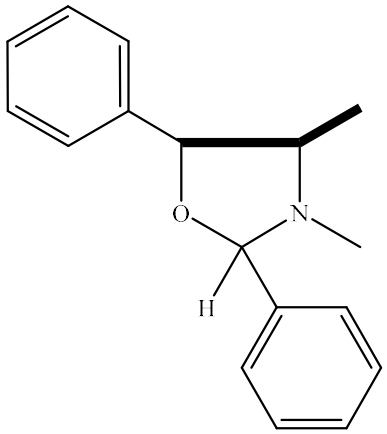






HSQC-TOCSY


10.0
20.0
30.0
40.0
50.0
60.0
70.0
80.0
90.0
100.0
110.0
120.0
ppm
5.0
4.0
3.0
2.0
1.0
ppm


O
N
H

FAST NMR experiments

ASAP, NOAH, NUS

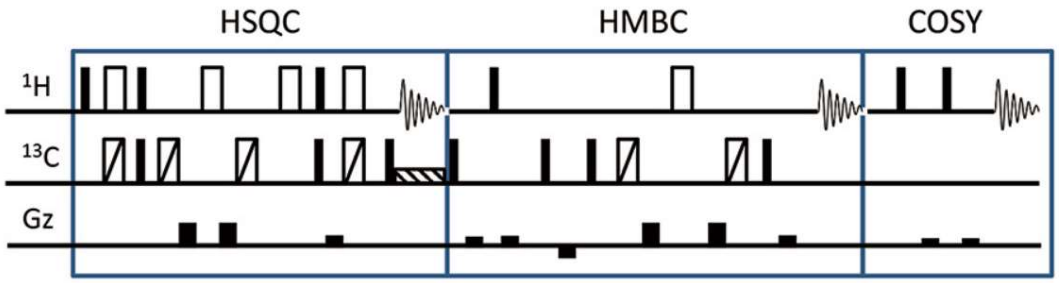


Fig. 1: Pulse sequence of NOAH-3

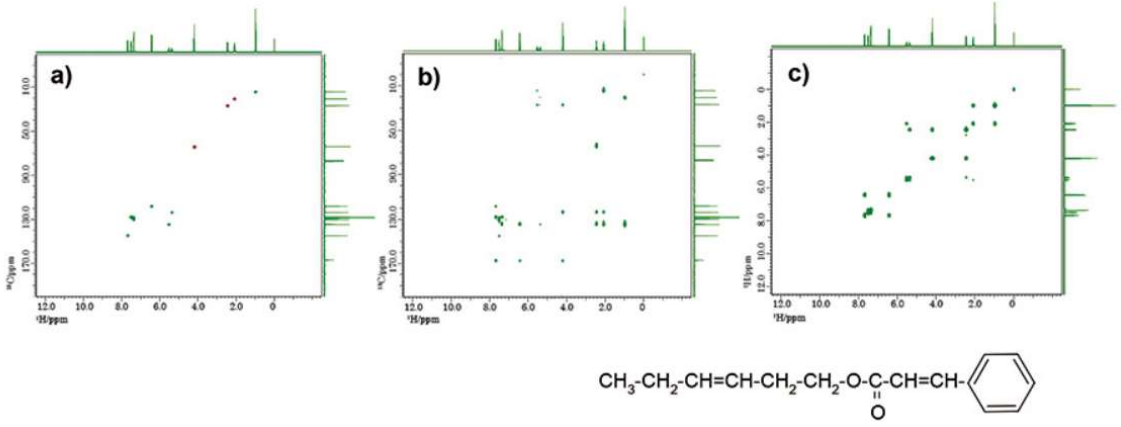
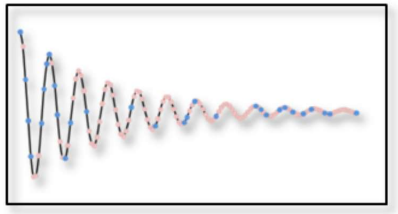


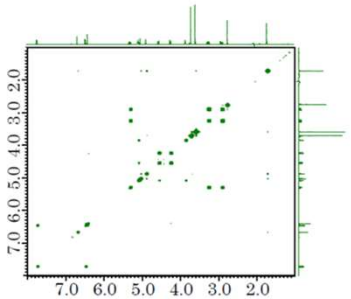
Fig. 2: NOAH-3: a) HSQC, b) HMBC, c) COSY

Number of scans: 1, Y points: 256, NUS density: 25%, exp. time: 5 min

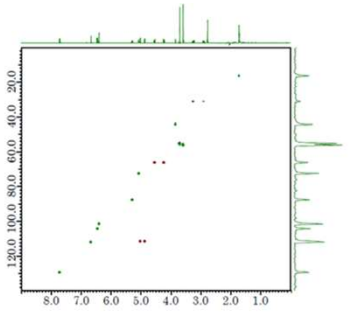


- Sampling points
- Reconstructed points

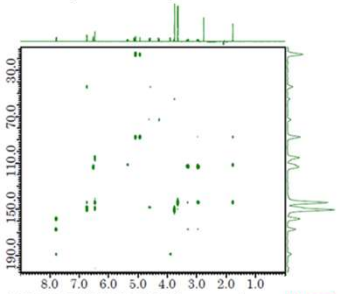
Fig.9 NUS + reconstruction (25% data collection)



COSY (NUS25%) ext time: 4 min

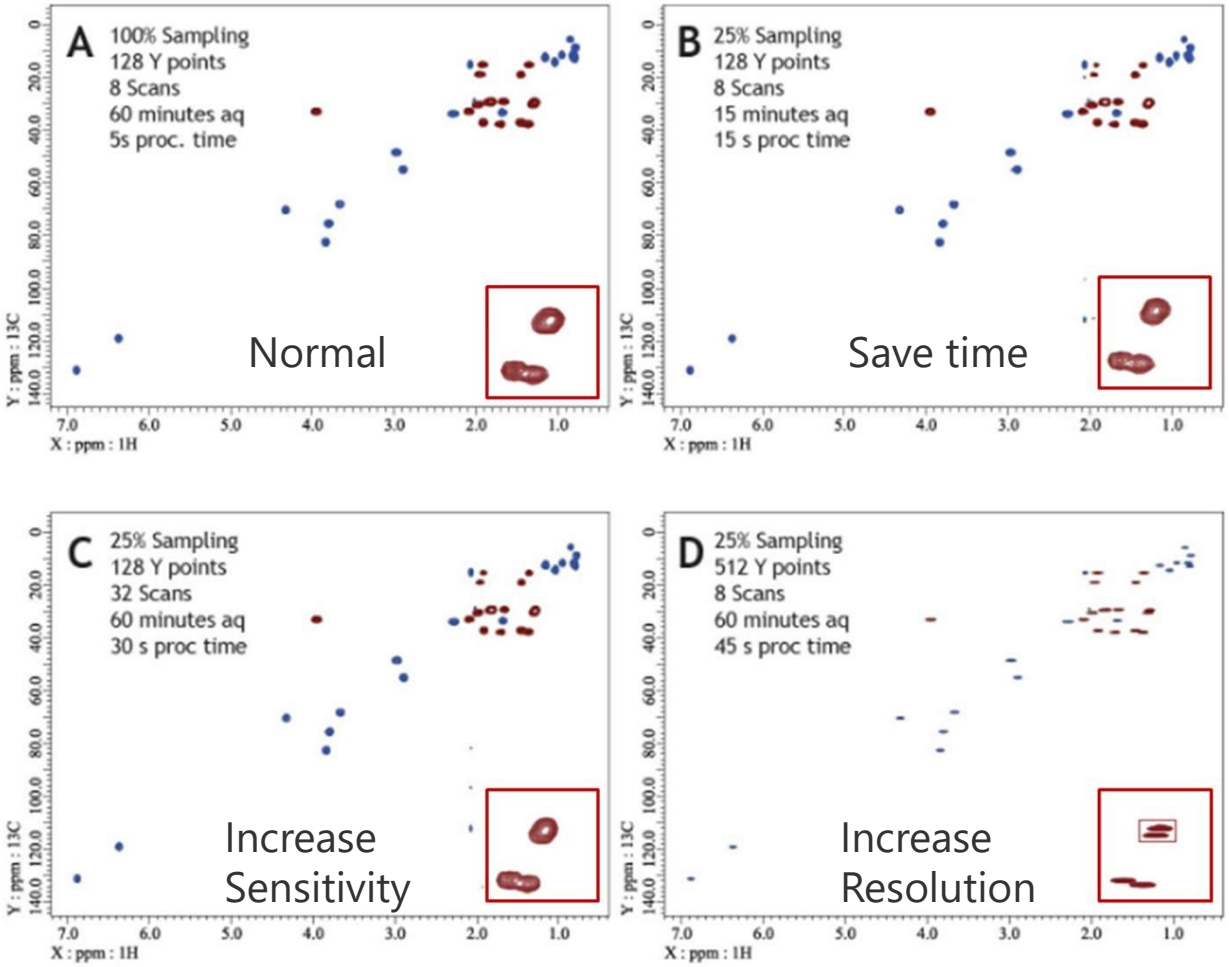


HSQC (NUS25%) ext time: 15 min

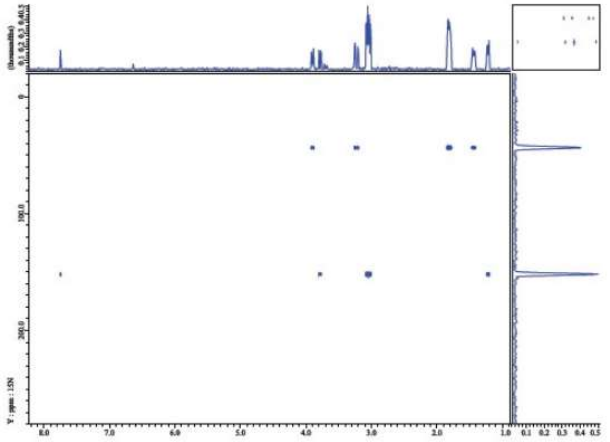


HMBC (NUS25%) ext time: 9 min

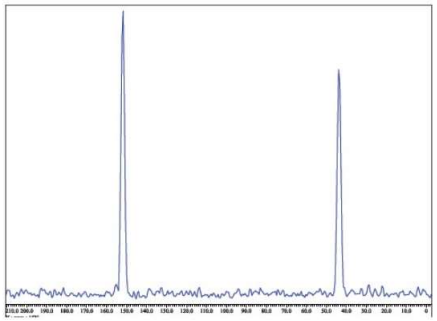
Fast experiment application



H-N gHMBCAD of brucine (50 mg, chloroform-d).

