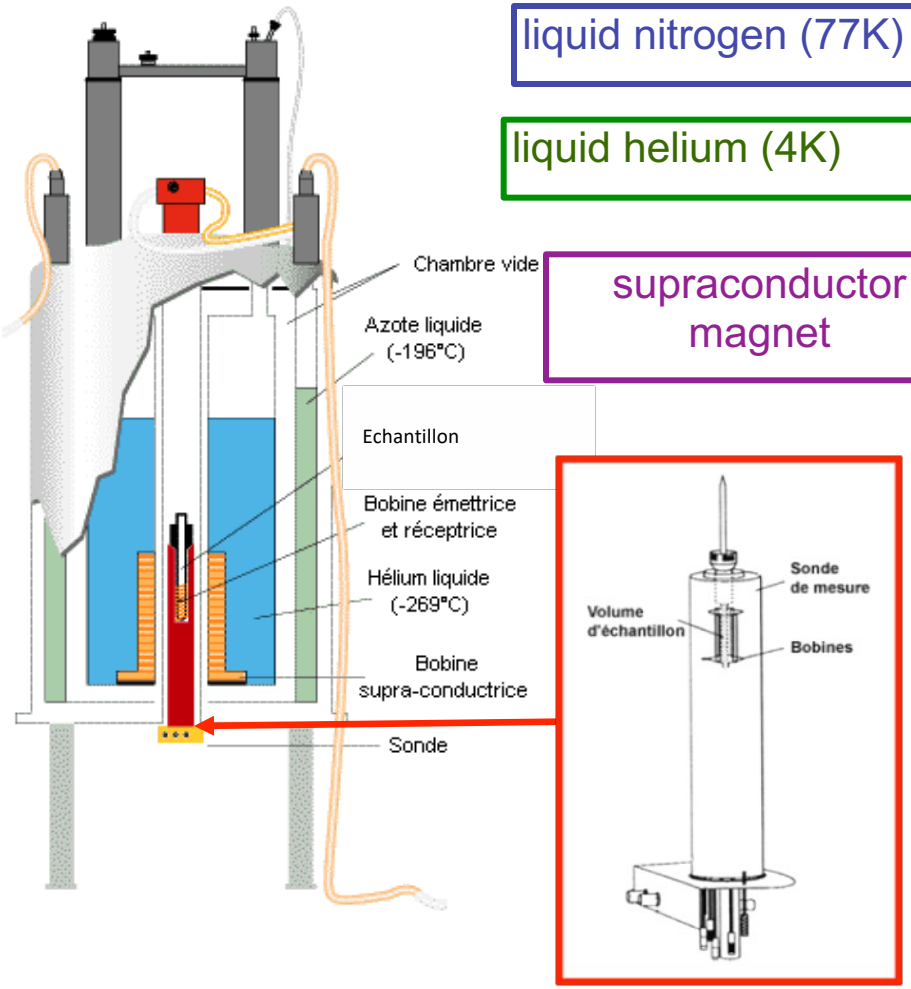
User training session
Liquid state NMR

Sandrine DENIS-QUANQUIN – 2022

Magnet



liquid nitrogen (77K)

liquid helium (4K)

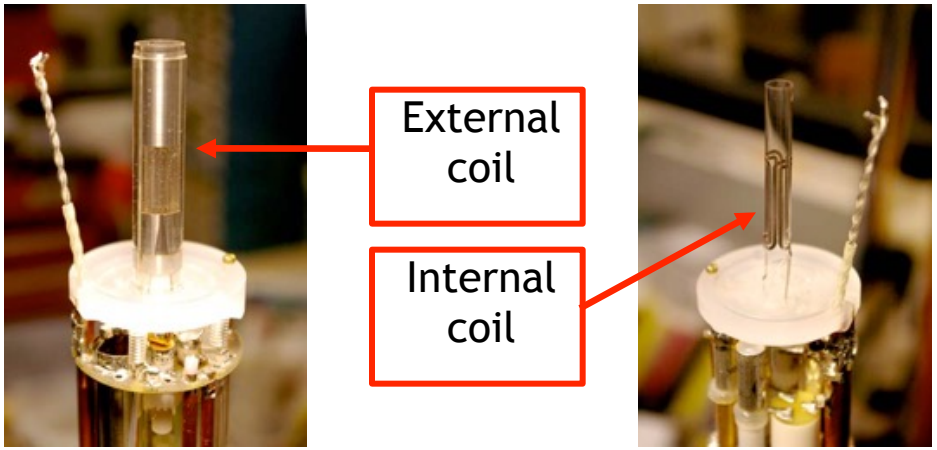
supraconductor magnet

Illustration from http://www.astrosurf.com/luxorion/technologies-futur7.htmet
and photo from JEOL http://www.jeolusa.com

Probes

300 MHz spectrometer is equiped with a BBFO probe (external ^{1}H coil and internal coil for observation of ^{19}F and all nuclei between ^{31}P and ^{15}N).

400 MHz spectrometer is equiped with a cryogenic probe, there is less electronic noise at low temperature and thus the probe is much more sensitive than a « warm » one .



NMR tubes

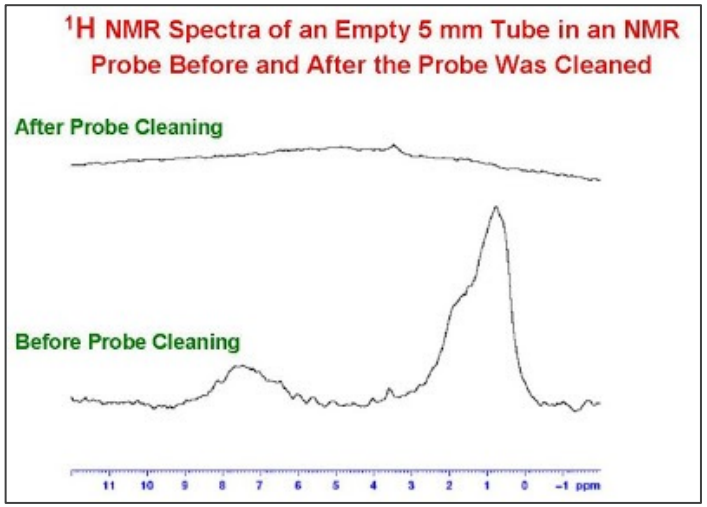
If you need specific tubes just ask me.

