ULSAN NATIONAL INSTITUTE OF SCIENCE AND TECHNOLOGY

Water suppression NMR

Advanced Operation Training Course

Solvent suppression

ULSAN NATIONAL INSTITUTE OF SCIENCE AND TECHNOLOGY

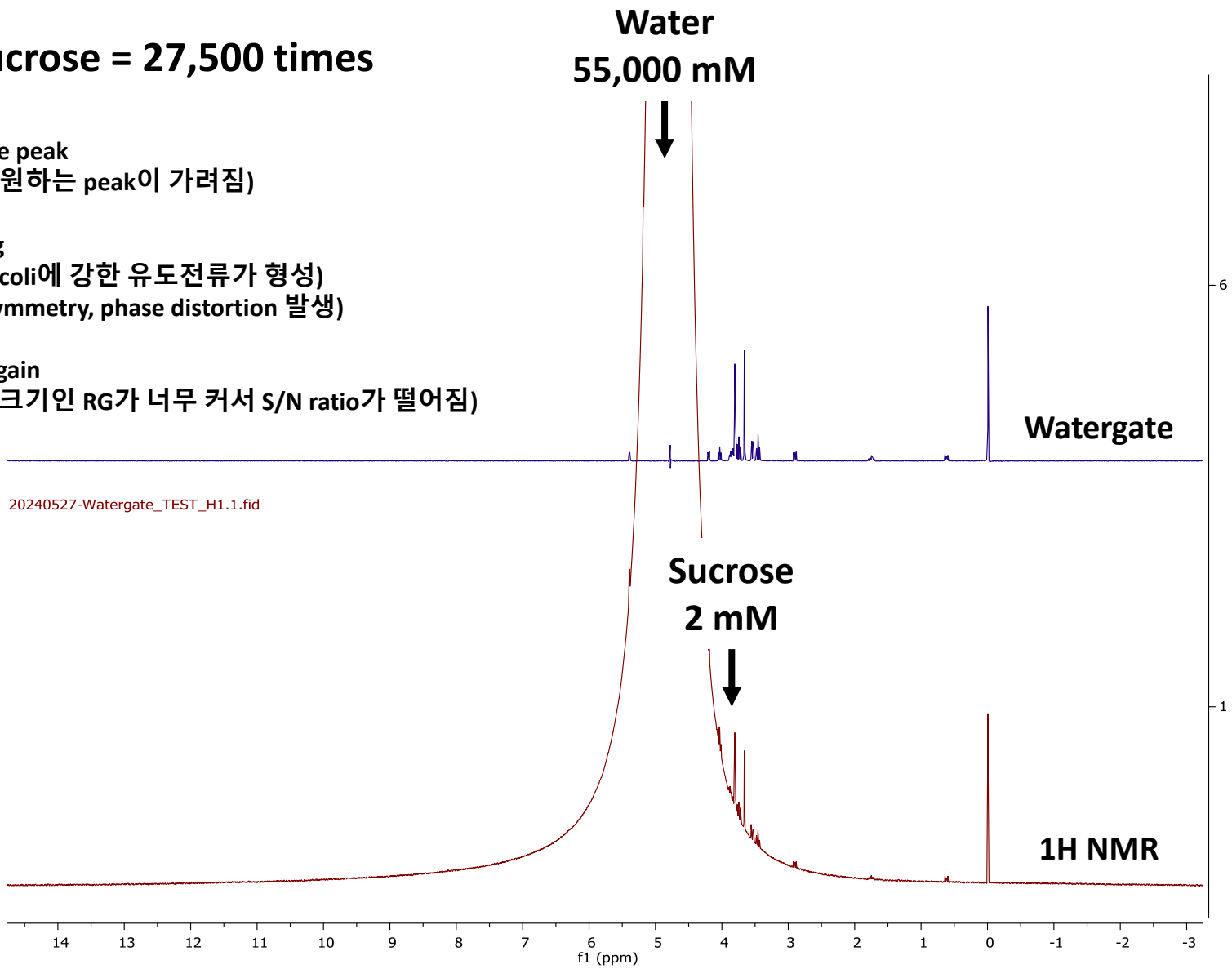
Why need to suppress solvent?

Water / sucrose = 27,500 times

1) Overlapped sample peak
(peak간 겹침으로 원하는 peak이 가려짐)

2) Radiation damping
(고농도로 인하여 coli에 강한 유도전류가 형성)
(-> broadening, asymmetry, phase distortion 발생)

3) Too high Receiver gain
(Detector window 크기인 RG가 너무 커서 S/N ratio가 떨어짐)



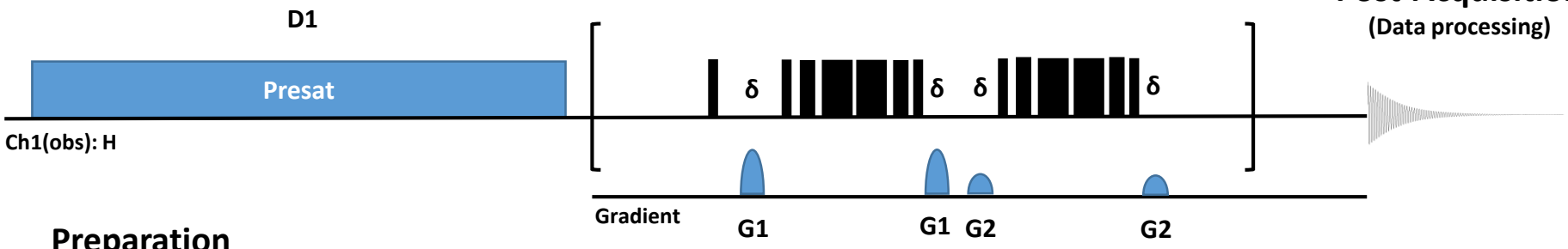
Type of solvent suppression

Excitation

1) Jump-Return
2) Watergate (WG) (+ Field gradient)
3) Pulsed Gradient Spin-Echo (PGSE) through T2*
4) Pulsed Gradient Stimulated-Echo (PGSTE) through T1

Post-Acquisition

(Data processing)



Preparation

1) Pre-saturation
2) WET (Water suppression Enhanced through T1 effects)
3) Flip-back Pulse(FP)

Post-Suppression

1) Excitation Sculpting (ES)
2) ES-W5 (W5)

https://ethz.ch/content/dam/ethz/special-interest/biol/mol-biol/bnsp-dam/NMRSeminar2018HS/Water_suppression_pwu_20181017.pdf