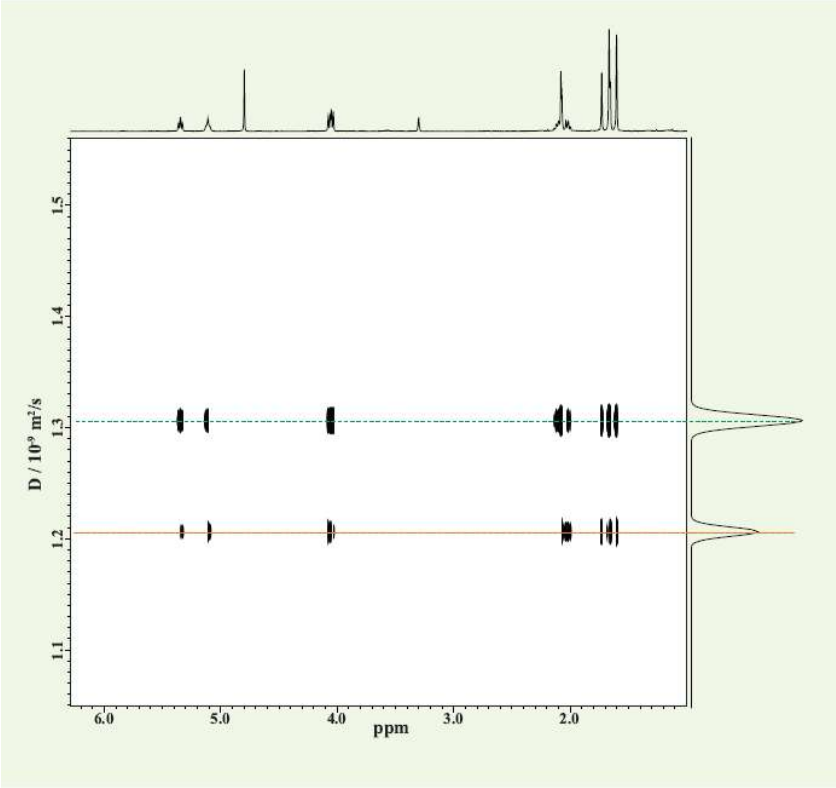


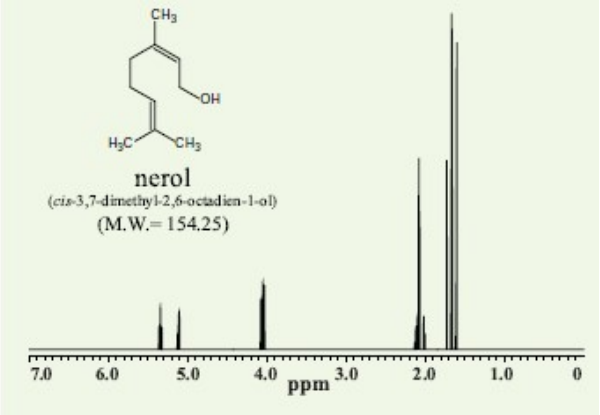
Projection of the Y-dimension as a 1D 15N spectrum.

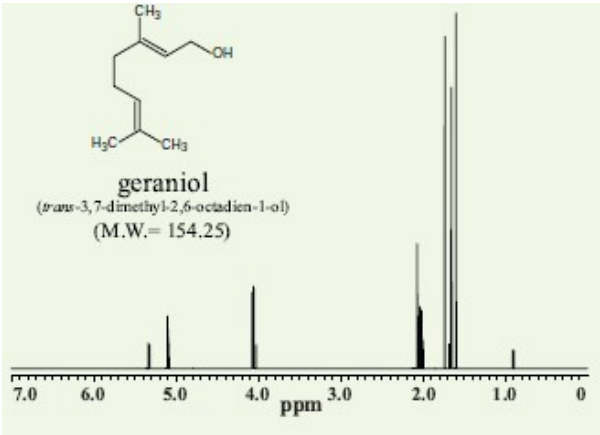


Solutions for Innovation JEOL

DOSY (Diffusion-Ordered Spectroscopy)



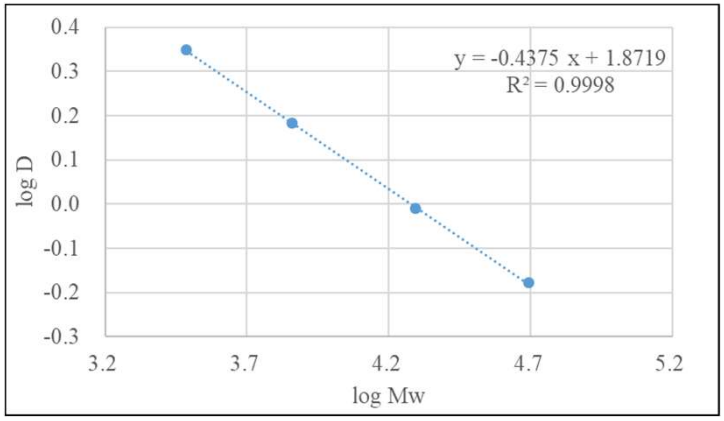




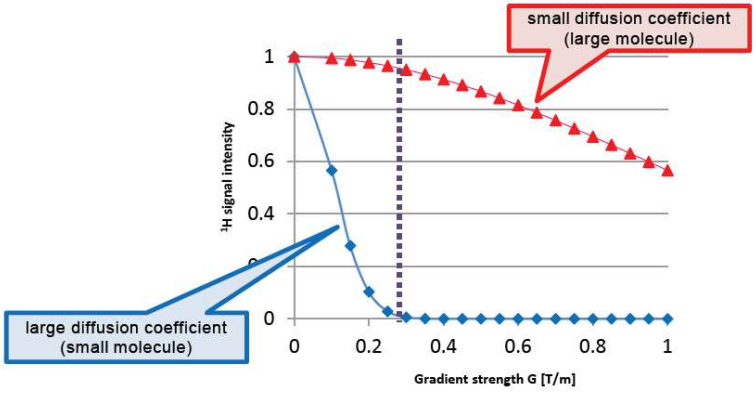
DOSY -molecule weight (Using No-D NMR)

- *Standard
  - *Polystyrene
- *Solvent
  - *CH_2Cl_2
- *Experiment
  - *DOSY with Pre-saturation

Compound name	Mw	Log Mw	diffusion coefficieon (m2/Gs)	Log D	Results (Sample Mw)
Std-1 (Polystyrene)	7210	3.8579	1.524	0.1830	
Std-2 (Polystyrene)	19600	4.2923	0.977	-0.0101	
Std-3 (Polystyrene)	49200	4.6920	0.664	-0.1778	
Std-4 (Polystyrene)	3070	3.4871	2.230	0.3483	
Sample-1		4.4553	0.837	-0.0773	28527
Sample-2		4.4435	0.847	-0.0721	27763
Sample-3		3.5704	2.041	0.3098	3719



Filter experiments in NMR -Diffusion filter
- small molecule suppression



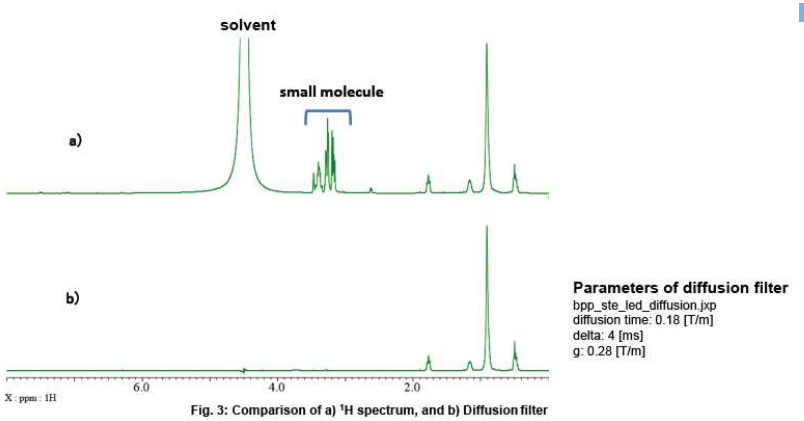
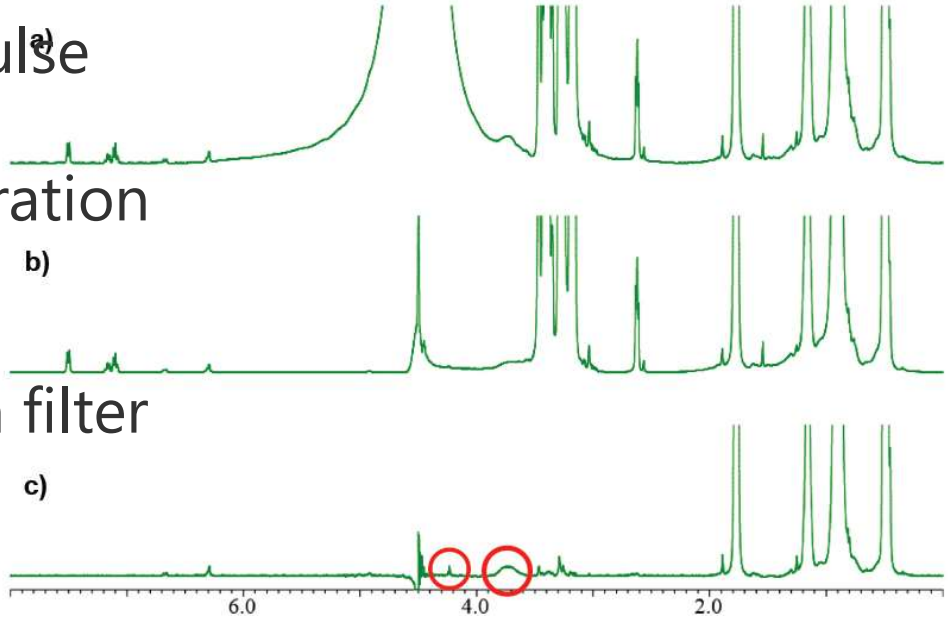