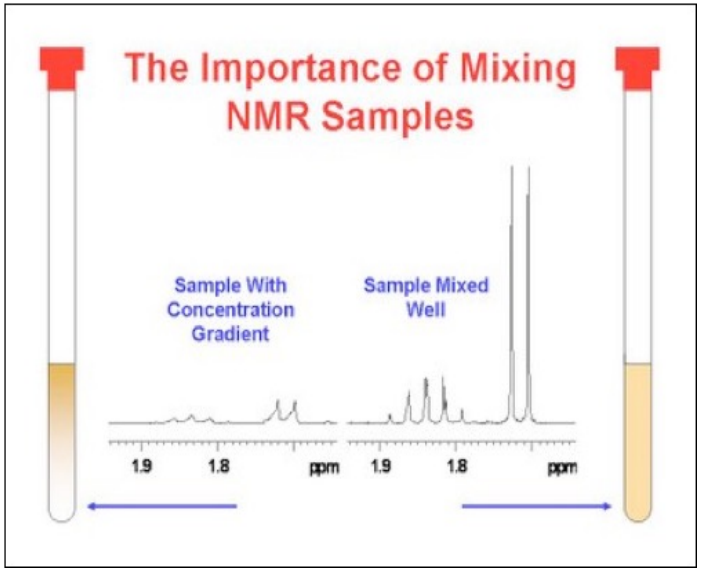
Always wipe your tube before putting it in the spectrometer! Dirt from your hands may go in the probe!

- small volume
- poor solubility
- wrong tube
- dirty or scratched tube
- paramagnetic impurities

bad shims

To clean tubes:
- don't use a brush (might damage the tubes)
- use water and acetone
- if very dirty use nitric acid or ultrasounds
- dry tubes flat at 125° C max, 30 min



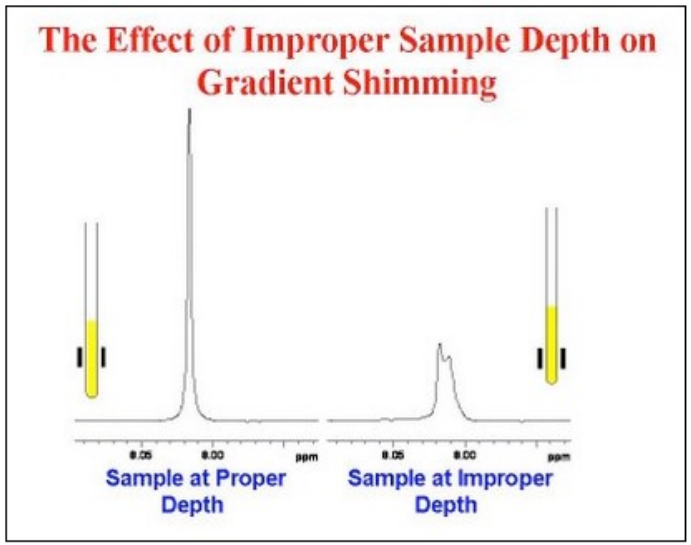


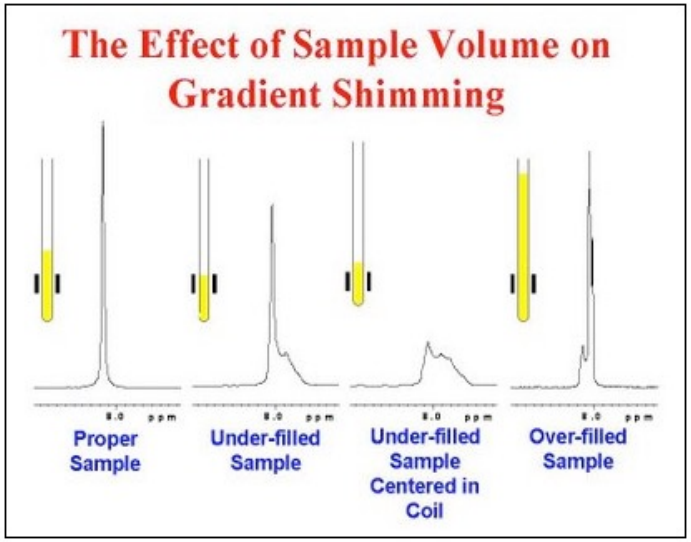
The sample

Sample quantity depends on the spectrometer and the considered experiments (1 to 10mg is usually ok).
Check your sample solubility!

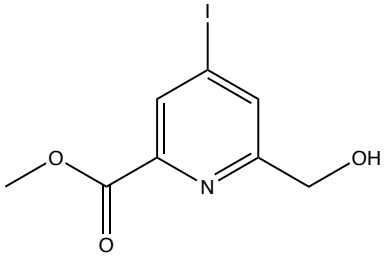
Volume: 500-600µL in a deuterated solvent ($CDCl_3$, D_2O, DMSO-d_6...).

The volume and the height of the tube in the spinner have a strong effect on shims quality.





Sensitivity: 300 MHz vs 400 MHz prodigy



Small molecule, MW = 293 g.mol^{-1}
First tube with 50 mg in 500 µL (c = 340 mM) then dilution 1/10 3 times
Experiments tested: ^{1}H, ^{13}C and HSQC

300 MHZ

c	^{1}H	^{13}C	HSQC
340 mM	NS = 1	NS = 50 3 min	NS = 2 7 min
34 mM	NS = 16 1 min	NS = 500 34 min	NS = 2 7 min
3.4 mM	NS = 16 1 min	not tested	NS = 4 14 min
0.34 mM	NS = 64 4 min	not tested	NS > 16