Building blocks

Preparation blocks

Excitation blocks

Readout blocks

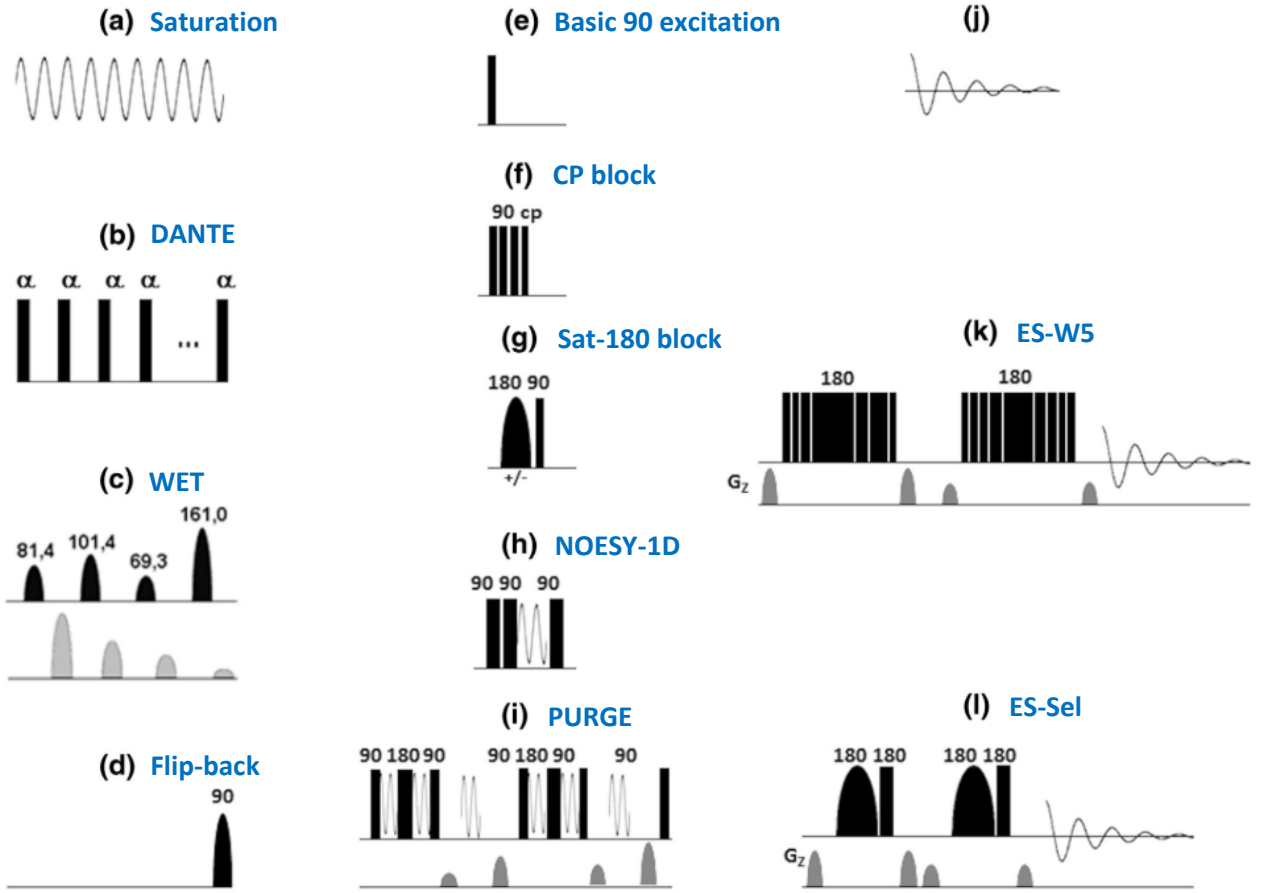


Fig. 1 Basic building blocks constituting water suppression sequences. **a–c** Preparation blocks, **e–i** excitation blocks and (**j–k**) readout blocks. **a** Sat block, **b** DANTE block, **c** WET block, **d** Flip-back block, **e** basic 90° excitation, **f** CP block, **g** Sat-180 block, **h** NOESY-1D block, **i** PURGE block, **k** ES-W5 block and **l** ES-Sel block

Optimizing water suppression for quantitative NMR-based metabolomics: a tutorial review

Pre-suppression

Presat (Pre-saturation)

zgpr: conventional presaturation

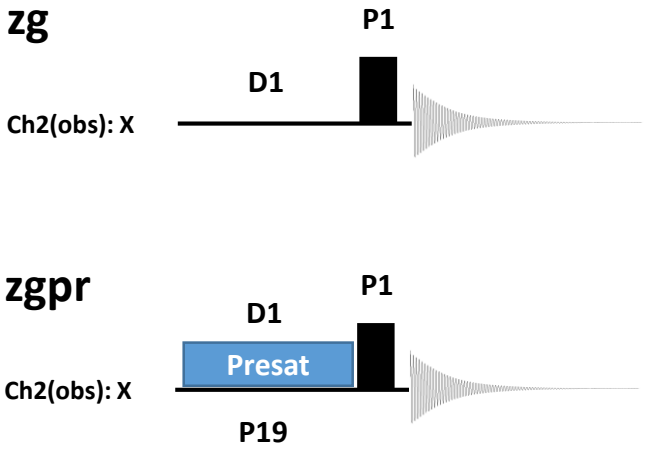
- pr (presaturation):
1) P1(RF pulse) 이전인 D1 동안 H2O만 선택적으로 CW irradiation을 가하여 Excitation (Saturation)
2) Saturation된 H2O은 Net magnetization이 감소하여 signal이 작아짐

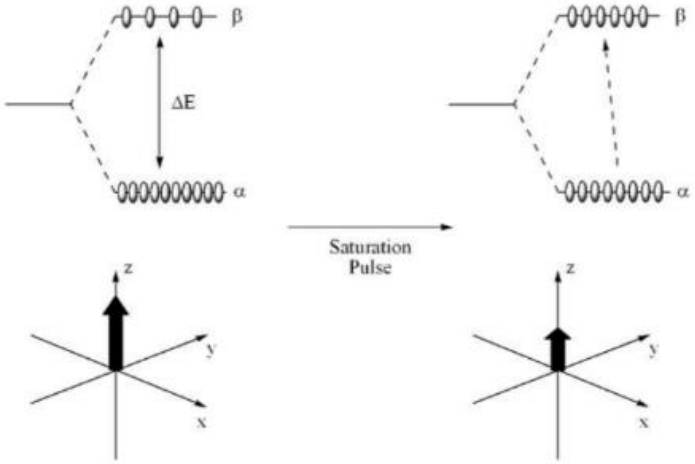
- Ernst angle: 주어진 D1 동안 최단시간에 최대 S/N ratio를 얻을 수 있는 Flip angle

- p19 (Presat power를 가하는 시간)
- plw9 (Presat power level)

- plw9의 값을 조절하여 Saturation peak이 최대한 작아지는 방향으로 조절
- Baseline 기준으로 대칭에 가깝도록!
- Saturation peak이 작아질 수록 RG(Receiver Gain)을 열어 S/N 를 증가시킬 수 있음

- 최소값
plw9 ≤ 0.0003 W (0.3 mW)
(pldb9 ≥ 35 –dBW)
plw9과 pldb9은 서로 연동 되어있는 값





Pre-suppression

Presat (Presaturation) Origin technique

zgpr: conventional presat.
zg0pr: find ernst angle
zgcppr: presat. with cp(composite pulse)

- PR (PResaturation):
1) P1(RF pulse) 이전인 D1 동안 H2O만 선택적으로 CW irradiation을 가함
2) Saturation된 H2O은 Net magnetization이 감소하여 signal이 작아짐

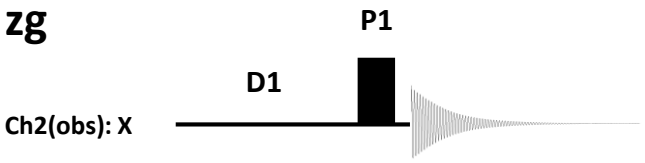
- CP (Composite Pulse): 연속적인 RF 입력보다 pulse 형식으로 여러 번 쪼개서 RF pulse를 가하여 B1 field의 불균일성을 보정하고 더 완벽한 H2O peak 포화(제거)를 할 수 있음

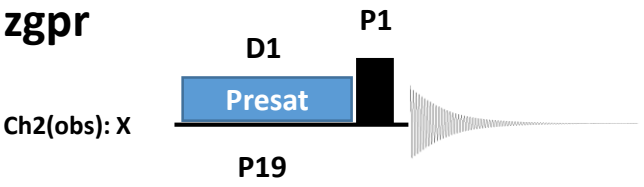
- Ernst angle: 주어진 D1 동안 최단시간에 최대 S/N ratio를 얻을 수 있는 Flip angle

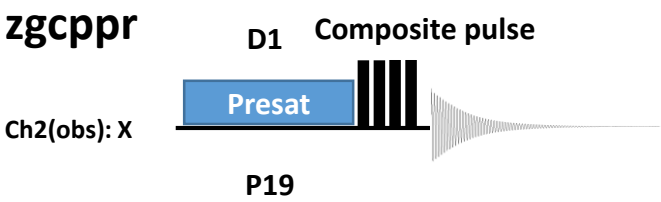
- P19 (Presat power를 가하는 시간)
- plw9 (Presat power level)

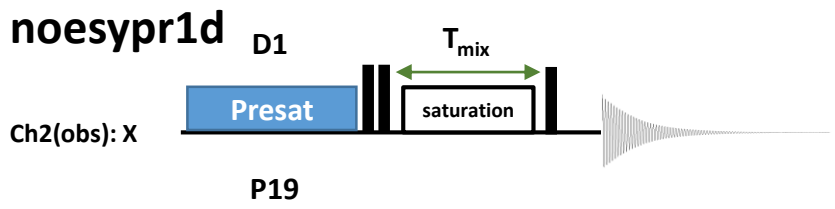
- plw9의 값을 조절하여 Saturation peak이 최대한 작아지는 방향으로 조절
- Baseline 기준으로 대칭에 가깝도록!
- Saturation peak이 작아질 수록 RG(Receiver Gain)을 열어 S/N 를 증가시킬 수 있음

- Minimum Value (최소값)
plw9 ≤ 0.0003 W (0.3 mW)
(pldb9 ≥ 35 –dBW)
plw9과 pldb9은 다른 단위로 연동 되어있는 값