Fig. 3: Comparison of a) ¹H spectrum, and b) Diffusion filter

Single pulse

Pre-saturation

Diffusion filter



Enantone analysis

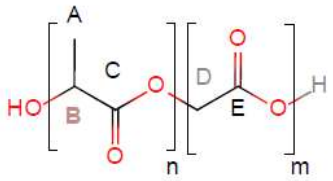
1H

ENANTONE

End group: N/D

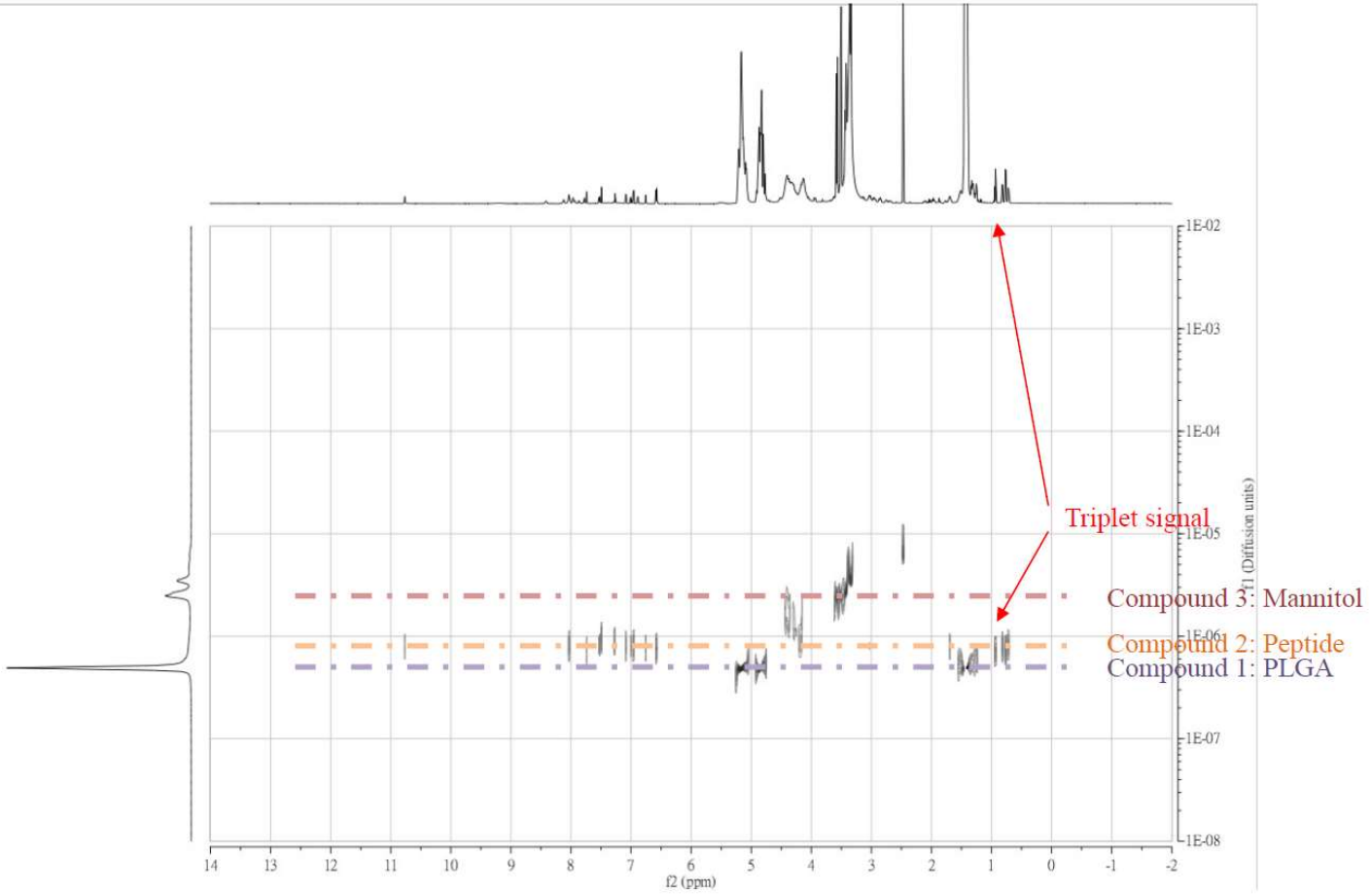
Mn: N/A

LA (n): GA (m)= 3:1



Enantone analysis

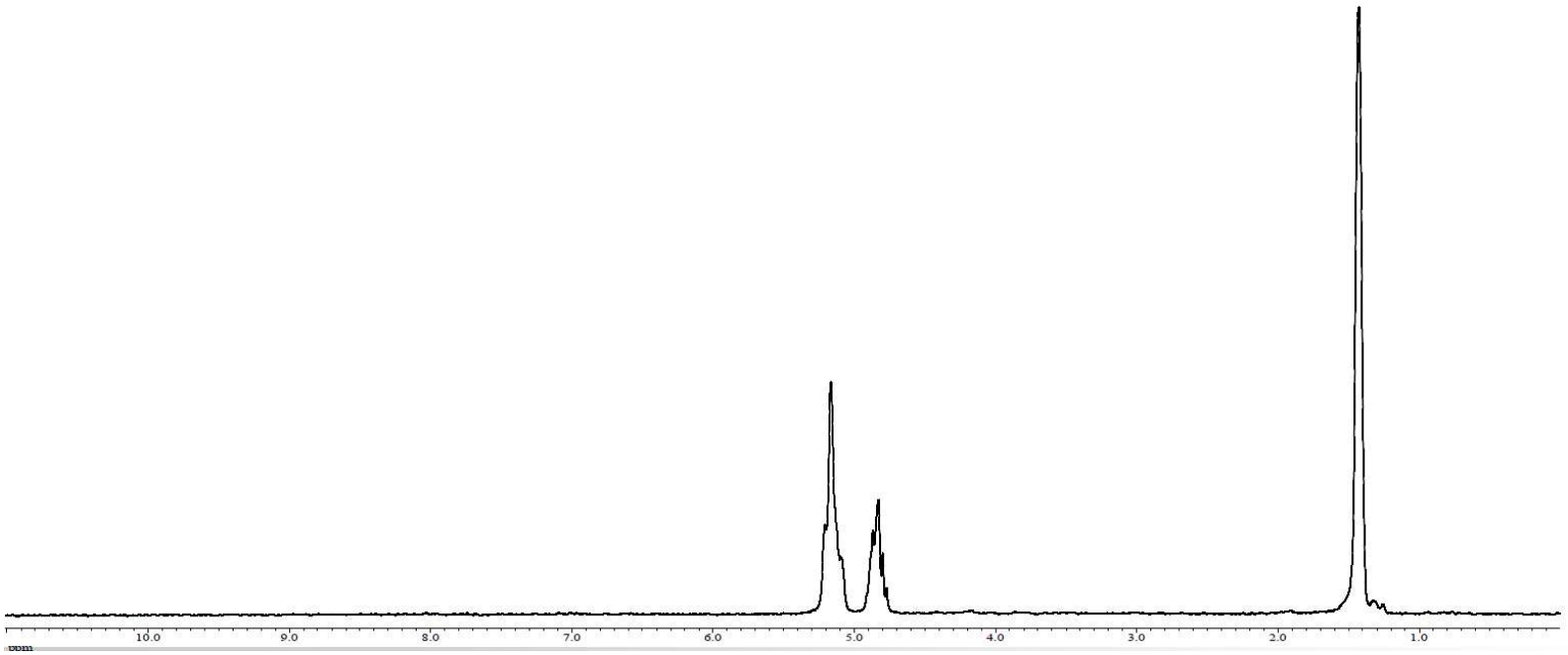
DOSY



Enantone analysis

diffusion filter

Polymer signal



Filter experiments in NMR -T2 filter
- large molecule suppression

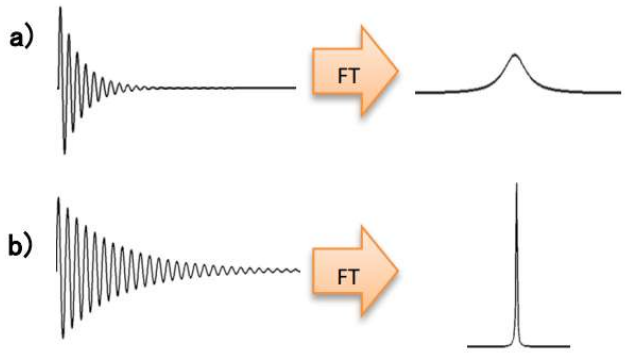


Fig. 1: Decay time and line width of a) fast decaying signals, and b) slow decaying signals

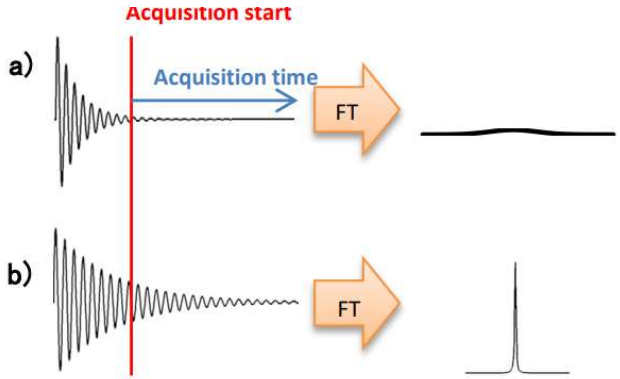


Fig. 2: Effect of T_2-filter on a) fast decaying signals, and b) slow decaying signals