400 MHZ, prodigy probe

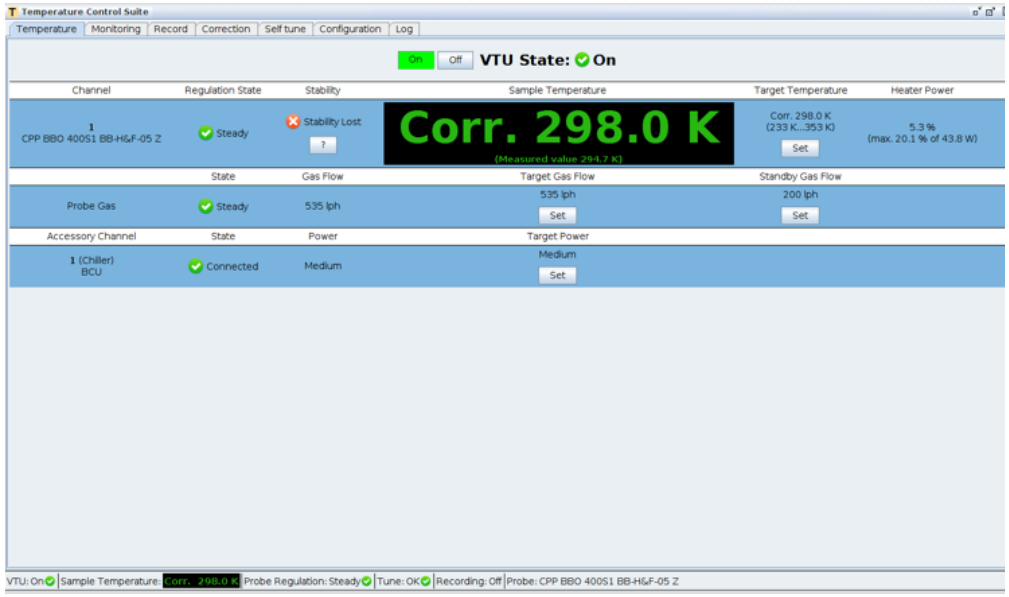
c	^{1}H	^{13}C	HSQC
340 mM	NS = 1	not tested	not tested
34 mM	NS = 1	NS = 50 3 min	NS = 2 7 min
3.4 mM	NS = 16 1 min	NS = 500 34 min	NS = 2 7 min
0.34 mM	NS = 64 4 min	NS = 12000 12 h	NS = 16 1 h

Variable temperature

400MHz spectrometer is equipped for variable temperature experiments.

check boiling and freezing points for your solvent!

For long experiments and high/low temperature use the specific spinner and even a pyrex tube.

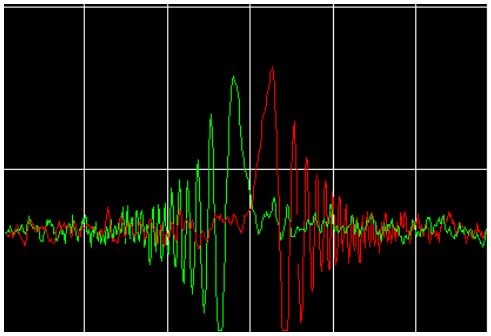


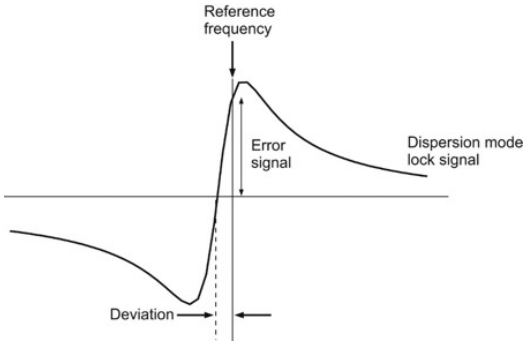
Solvent	^{1}H Chemical Shift* (ppm from TMS) (multiplicity)	Melting point (°C)***	Boiling point (°C)***
Acetic acid D_4	11.65 (1) 2.04 (5)	16.7	118
Acetone D_6	2.05 (5)	-94	56.5
Acetonitrile D_3	1.94 (5)	-45	81.6
Benzene D_6	7.16 (1)	5.5	80.1
Chloroform D	7.24 (1)	-63.5	61-62
Cyclohexane D_{12}	1.38 (1)	6.47	80.7
Deuterium oxide	4.80 (DSS) 4.81 (TSP)	3.81	101.42
N,N Dimethyl-formamide D_7	8.03 (1) 2.92 (5) 2.75 (5)	-61	153
1,2 Dichlorobenzene D_4	6.93 (1) 7.19 (1)	-17	181
Dimethyl sulfoxide D_6	2.50 (5)	18.45	189
1,4 Dioxane D_8	3.53 (m)	11.8	101.1
Ethanol D_6	5.19 (1) 3.56 (1) 1.11 (m)	-114.1	78.5
Hexafluoroisopropanol D_2	4.41 (m) 4.86 (1)	-4	59
Isopropanol D_8	1.1 (1) 3.89 (1) 5.27 (1)	-89	83
Methanol D_4	4.78 (1) 3.31 (5)	-97.8	64.7
Methylene chloride D_2	5.32 (3)	-95	39.75

The magnet is not parfect and the magnetic field drifts by a few herz everyday. Lock is used to compensate this drift. It garantees a **stable magnetic field** during the experiments.

The lock system is dedicated to the observation of 2H. The system compares the solvent deuterium signal frequency with a theoretical value and corrects the magnetic field strength accordingly. This correction is used for proton and X nuclei as well.

This correction is repeated as long as the system is locked, thus compensating for magnetic field fluctuations.