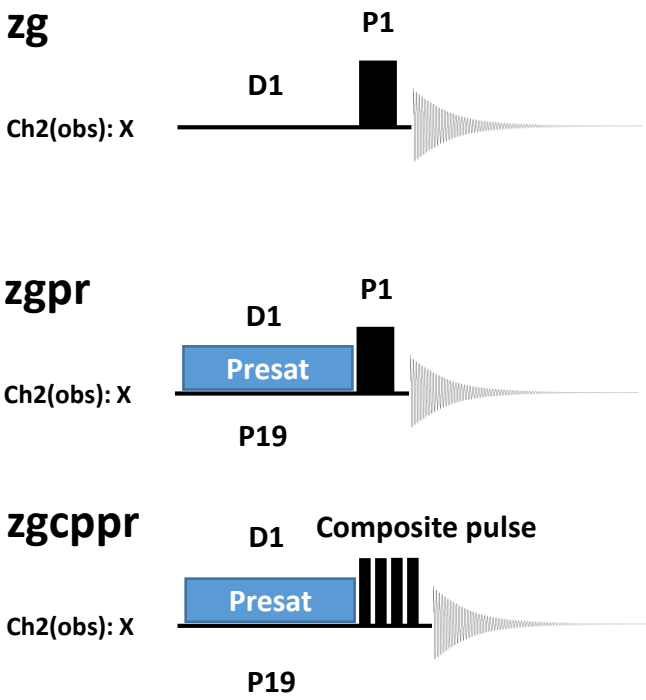




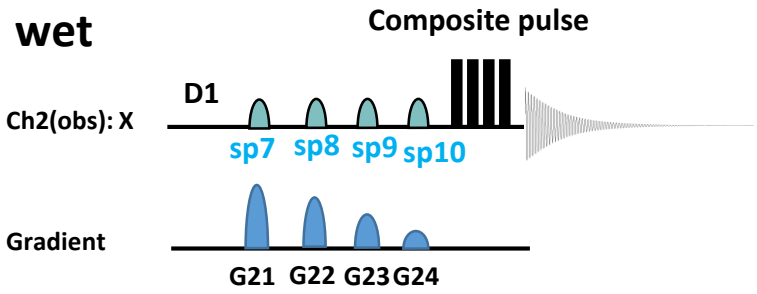


https://www.researchgate.net/profile/Soren-Engelsen-2/publication/277889806_The_Quantitative_Impact_of_Water_Suppression_on_NMR_Spectra_for_Compositional_Analysis_of_Alginates/links/5577dfc908ae753637539698/The-Quantitative-Impact-of-Water-Suppression-on-NMR-Spectra-for-Compositional-Analysis-of-Alginates.pdf

WET (Water suppression Enhanced through T1 effects)



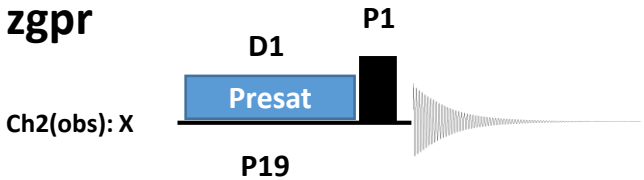
wet: Pre-saturation with gradient + CP (Composite Pulse)
Minimizing reduced exchangeable proton effect
Need to control sp7 / sp10

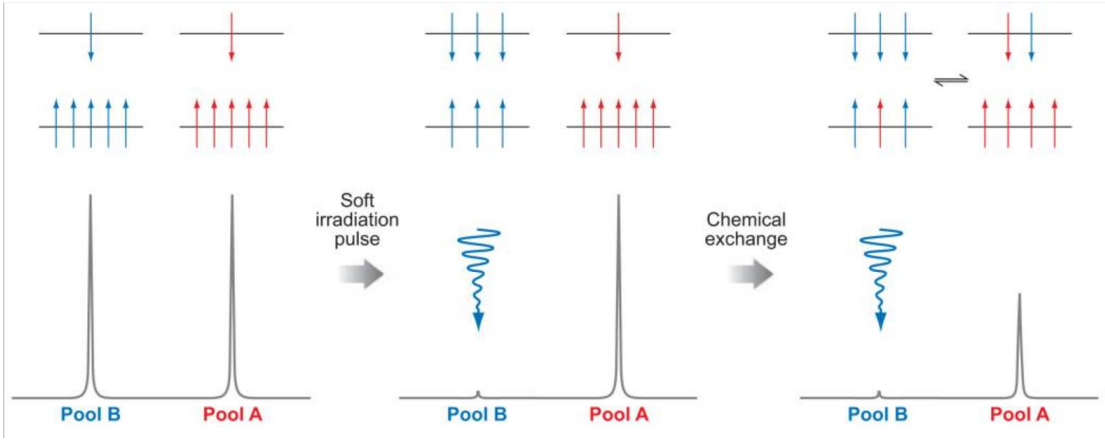


Exchangeable proton

Signal attenuation effect

- Saturation된 H2O의 H(Proton)이 다른 OH/NH group과 exchange 되어 해당 peak의 신호 감소 효과를 가져옴
- Protein sample의 경우 pH를 7.5이하로 낮춰 Exchange rate을 낮출 필요가 있음





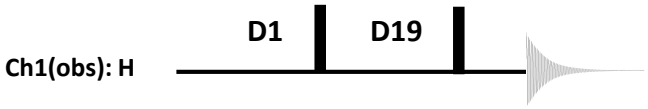
https://www.nmr.sinica.edu.tw/Cours/Course20030929-30/Lecture_1_NMR_Methods.pdf
https://www.frontiersin.org/journals/psychiatry/articles/10.3389/fpsyt.2020.532606/full

Pulse sequence

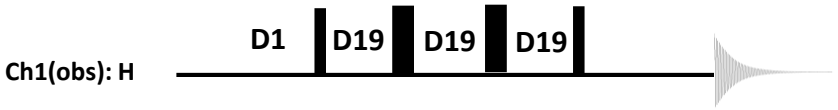
Jump-Return

P11 (1-1 scheme)
P1331 (1-3-3-1 scheme)