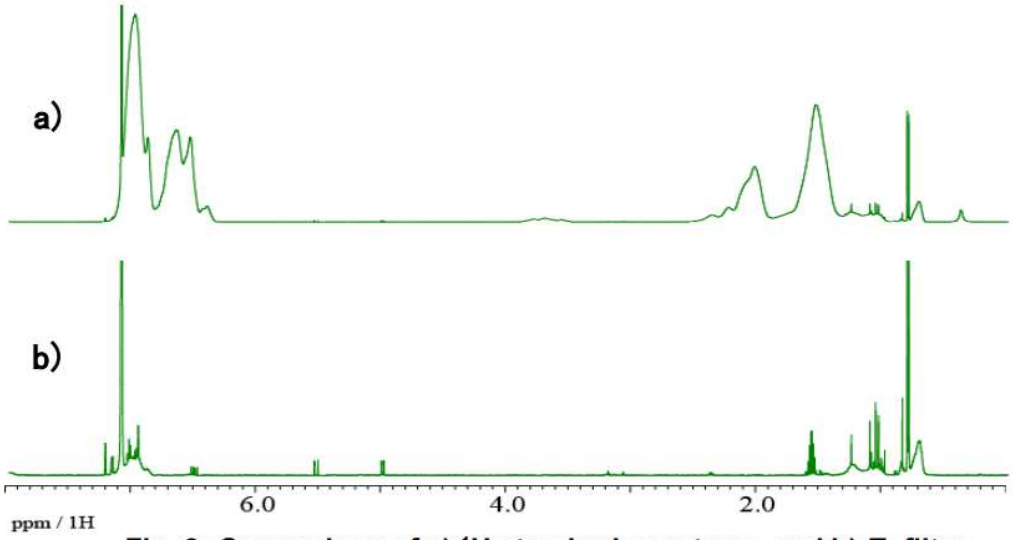


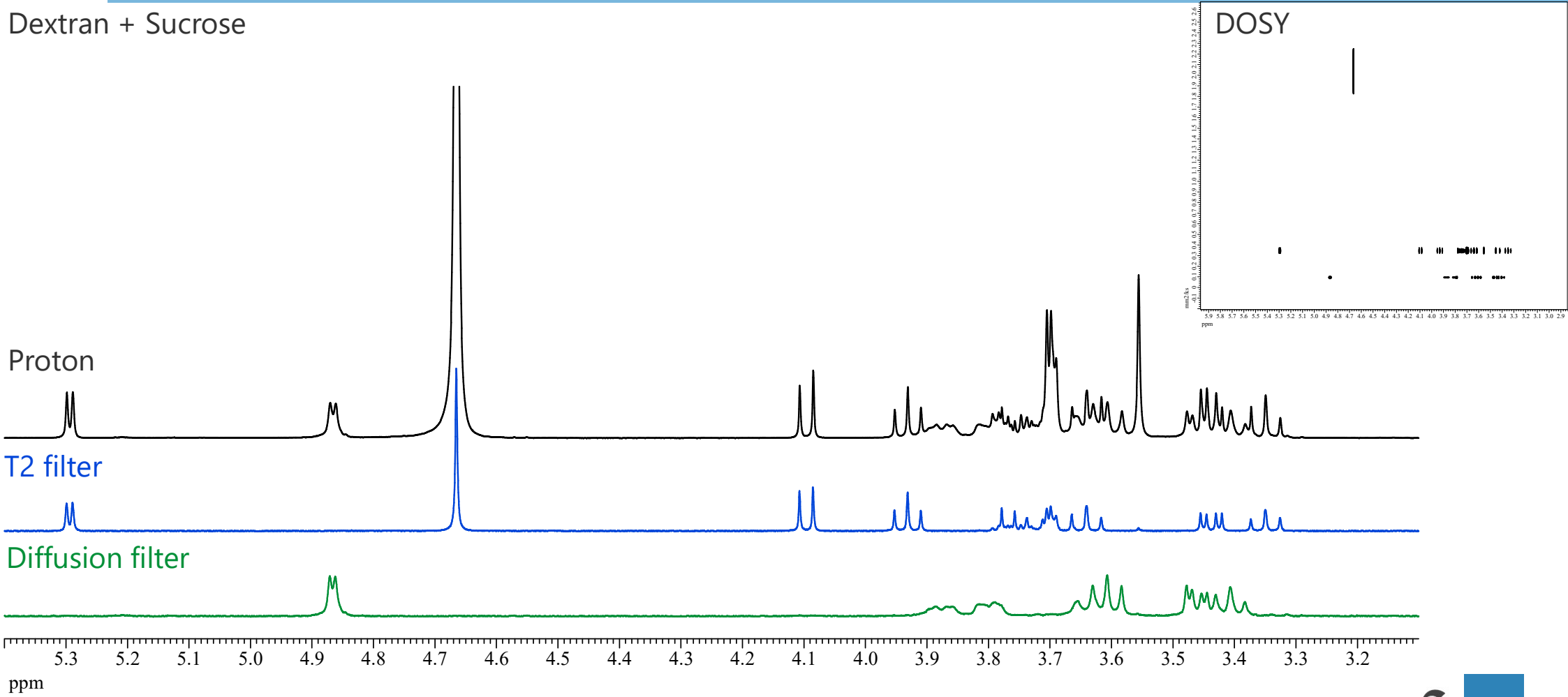
Fig. 3: Comparison of a) ^{1}H standard spectrum, and b) T_2-filter.

Parameters of T_2-filter

Experiment: cpmg_project.jxp
Scans: 32
Delay_list: 2.5 [s]
Tau_step: 10 [ms]

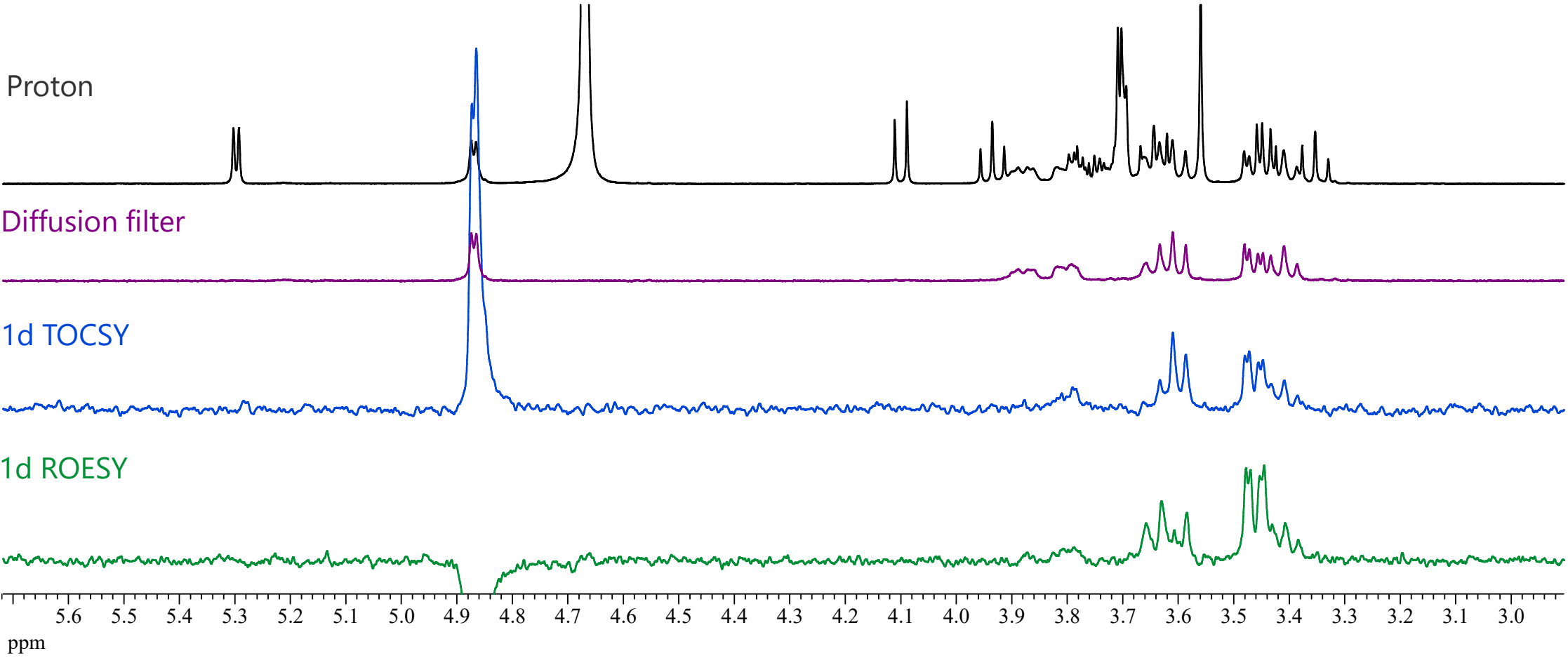
1D V.S. 2D

- Suppression, Selective & filter in NMR



1D V.S. 2D

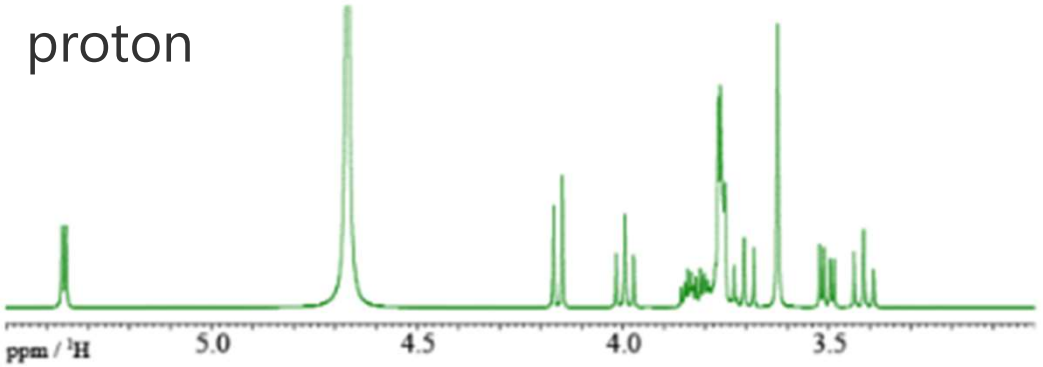
- Suppression, Selective & filter in NMR