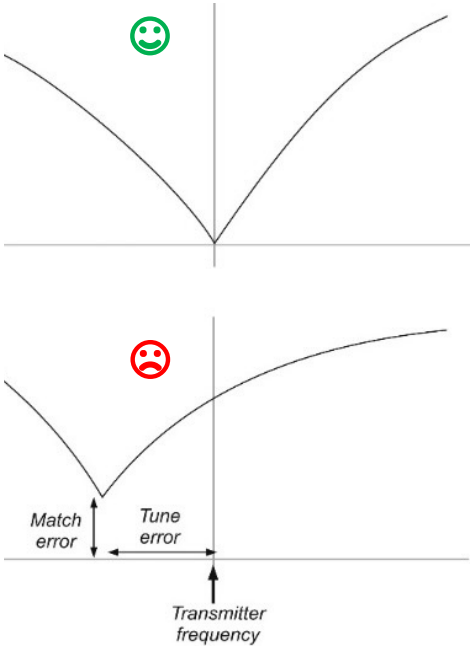


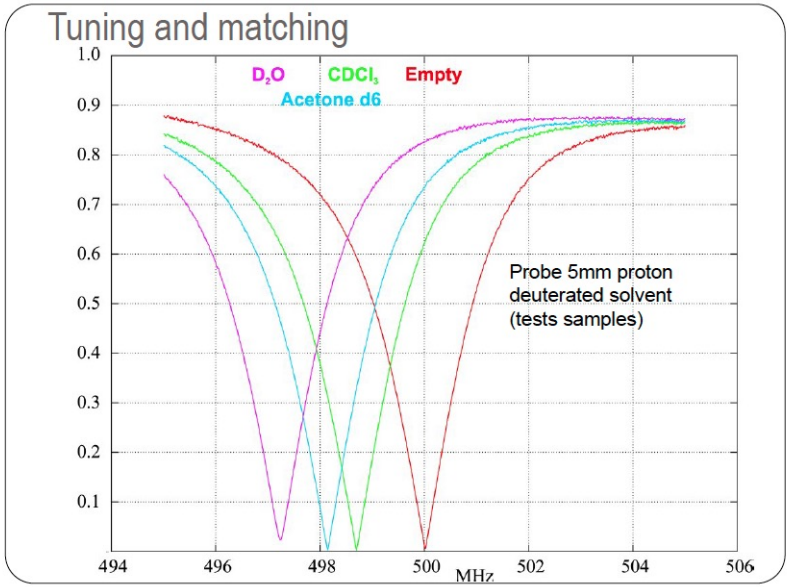


The probe is tuned for each observed nucleus. It is an electronic optimization procedure for a maximum transmission of the signal between the probe and the receiver system at the desired frequency (like tuning the radio in your car).

If you run a carbon experiment and forget to tune, if the probe is still tuned for ^{31}P you will acquire only noise.

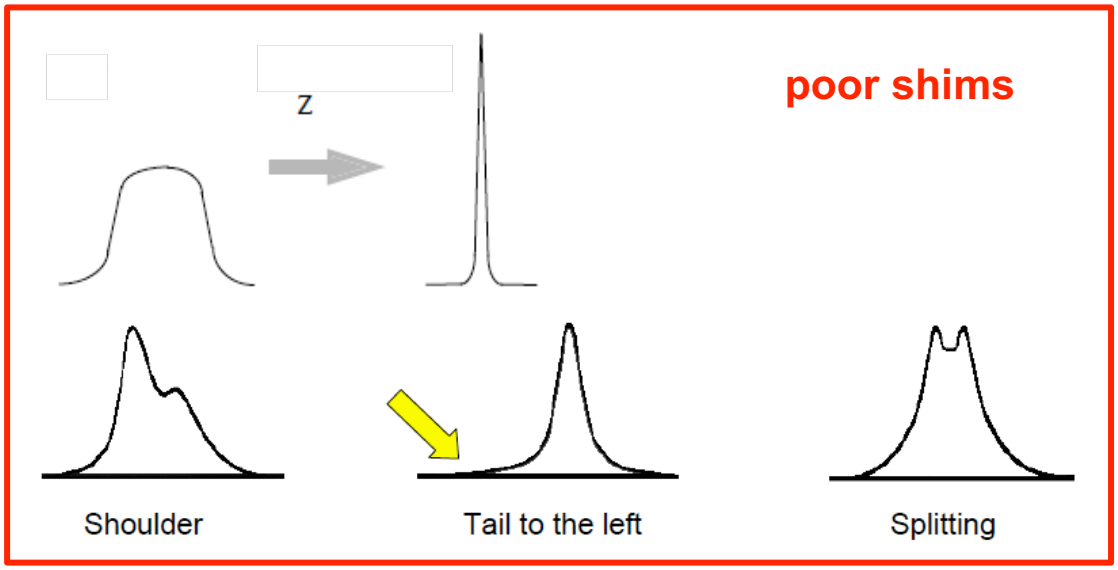
Proton experiment: the frequency depends on the dielectric constant of the sample (importance of the solvent)

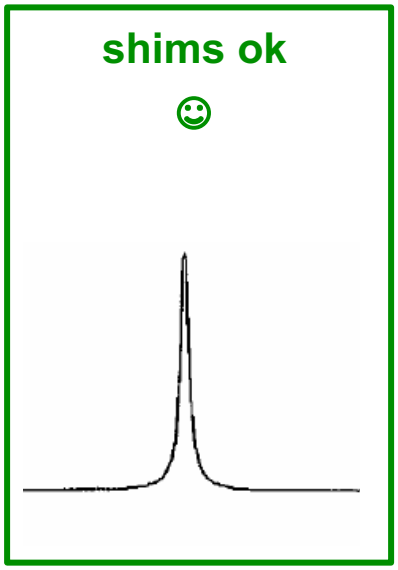




Shim coils around the probe are used to compensate for B_0 field inhomogeneities.

Topshim is an automatic shimming procedure that works well as long provided that the conditions are not too bad: no miracle with a non soluble sample, a damaged tube...





NMR signal is weak and must be amplified by the receiver. The receiver gain (RG) is automatically optimized. A small value means the sample is quite concentrated and needs little amplification. RG is between 1 and 203.