P11

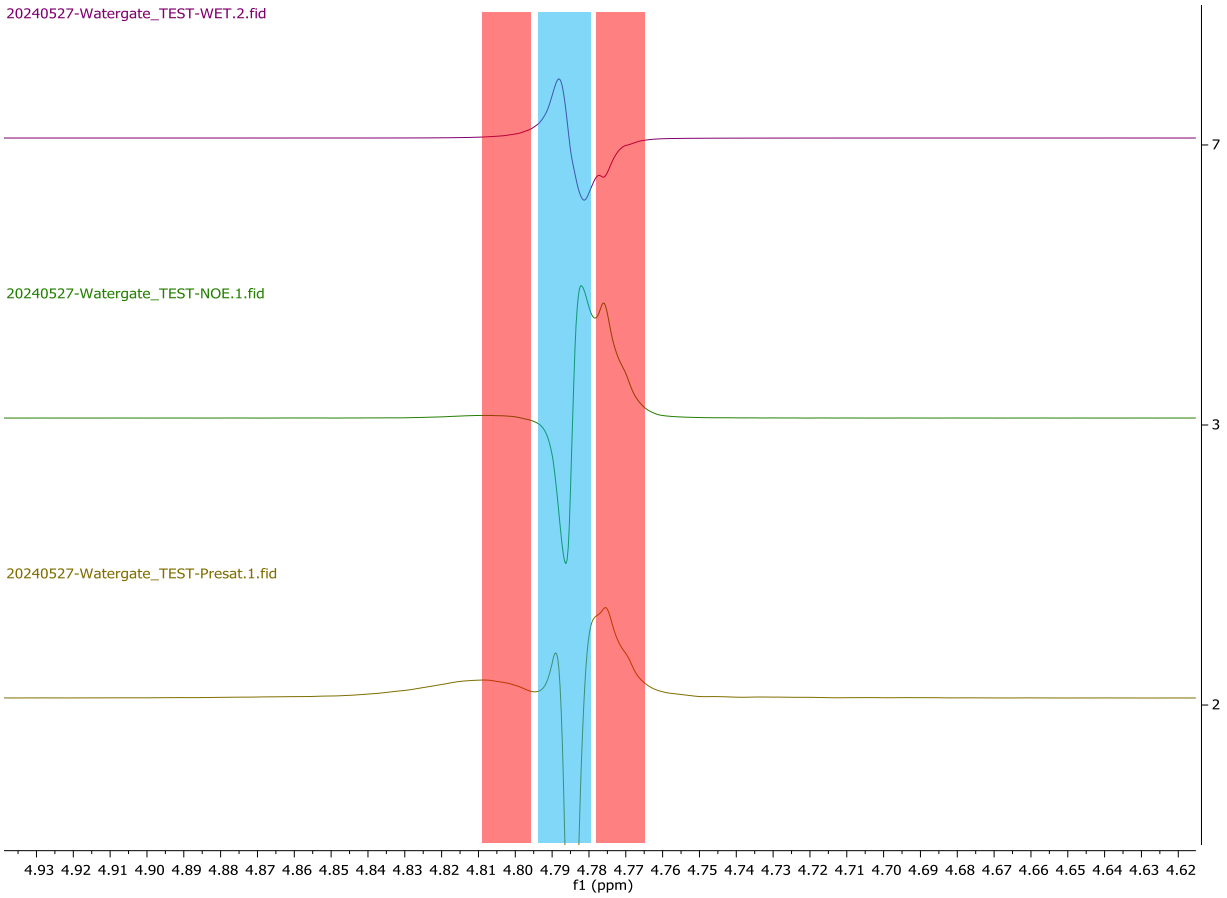


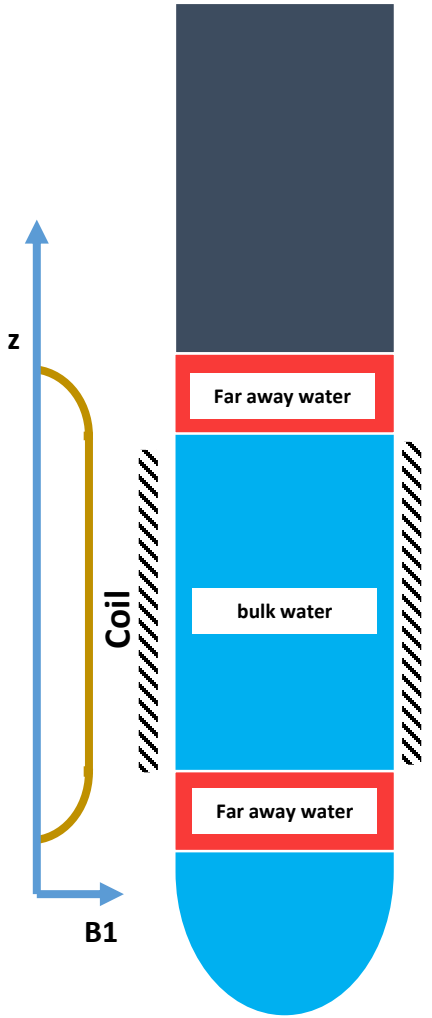
P1331



Far away water

- **Far way water:**
  Coil center 에서 멀어질수록 B1 field(직접 가해주는 RF pulse의 영향이 감소하므로 Residual peak이 나타남

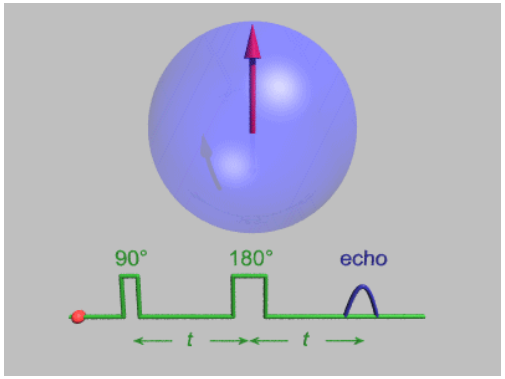




https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2662483/pdf/nihms38883.pdf

Spin echo (Hahn-echo) vs Gradient echo

SE (Spin Echo)

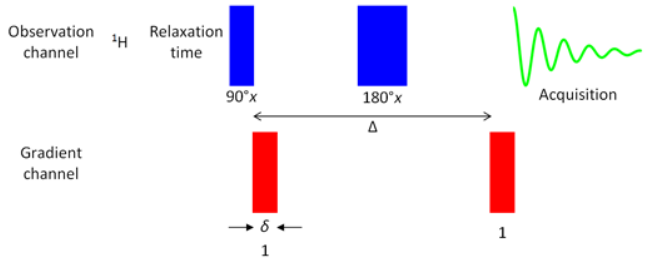


- Dephasing/Refocusing
  : Related T2
- Experiment time: Long

T2 time을 따라 dephasing이 일어나므로 실험시간이 길어짐

Origin pulse sequence of DOSY

PGSE (Pulsed Gradient Spin Echo)



- Dephasing/Refocusing
  : Use Gradient
- Experiment time: Middle
- Limited Diffusion Delay (T2*)
  T2* = T2 in rotating frame

Gradient를 이용하여 강제로 Dephasing / Refocusing을 진행하므로 실험시간이 짧음,
대신 T2*시간이내로 Diffusion delay가 제한됨

STE (STimulated Echo)

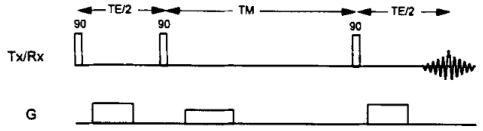


Figure 3 Pulse sequence used to generate a stimulated echo.

- Dephasing/Refocusing
  : Use Gradient
- Experiment time: Short
- Limited Diffusion Delay (T1)

3개의 90도 펄스와 Gradient를 이용하여 T2보다 상대적으로 긴 T1 을 Diffusion delay로 사용

Jump-return → **Watergate** → **PGSTE**

https://onlinelibrary.wiley.com/doi/epdf/10.1002/%28SICI%291099-0534%281996%298%3A4%3C269%3A%3AAID-CMR3%3E3.0.CO%3B2-X

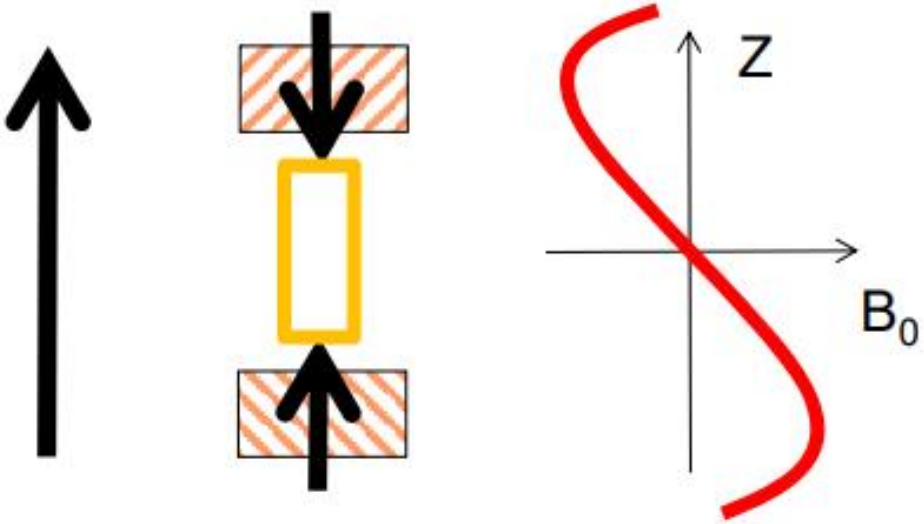
https://magritek.com/2016/07/18/gradients-in-nmr-spectroscopy-part-5-the-pulsed-gradient-spin-echo-pgse-experiment/

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



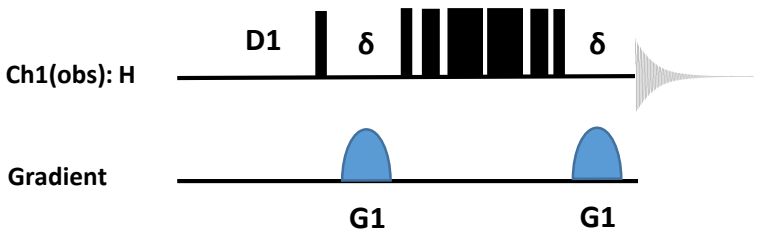
Post suppression

WATERGATE (**WATER** suppression by **G**r**A**dient **T**ailored **E**xcitation)