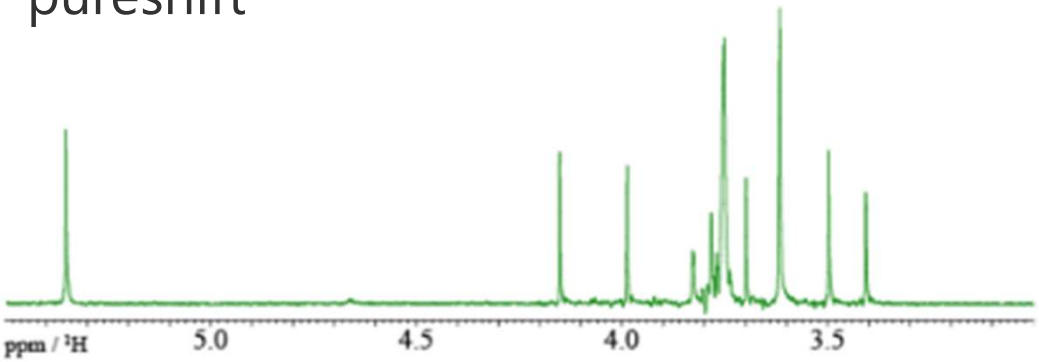
Eliminate coupling effect experiments

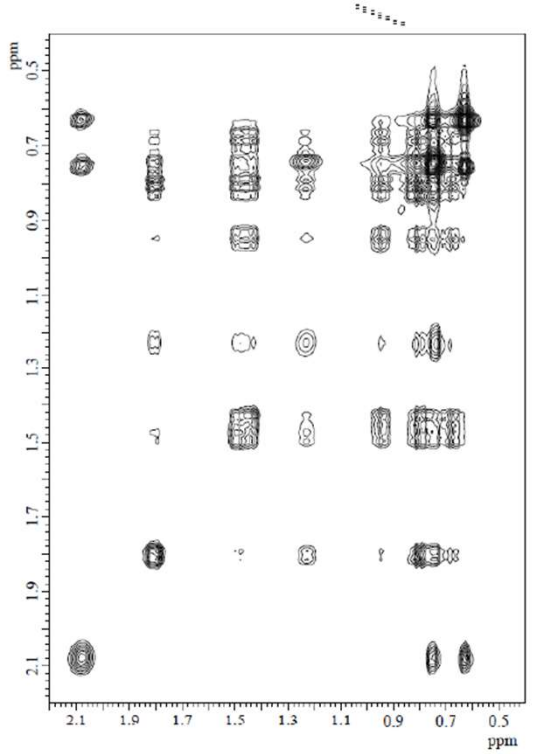
Pureshift

proton

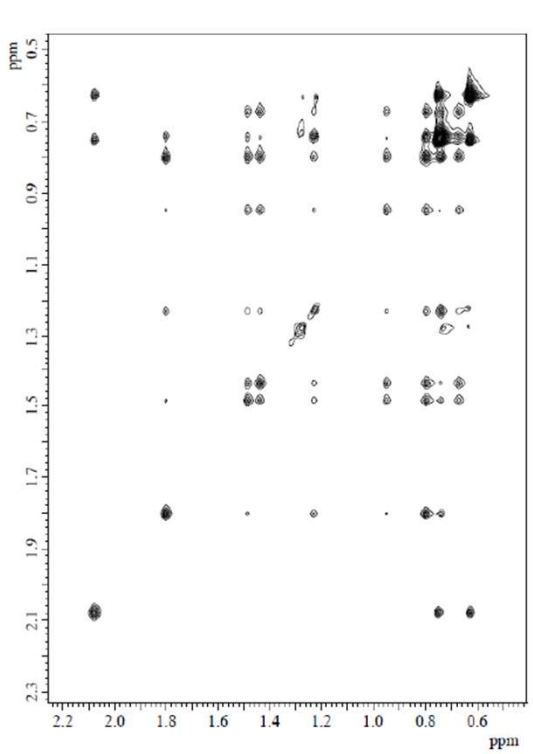


pureshift



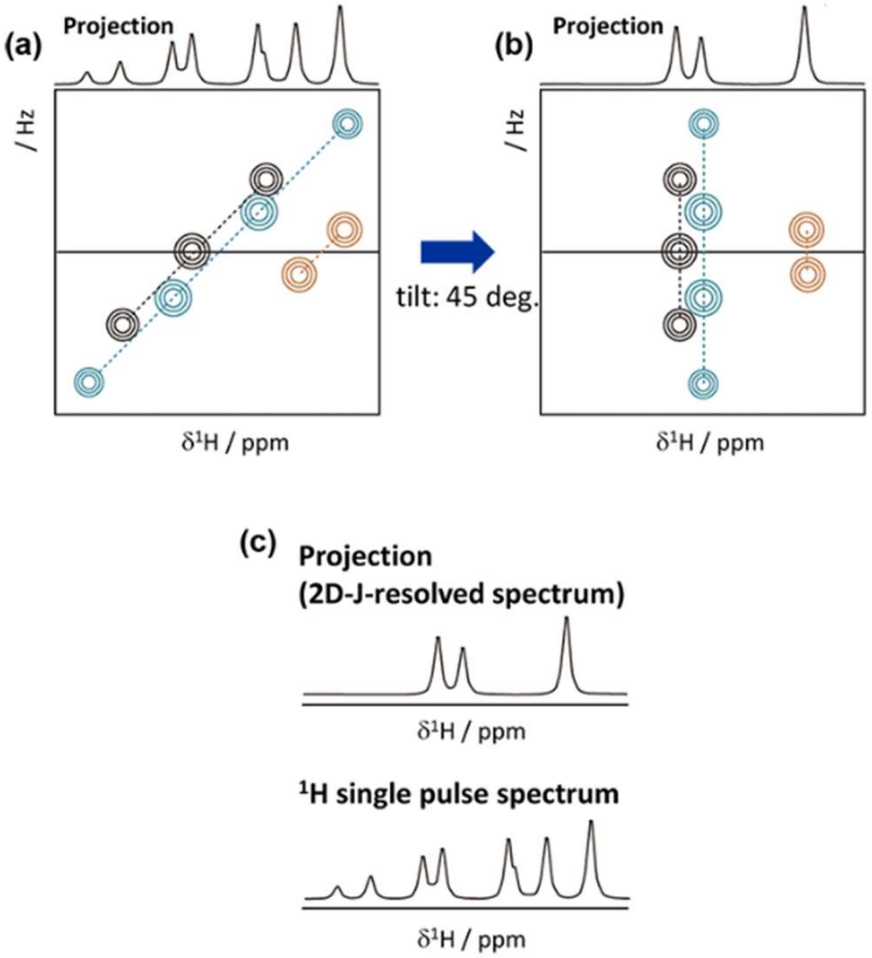


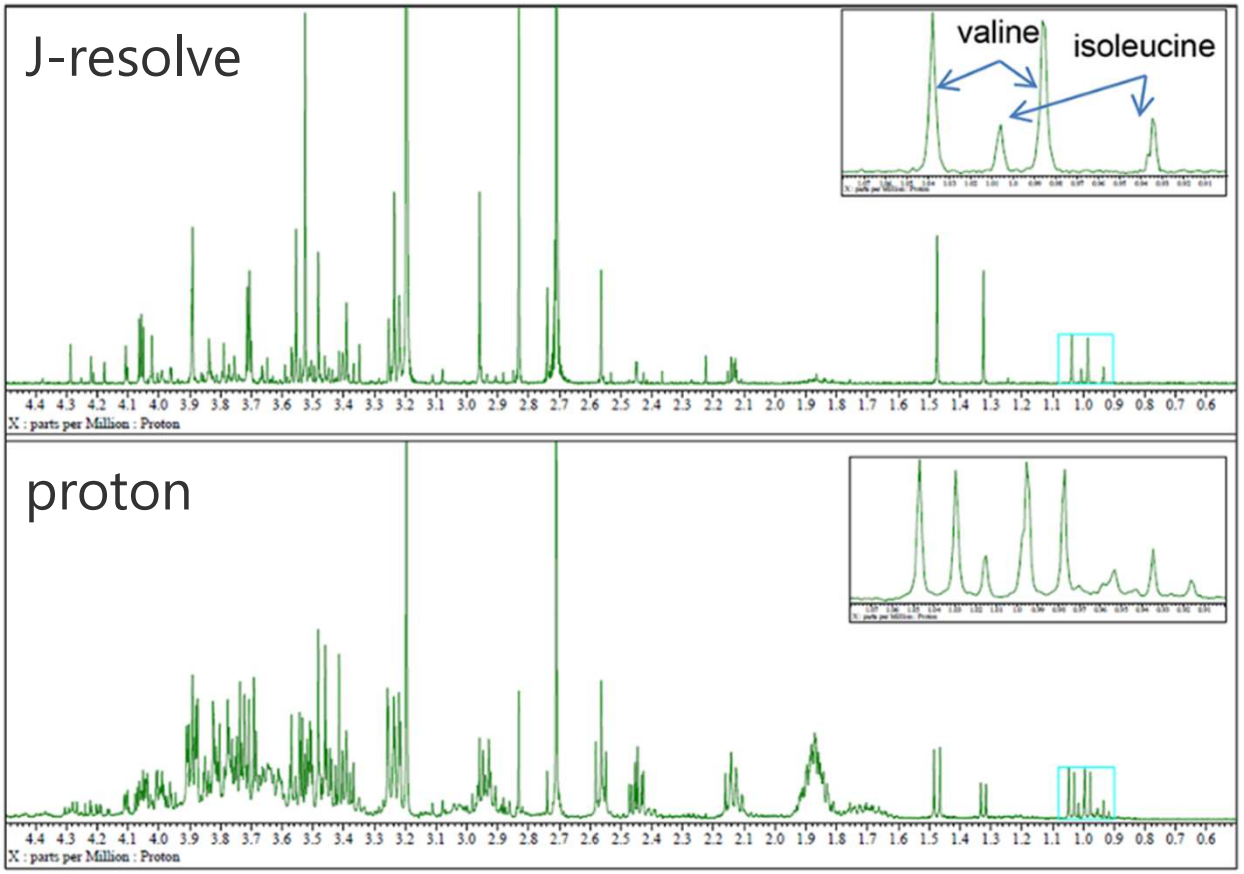
TOCSY



Pureshift-TOCSY

J-resolve


(a)
Projection
/ Hz
δ¹H / ppm
tilt: 45 deg.
(b)
Projection
/ Hz
δ¹H / ppm
(c)
Projection
(2D-J-resolved spectrum)
δ¹H / ppm
¹H single pulse spectrum
δ¹H / ppm


J-resolve
valine
isoleucine
X : parts per Million : Proton
proton
X : parts per Million : Proton