Be careful

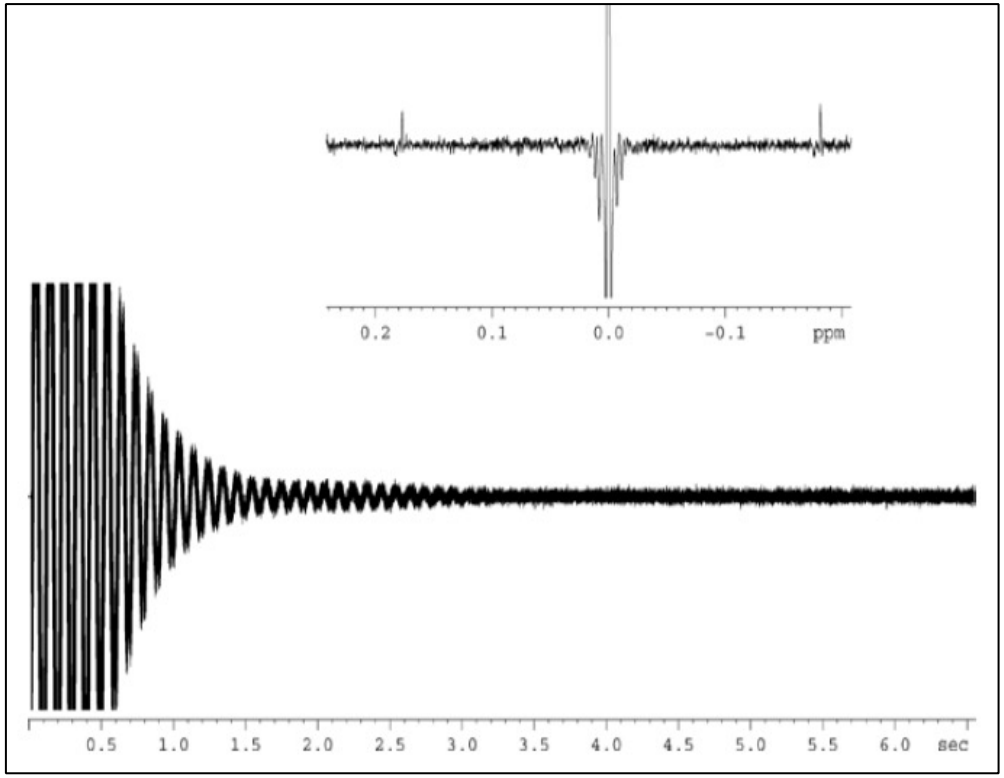
- RG too low

  ⇒ sensitivity loss

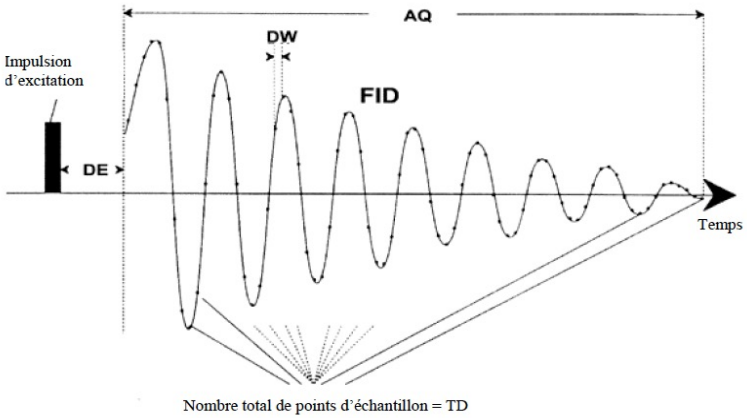
- RG too high

  ⇒ receiver is saturated

  ⇒ wiggles



1D acquisition

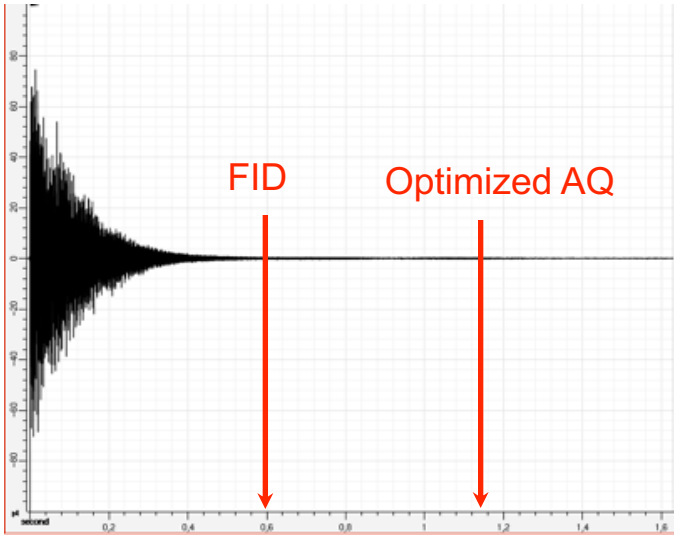


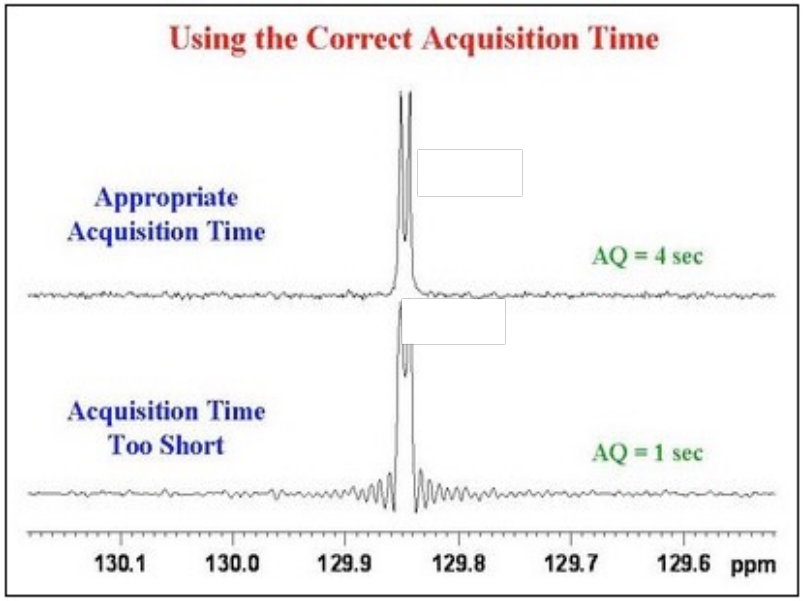
- AQ = acquisition time
- TD = number of points
- DW = sampling time (between 2 points)

$$AQ = TD \times DW \quad \text{with} \quad DW = \frac{1}{2 \times SWH}$$

$$TD = 2 \times AQ \times SWH$$

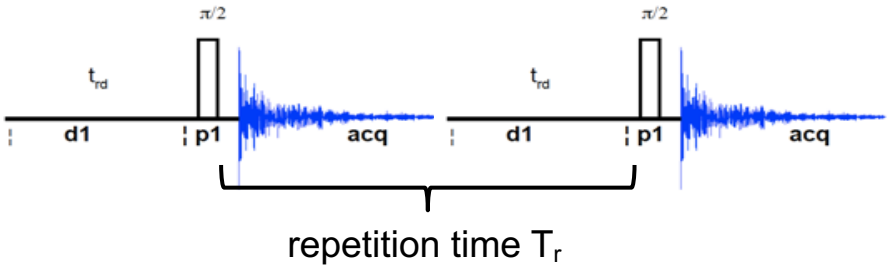
An optimized AQ is twice as long as the FID