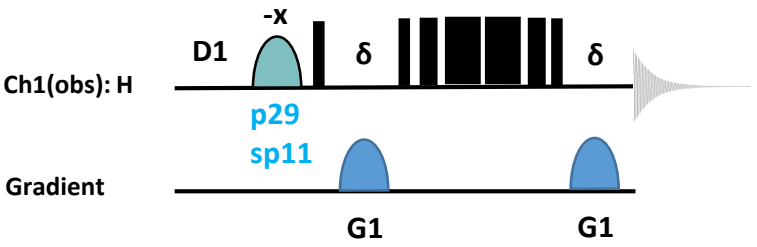
p3919gp: 3-9-19 Watergate + gp (gradient pulse)
p3919fpgp: 3-9-19 Watergate + fp (flip back pulse) + gp (gradient pulse)
zggpwg: 90 degree water selective pulse
zggpw5: W5 (Similar 3-9-19, but narrow notch)

▪ Flip back Pulse: 실험 전 단계에 –x 방향으로 water의 Mz 값만 선택적으로 이동시킴
Saturation Transfer에 의한 Exchangeable proton의 감소를 억제

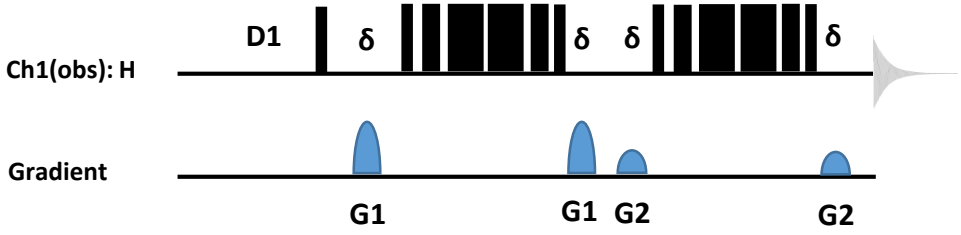
p3919gp



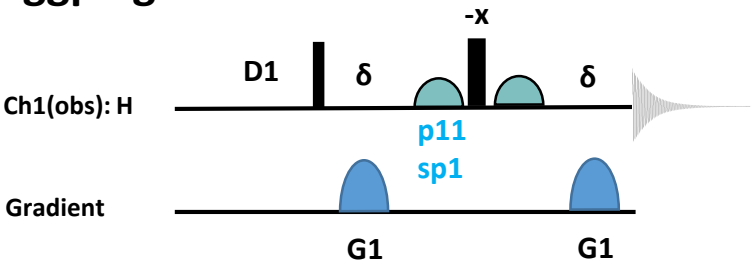
p3919fpgp



zggpw5



zggpwg

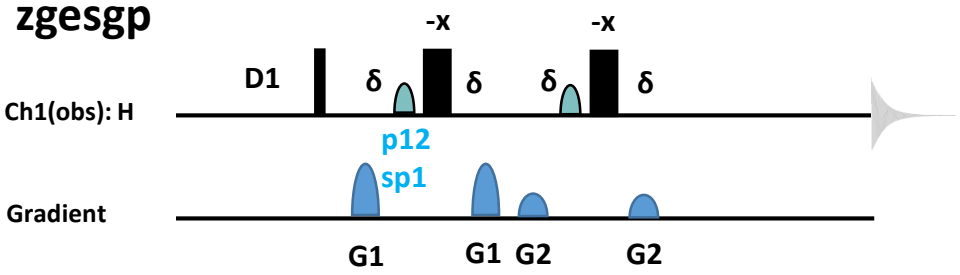


ES (Excitation Sculpting)

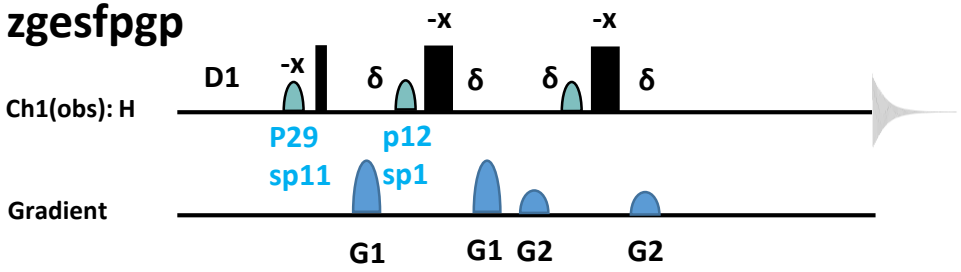
1D ES (Excitation Sculpting)

zgesgp: zg + es (excitation sculpting) + gp (gradient pulse)
zgesfpgp: zg + es (excitation sculpting) + fp (flip back pulse) + gp (gradient pulse)
zggpw5: W5 (Similar 3-9-19, but narrow notch)

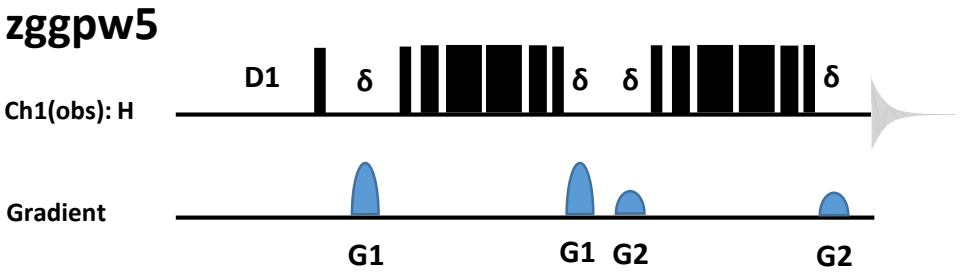
zgesgp



zgesfpgp



zggpw5



PGSTE-WATERGATE (2011)

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- Research Candidates
- Biomedical Magnetic Resonance Facility
- Research Projects

NMR Pulse Sequences

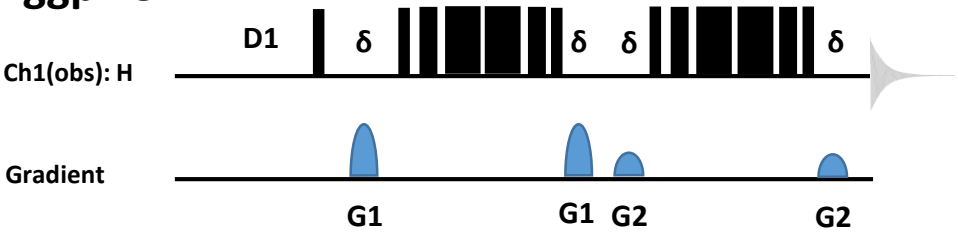
The following sequences are freely available for use. They have been developed by the Nanoscale Research Group for Bruker systems, although they can be ported to other spectrometer brands. It would be appreciated if you would cite the appropriate reference / references, when using any of the following sequences.

Expand all

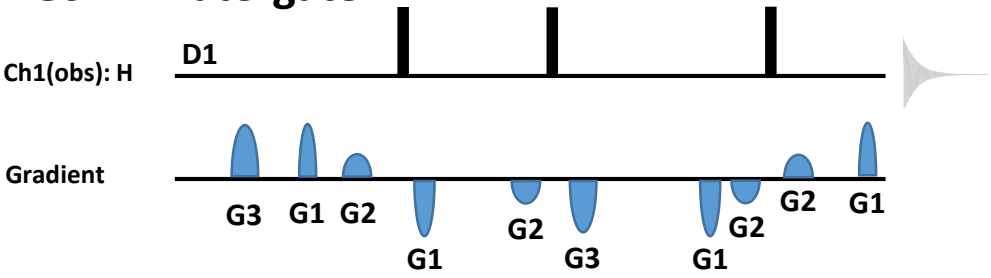
▾ PGSTE-WATERGATE: An STE-based PGSE NMR sequence with excellent solvent suppression

This is a stimulated-echo based pulsed gradient spin-echo NMR diffusion sequence incorporating selective inversion based solvent signal suppression. The sequence provides superb solvent suppression without any phase distortion. The sequence is simple to set up and particularly suited to measuring diffusion coefficients in aqueous solution such as is commonly required in biomolecular NMR experiments (e.g., probing drug-protein interactions).