Application of 1D-NMR

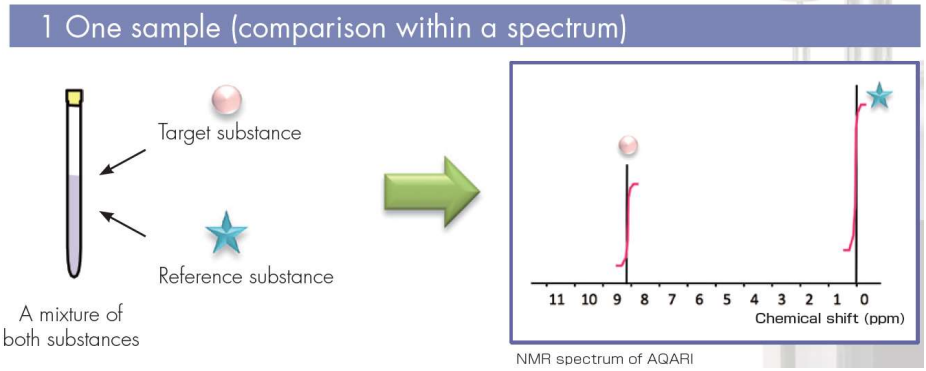
qNMR

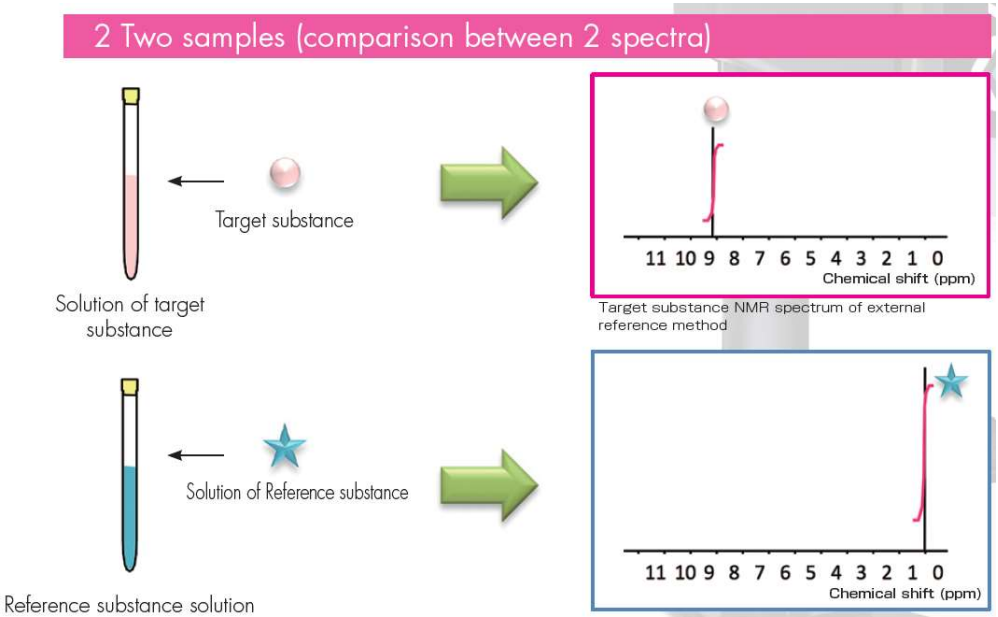
Parameter	Normal Condition*	Quantitative Condition**
Flip Angle	45°	90°
Repeating Time(recycle delay)***	7sec	64sec
Resolution	approx. 0.45Hz	0.25Hz or more
Sample spining	ON	OFF
^{13}C Decoupling	OFF	ON (or OFF)
Digital Filter	ON	ON
Number of Integration****	8	8
Observation Range	15 ppm	20 ppm
Measurement Temperature	Room temperature (22~23℃)fixed	Room Temperature (22~23℃)fixed



* Parameter setting on JEOL's NMR instrument
** Condition of joint test for uncertainty1% or lower is referred to.
*** Acquisition time + relaxation delay = pulse repeating time
**** In case of quantification, SNR of 100-200 is desirable

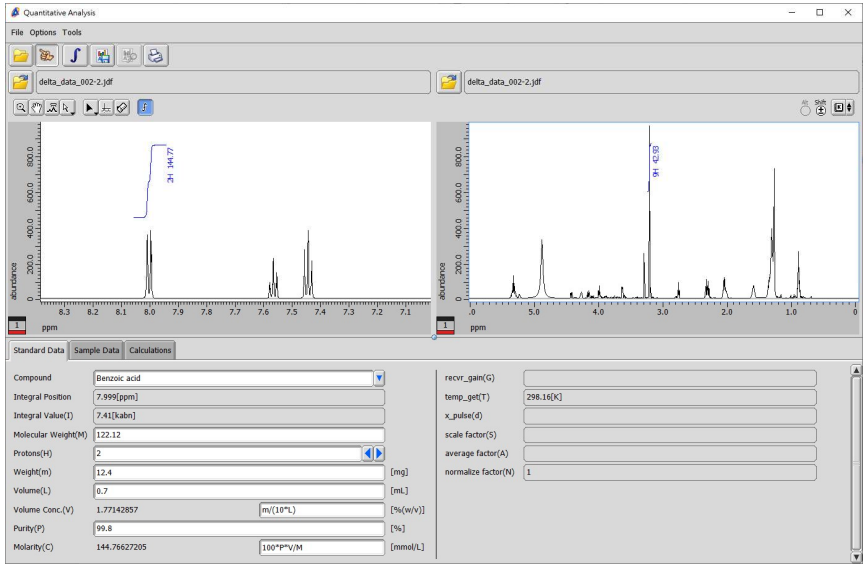
qNMR methods





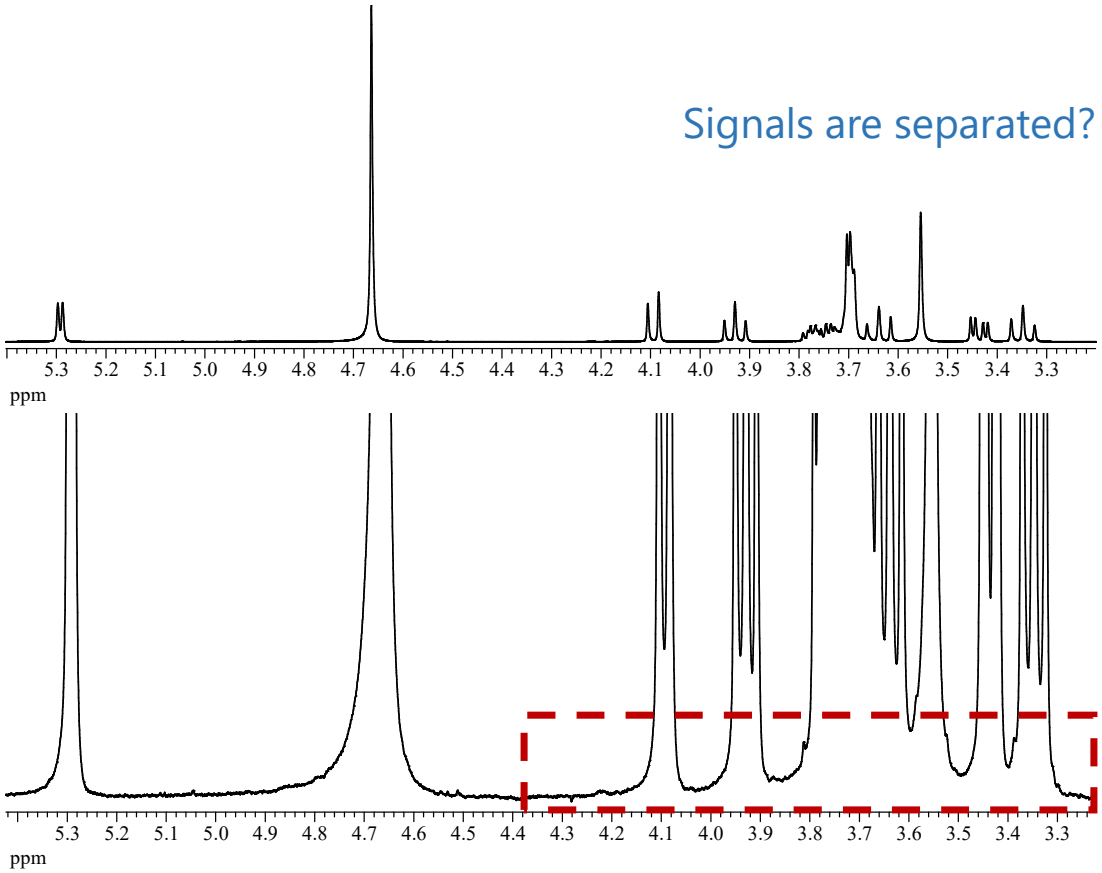
Internal Standard method
AQARI
(Accurate quantitative NMR with internal reference substance)

External standard method (automatic calibration)
PULCON
(Pulse length-based concentration measurement)

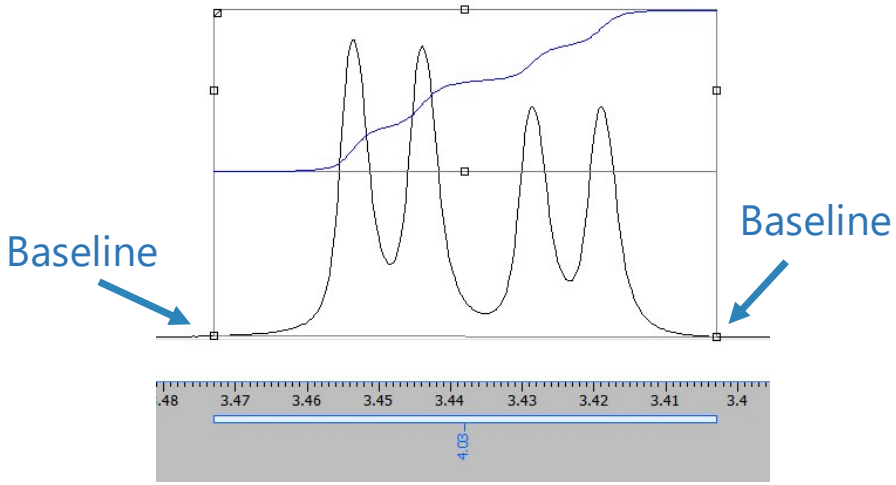


Baseline determination

-Error 1. overlap peak



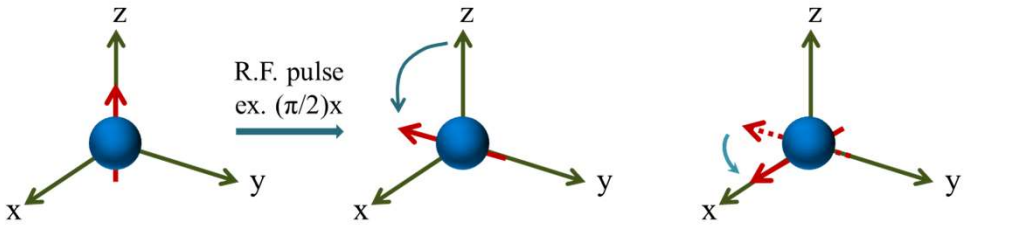
Signals are separated?