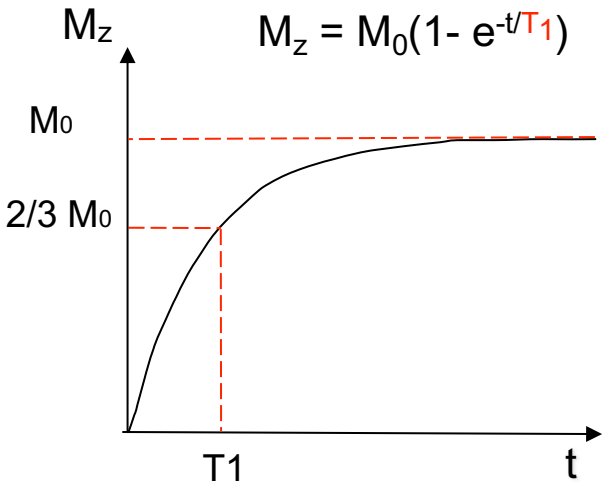


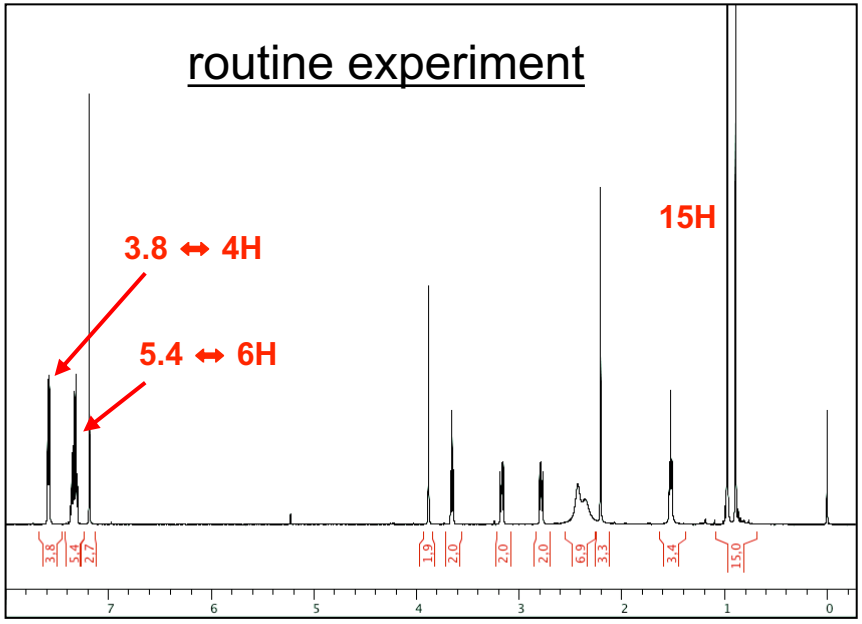


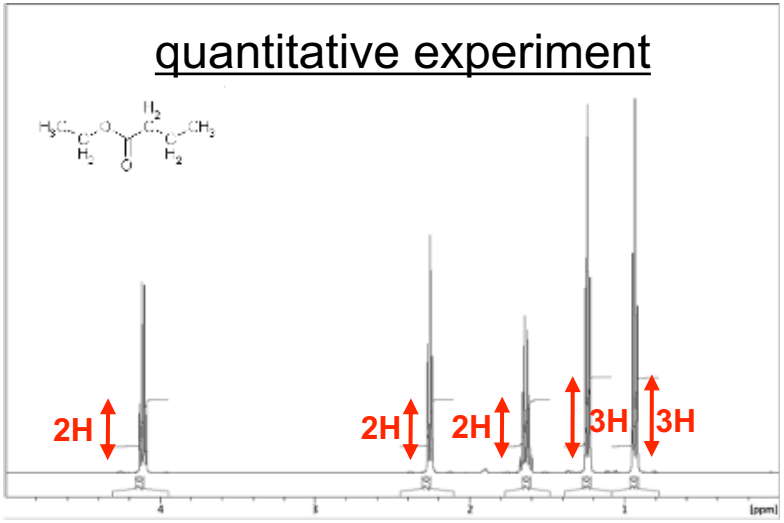
Integration and T_1 relaxation

For a quantitative spectrum use at least $T_r = 3x\ T_1$ between 2 pulses. T_1 is the time required for the recovery of ⅔ of M_0.







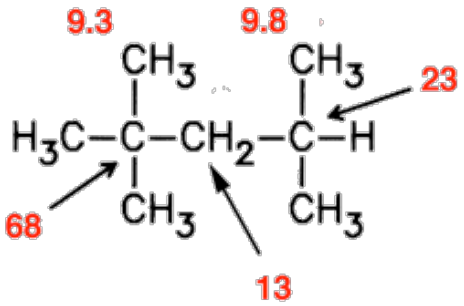


T_1 relaxation

For small molecules in solution:

- protons relaxation time T_1 varies from 1 to 5s. Aromatic protons relax slower than aliphatic ones.
- ^{31}P relaxation time T_1 varies from 2 to 20s
- ^{13}C T1 varies from 0.1s to severals tens of seconds. Quaternary carbons relax slower than others.

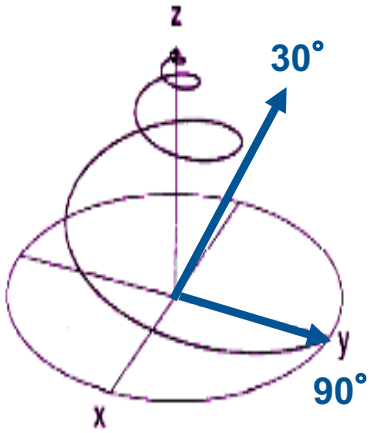
T1 relaxation for some ^{13}C



T_1 times can be really long with some nuclei (^{29}Si, ^{15}N…) leading to some issues regarding the experimental time.

Pulses and S/N

A pulse of duration t tilts the magnetization away from the z axis with an angle $\theta = 2\pi\nu_0 t$. For a maximum signal a 90° may be chosen but a 30° allows a shorter repetition time (T_r). The zg30 experiment is used for 1D routine.