zggpw5

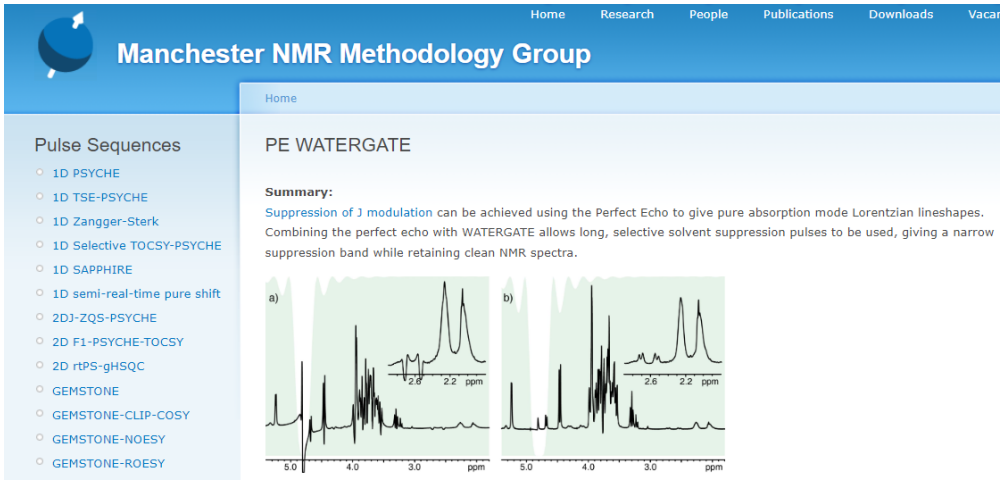


PGSTE-Watergate

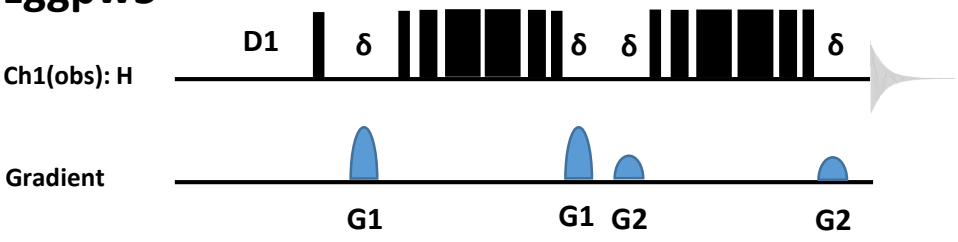


https://www.westernsydney.edu.au/nanoscale/nanoscale_organisation_and_dynamics/research_projects/NMR_sequences

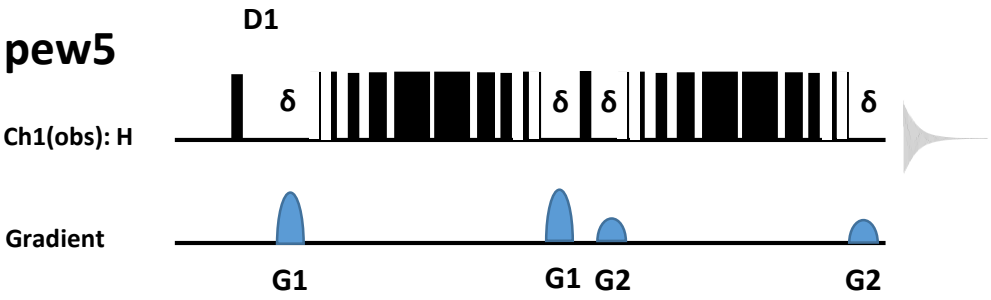
PEW5 (2012)



zggpw5



pew5



https://www.nmr.chemistry.manchester.ac.uk/?q=node/285

1D Solvent suppression

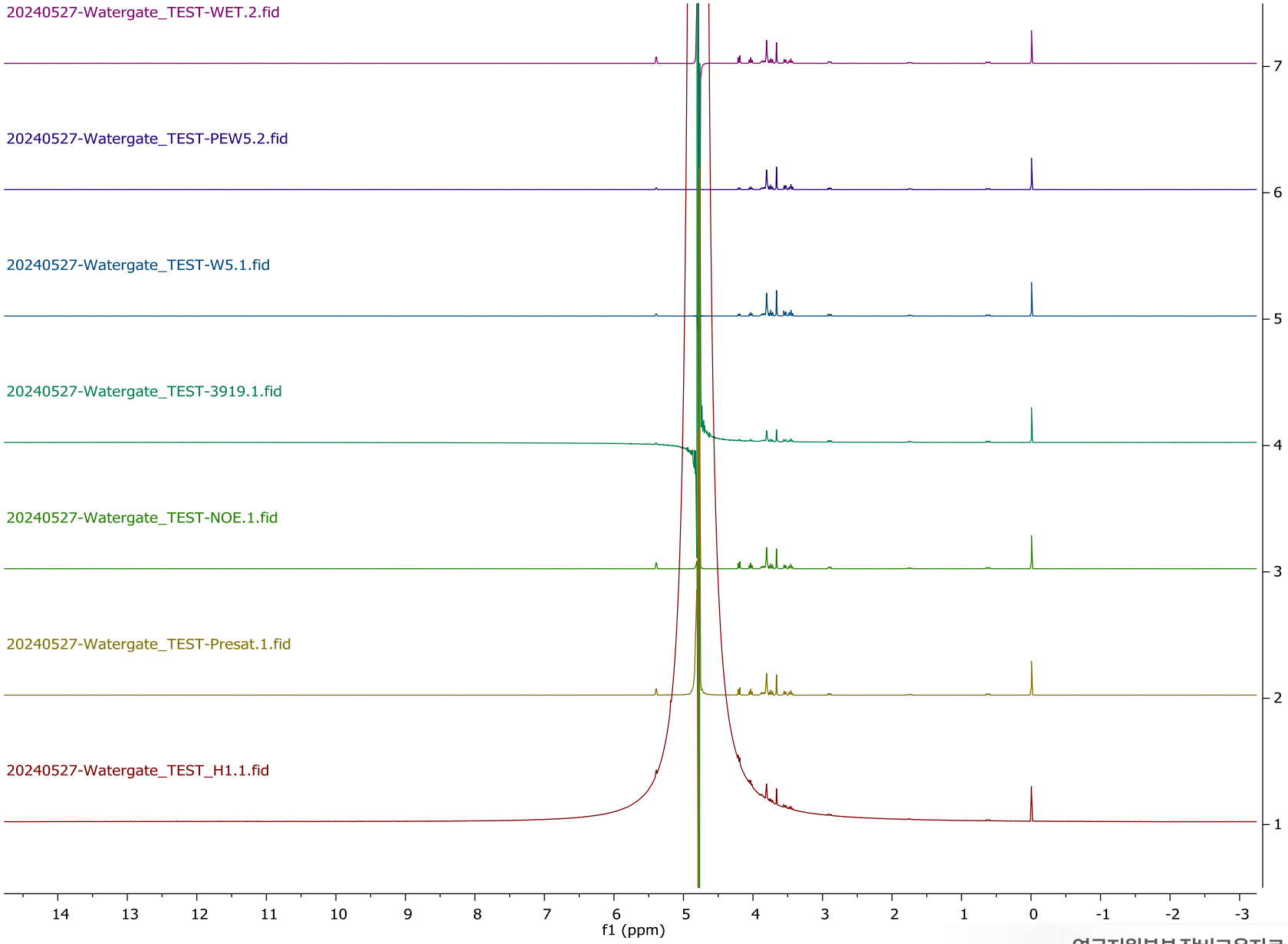
Pulse sequence	Advantage	Exchangeable Proton	Notch	Key paramter	Remark
Presat	▪ Cleanest ▪ Minimized saturation peak ▪ Possible multi suppression	Highly Reduced	Narrow	O1 plw9 ≤ 0.3mW	▪ Solvent suppression ▪ Follow T2
noesy1d (noesygppr1d)	▪ Clean baseline	Reduced	Narrow	O1 D8 ≤ 1ms	▪ Common purpose ▪ for metabolite
Watergate (3-9-19)	▪ Water suppression	No effect (+ Flip back)	Slightly Narrow		▪ for NH4+, Acid, Amine ▪ Check D19 (Delay for binomial water suppression)
Watergate (W5)	▪ Water suppression		Narrow		
Purge	▪ Quantitative				▪ for Quantitative NMR
WET	▪ Multi suppression	Possibly reduced			▪ Follow T1 effects
ES (Excitation Sculpting)	▪ Use Gradient/spin echo ▪ Excellent suppression		Broad		
PGSTE (Pulsed Gradient Stimulated Echo)	▪ Use Gradient/spin echo ▪ Excellent suppression	No effect	Excellent Narrow		▪ Advanced sequence
PEW5	▪ Clean spectra	No effect	Excellent Narrow		▪ for NH4+, Acid, Amine ▪ Advanced sequence

https://ethz.ch/content/dam/ethz/special-interest/biol/mol-biol/bnsp-am/NMRSeminar2018HS/Water_suppression_pwu_20181017.pdf