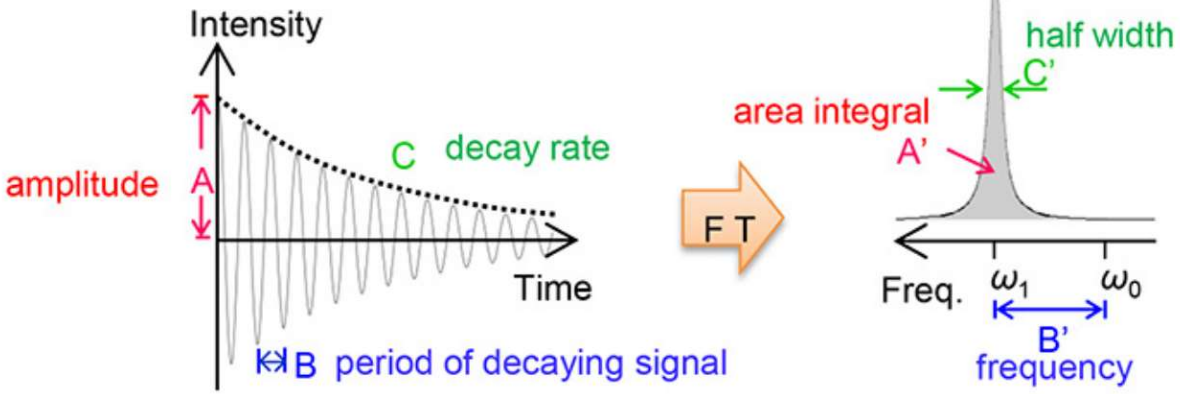


Delta software is easy to control integral baseline. It's good for each different application field.

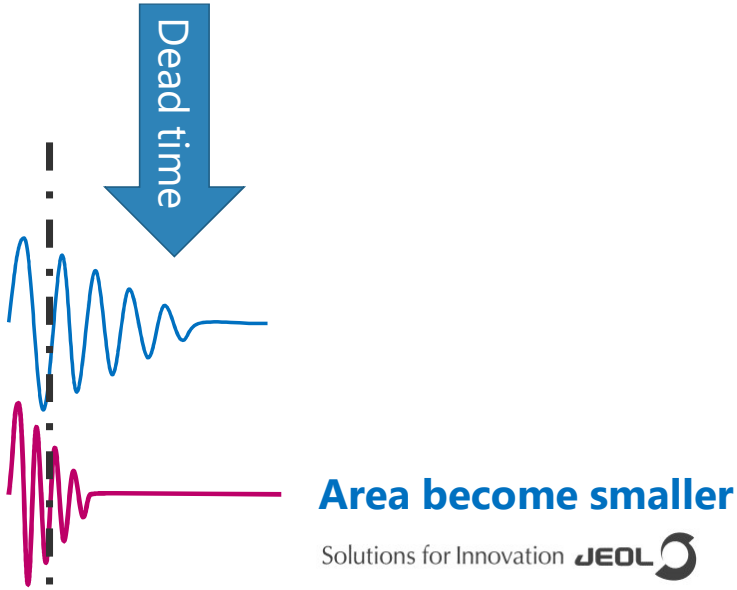
Data collection and transform

-Error 2. T2 effect







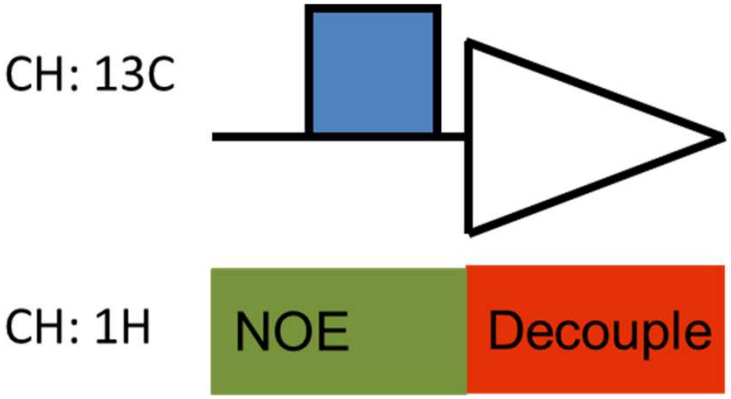


Data collection and transform
-Error 2. T2 effect

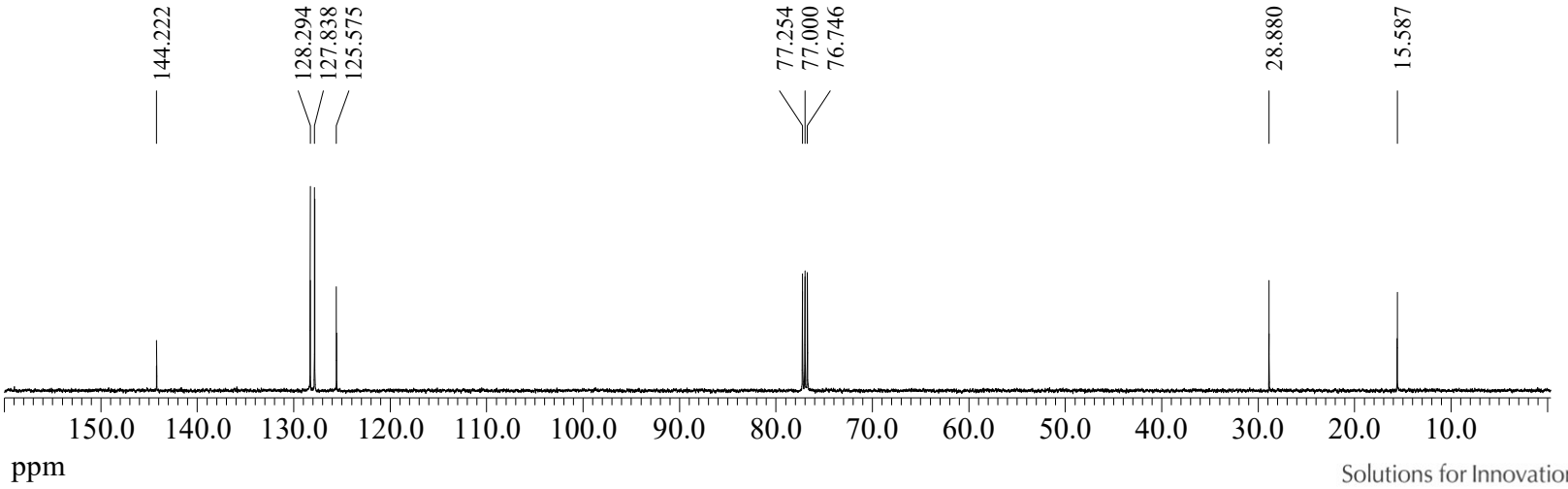
Reasons of T2 effect

- Not Suitable solvent
- Conformation change (rotation or exchange)
- Interaction between molecular
- Molecular weight
- Paramagnetic sample

Advanced application 1.
-heteronuclear qNMR



Area error is from NOE !!



Advanced application 1.
-heteronuclear qNMR

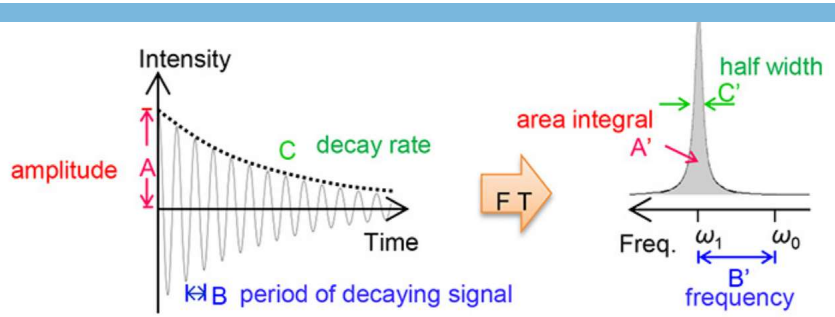
Long T1 and low sensitivity problems (Ex: 13C, 29Si…)

- Change to proton polarization experiments (Ex: RINEPT, POMMIE, qDEPT+)
- Use relaxation reagents (Ex: GdCl3)

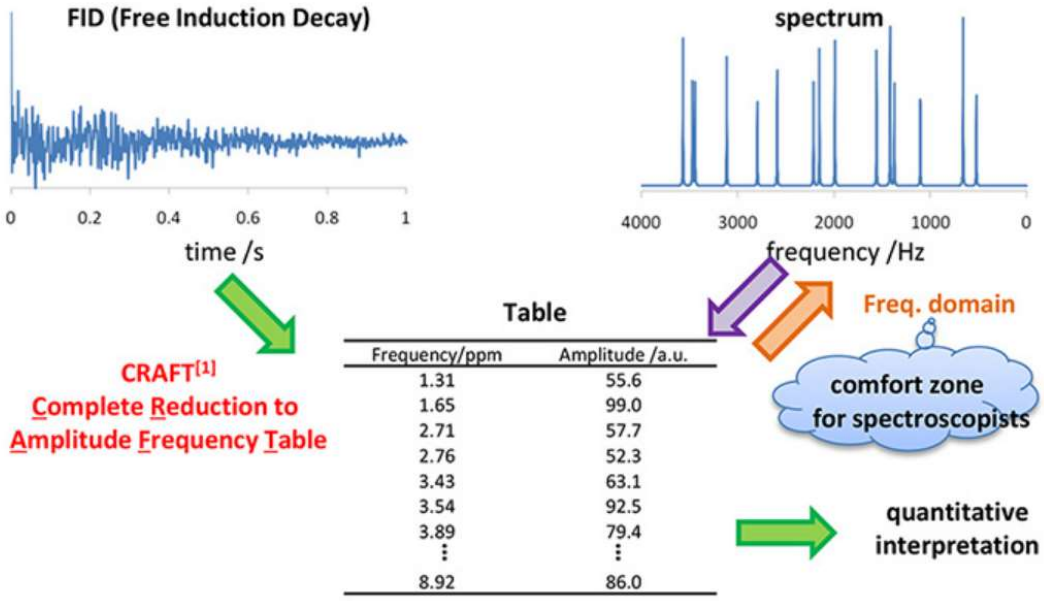
	Single pulse	With relaxation reagent	Polarization methods
Quantitative assay	Good	Good	Not include quaternary carbon
Spend time	Long (May take several hours)	Quick	Quick
Sampling	No change	System is changed	No change