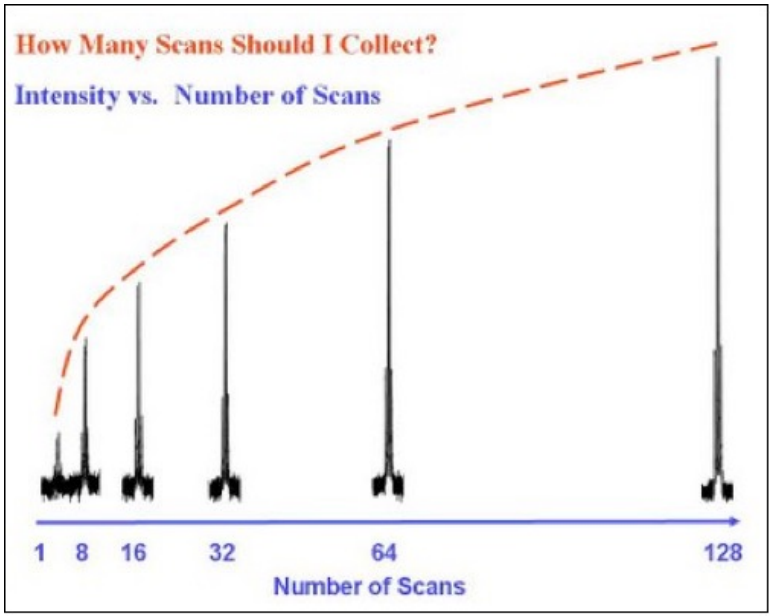


For an optimal S/N➔ Ernst angle $\cos\theta = \exp(-\frac{T_r}{T_1})$

With $\theta = 30°$ ⇒ short T_r ($\frac{1}{5}\ T_1$)

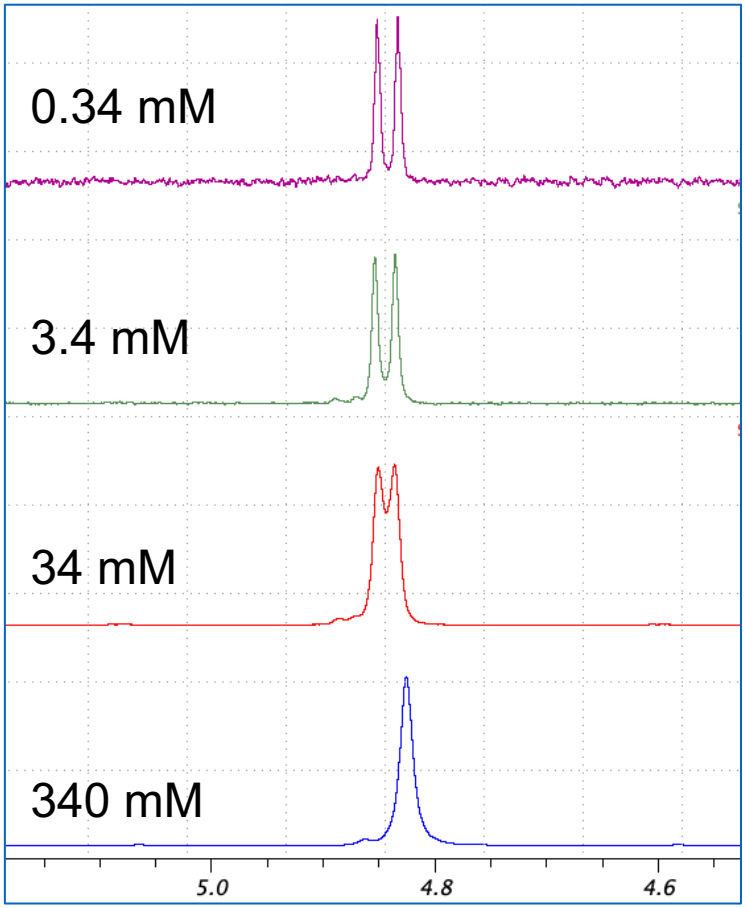
The experiment is not quantitative!

For an even better S/N increase the number of scans and remember that S/N ∝ √NS.



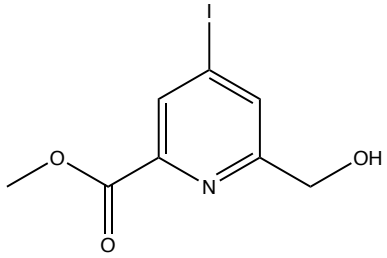
NS	S/N signal 1	S/N signal 2	time
4	5	-	12s
64	20	-	≈3min
256	40	1,5	≈13min
4096	160	6	≈3h25min