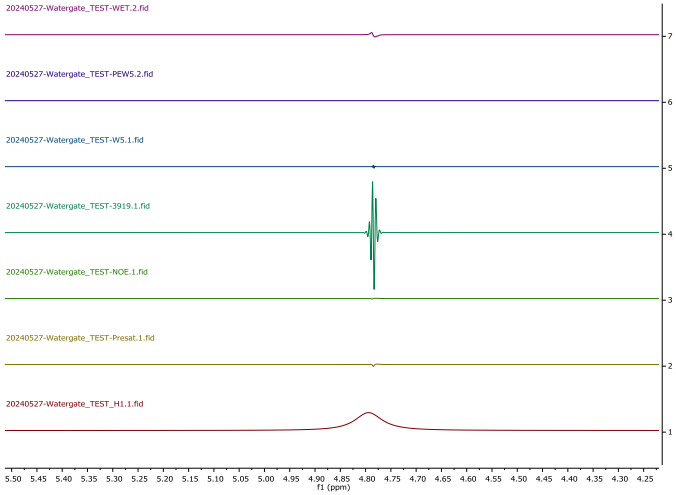
https://www.nmr.chemistry.manchester.ac.uk/?q=node/285

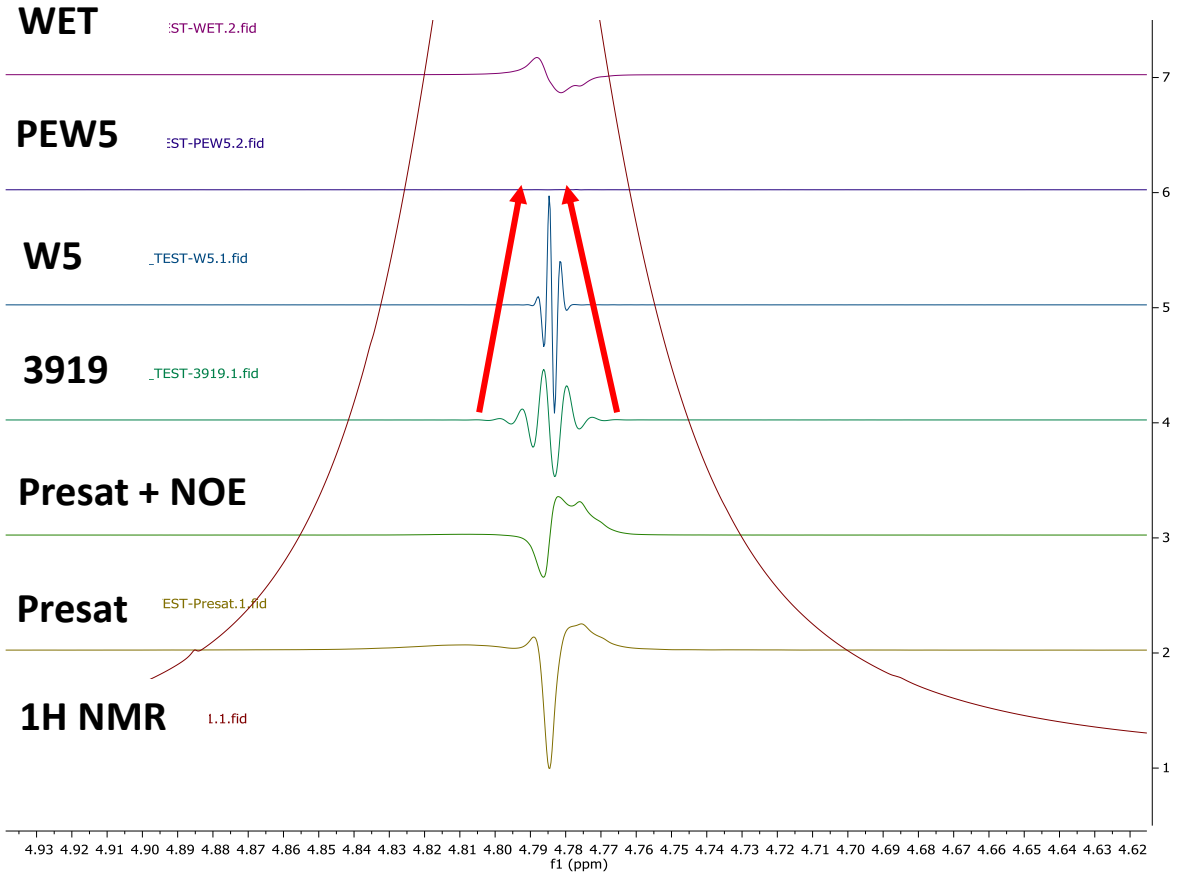
https://www2.chem.wisc.edu/~cic/nmr/Guides/Ba3vug/AV3_SolventSuppression.pdf