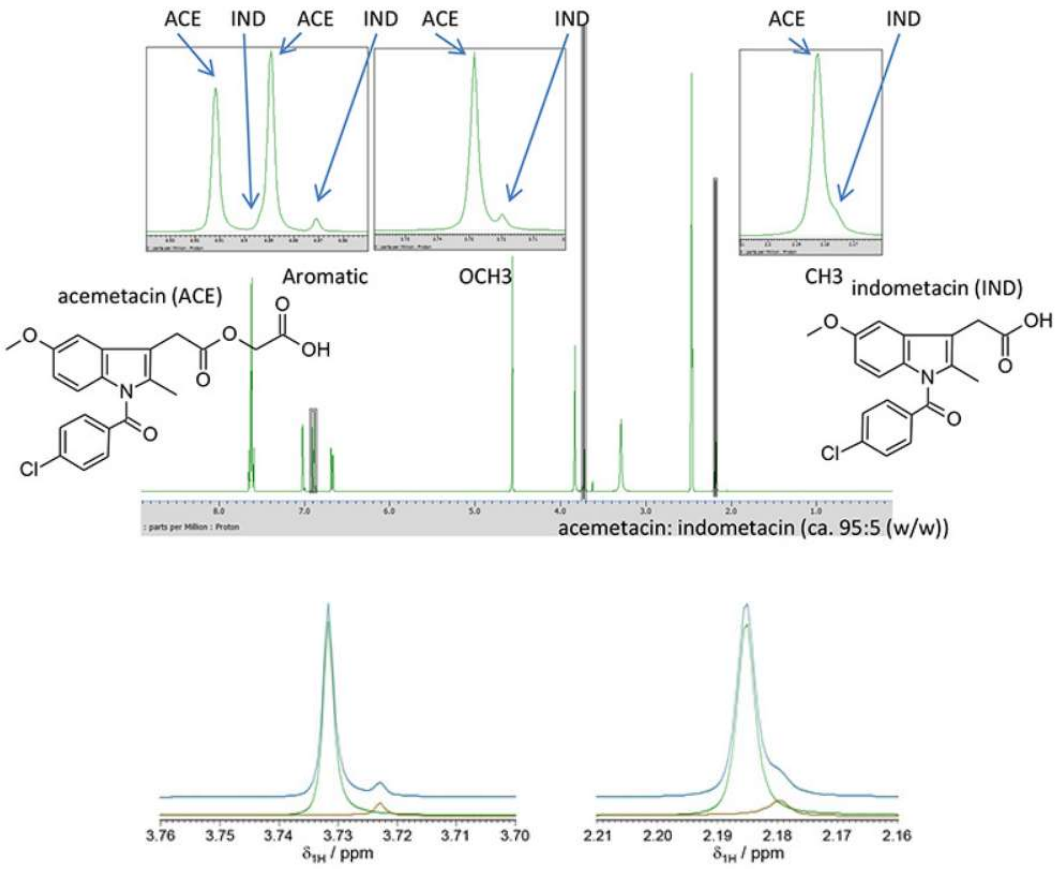
CRAFT

- overlap peak analysis



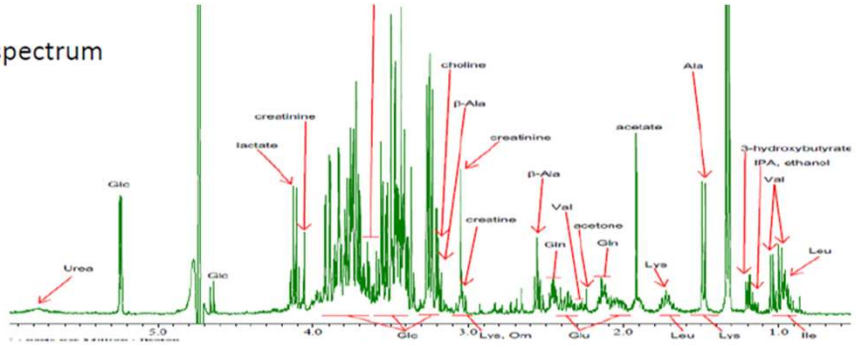
What is CRAFT?

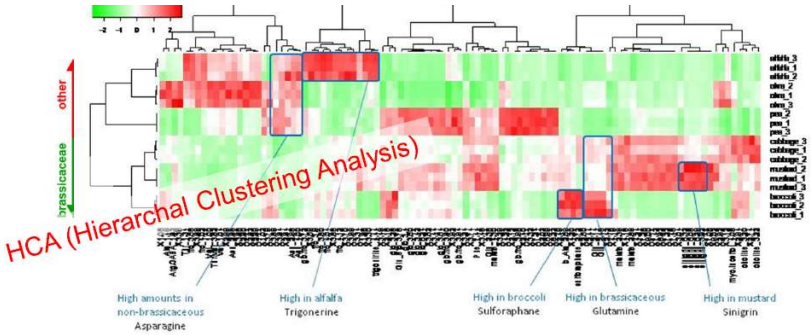




Metabolism NMR (Chemometric Tool)

ex. Serum ^{1}H-NMR spectrum



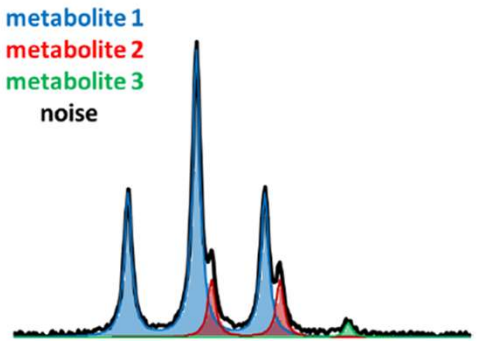


HCA (Hierarchal Clustering Analysis)

Target Analysis

-"Reductionism"

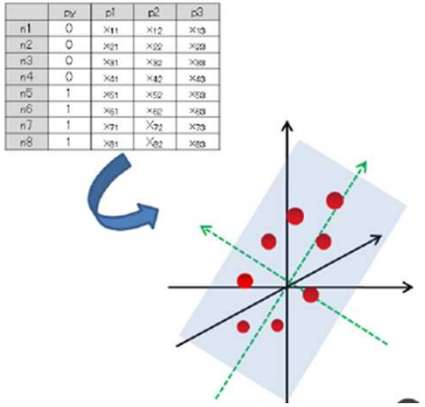
-Spectral library is required.

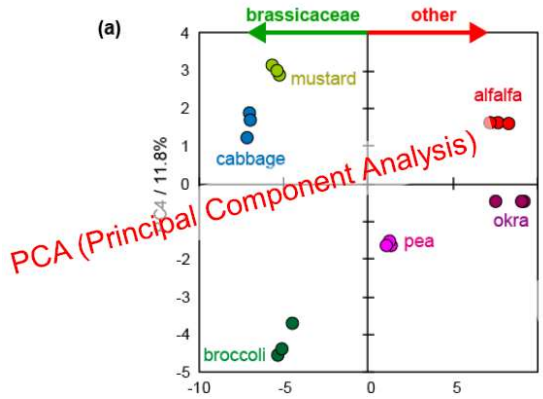


Non-Target Analysis

-"Holistic approach"

-Statistical analyses are typically used.



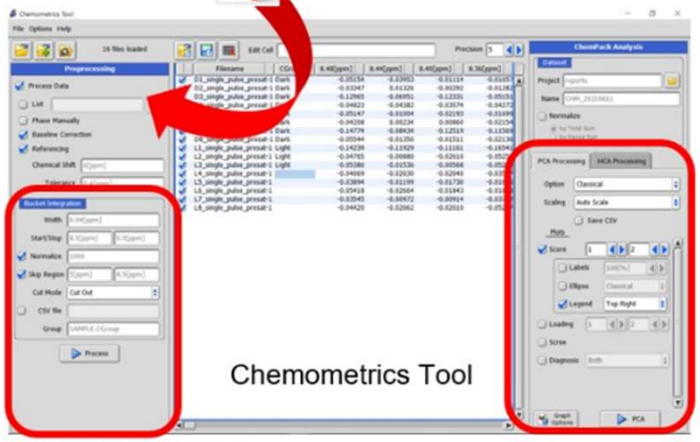


PCA (Principal Component Analysis)

Chemometric Tool



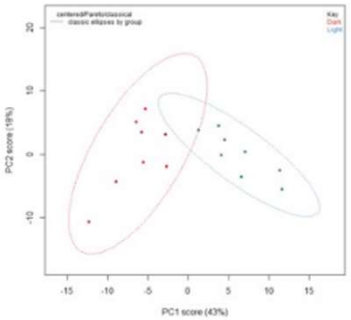
NMR data (jdf files)

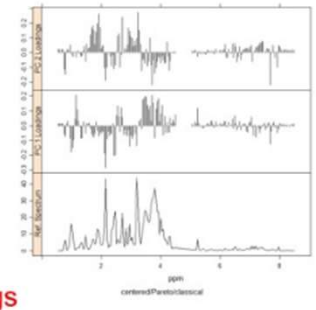


Bucket integration settings

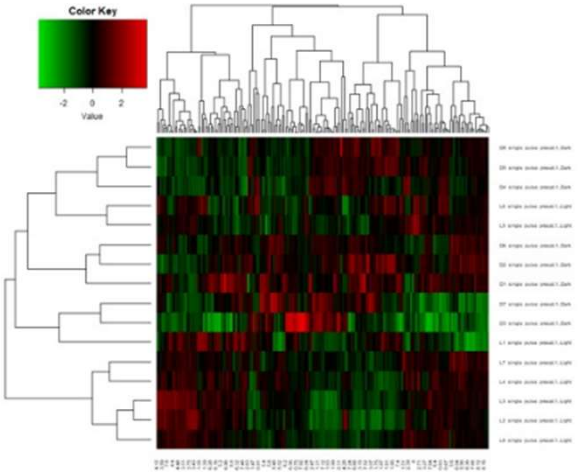
Multivariate analysis settings

Principal Component Analysis





Hierarchal Cluster Analysis



Thanks for your attention