1D Solvent suppression

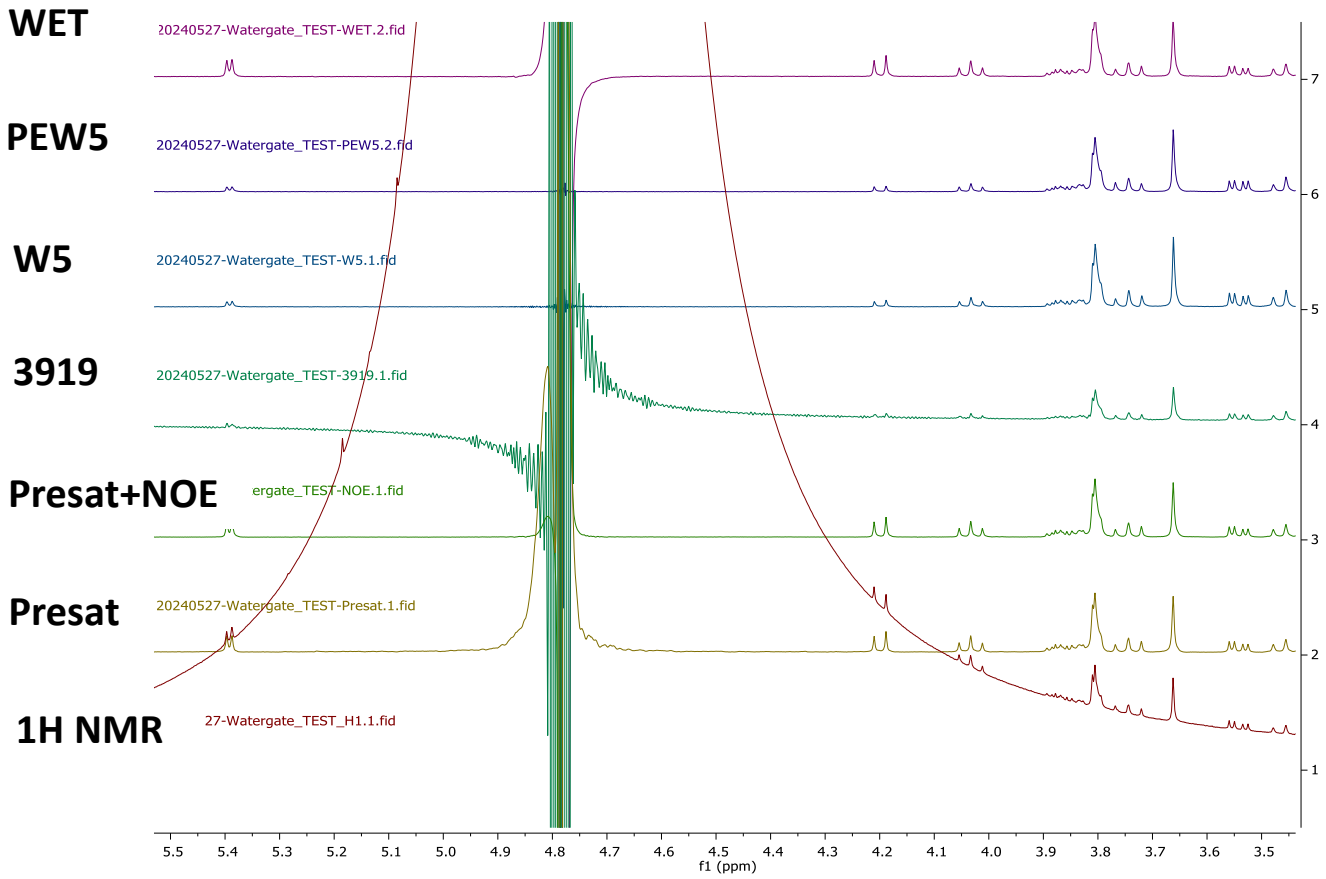


Spectrum for notch

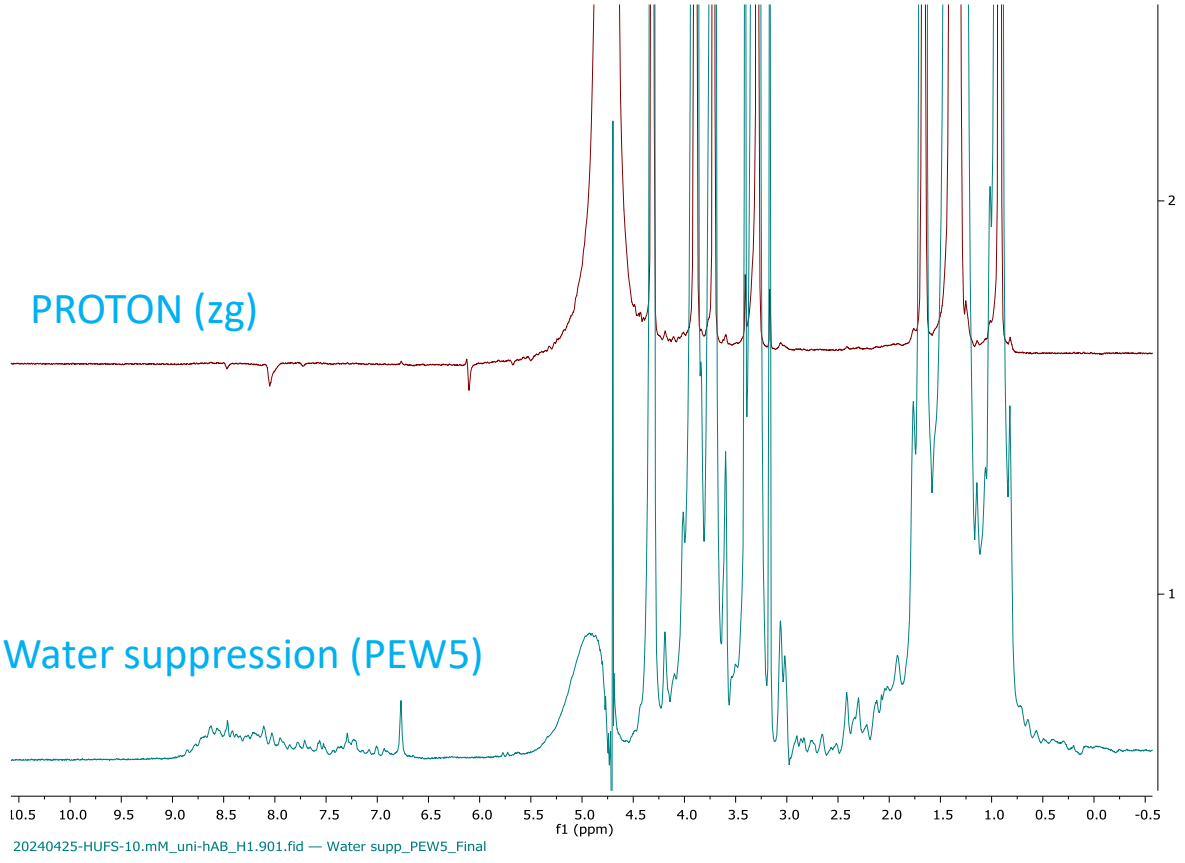




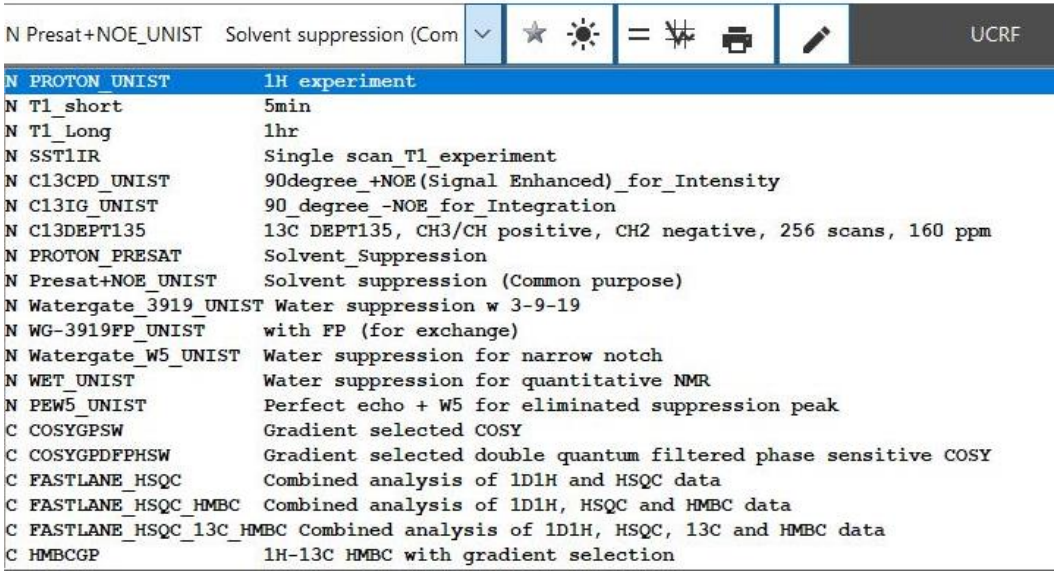
Spectrum for signal intensity



Protein for water suppression

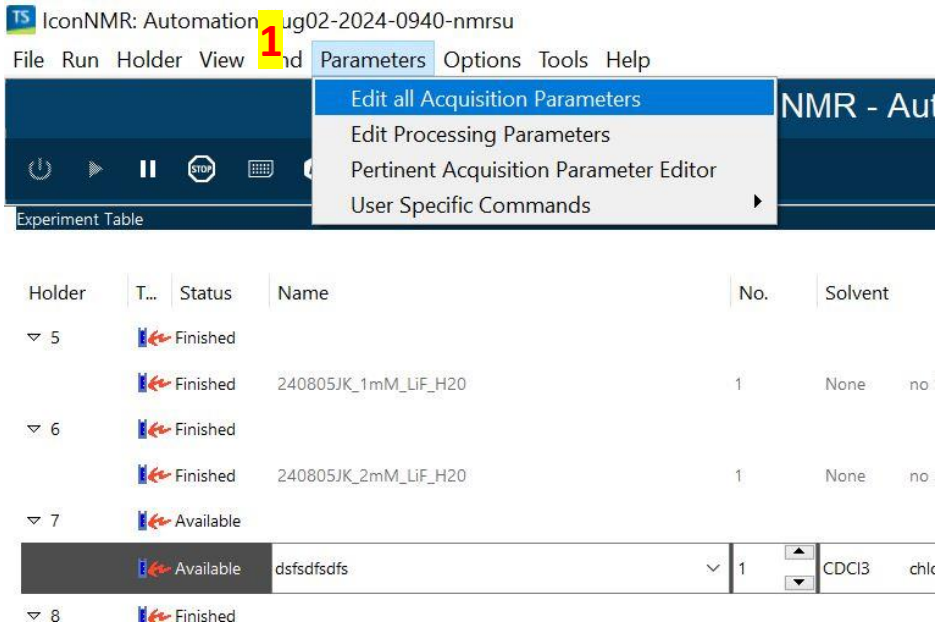


Pulse sequence in UNIST



Name in Bruker NMR	Pulse sequence	Purpose	Remarks
Proton_PRESAT	Presaturation	Solvent suppression (with water suppression)	Simple, any solvent, multi suppression
Presat+NOE_UNIST	noesy1d (noesygppr1d)	Solvent suppression (with water suppression)	More effective sequence
Watergate_3919_UNIST	Watergate (3-9-19)	Water suppression	
WG-3919FP_UNIST	Watergate (W5)	Water suppression	Flip back (No effect exchangeable H)
WET_UNIST	WET	Solvent suppression (with water suppression)	Good baseline, any solvent, multi suppression
PEW5_UNIST	Perfect echo W5	Water suppression	Smallest notch!

Important parameter (O1P)



au_watersc : acquisition program in auto
bt_calib=1 : Automatically find O1
pcal=1 : Automatically find P1
supp_opt=1 : Automatically find O1 and P1
standard set is supp_opt=1

O1P(O1 ppm) related to suppression effectivity!
[Solvent peak center = O1P]

Already known solvent chemical shift..

1) **Just set au_watersc**
2) **Directly change O1P (ex. H2O+D2O = 4.7 ppm)**