S/N and NS



S/N for ^{1}H spectrum

$$S/N \propto \sqrt{NS}$$

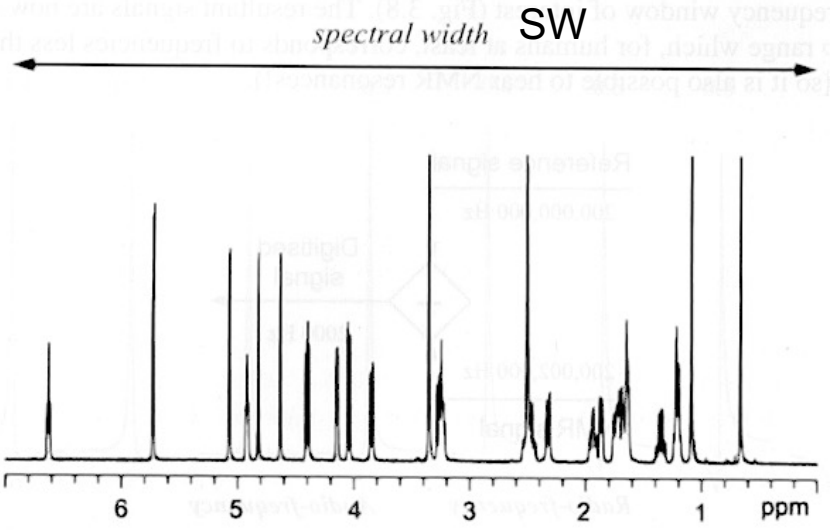


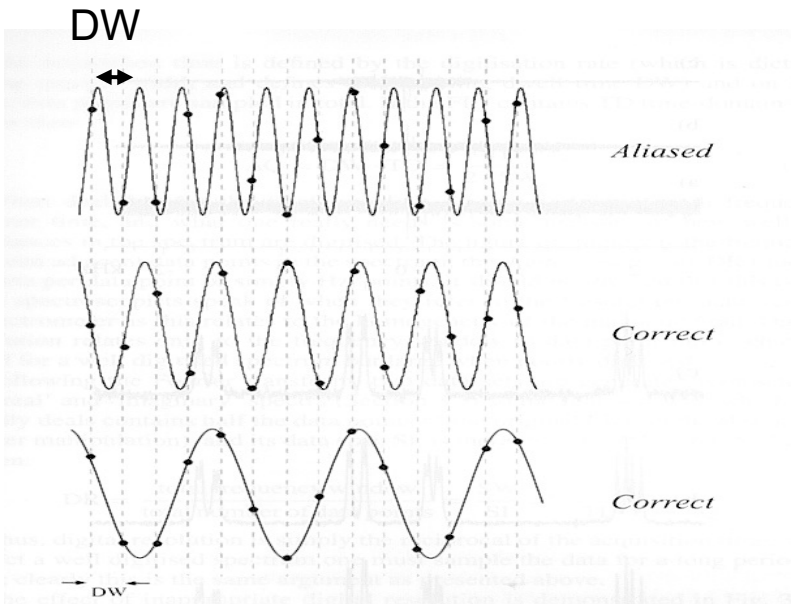
c	NS	300: S/N	400: S/N
340 mM	NS = 1	1240	4470
34 mM	NS = 16	550	2480
3.4 mM	NS = 16	105	510
0.34 mM	NS = 16	-	116
	NS = 64	47	227
	NS = 256	95	-

NS x4 ⇒ S/N x2

high concentration may affect the quality of the data!

FT: digitisation, sampling

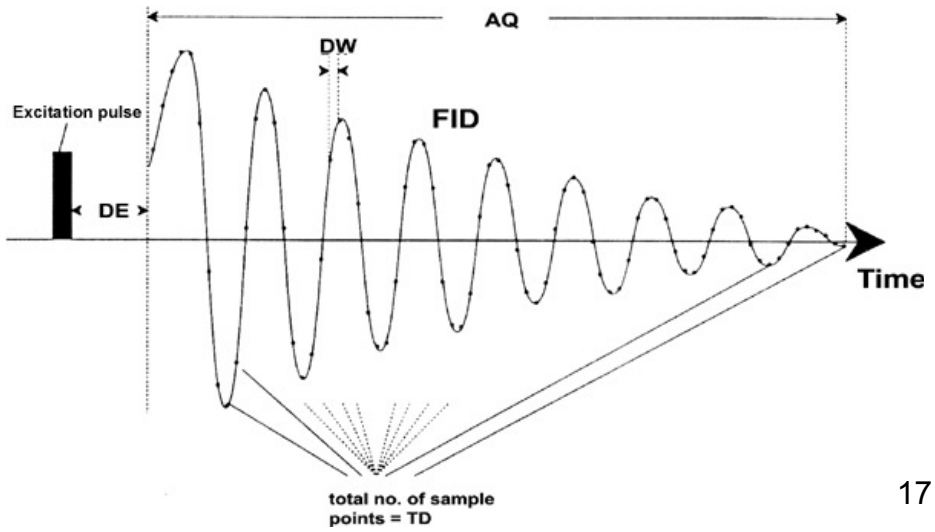




Nyquist theorem: at least 2 points per period are needed for a good representation of all frequencies.

Nyquist condition DW = 1/2SW

$$AQ = TD \times DW \text{ with } DW = \frac{1}{2 \times SWH}$$



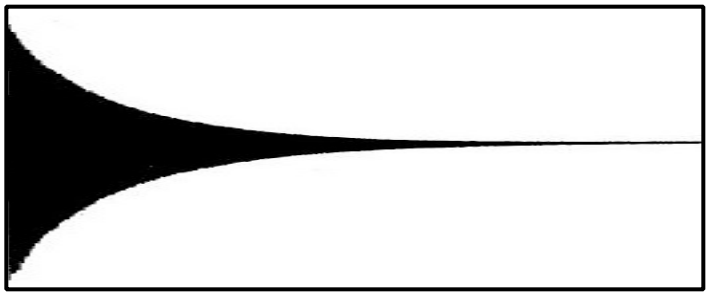
FID and T_2 relaxation

T_2 depends on:
- field inhomogeneities
- molecular interactions

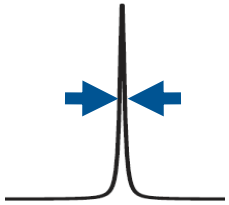
Shims!

We should talk about T2* : $1/T_2^* = 1/T_2 + 1/T_{2(\Delta B0)}$

The FID decreases as exp (-t / T_2^*): $M_x = e^{-t/T_{2^*}} \cos(\omega-\omega_o)t$



- Short T_2^*
- Fast relaxation



- Long T_2^*
- Slow relaxation

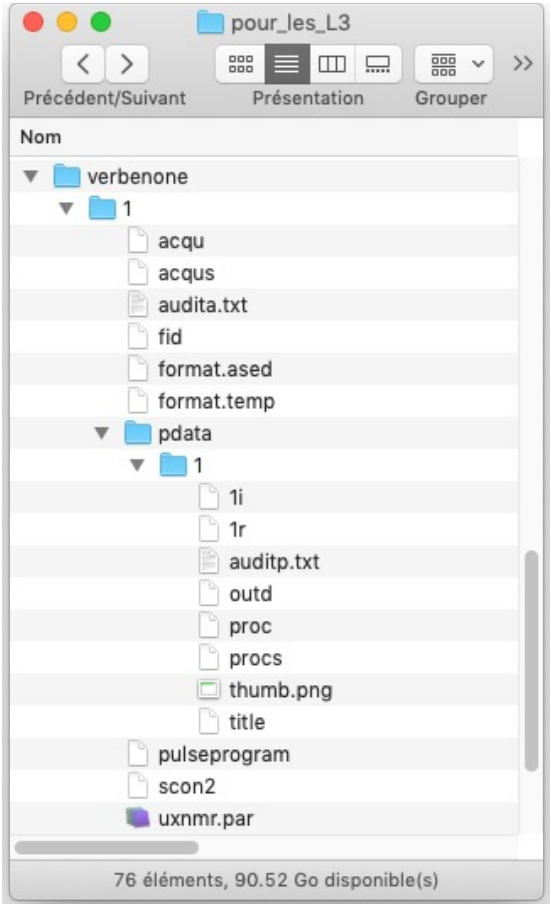
half height width: 1 / πT_2^*

Data processing

Some processing techniques help to get a nicer spectrum, to compensate for imperfections due to a non optimized acquisition.

The effect is only aesthetic, the data are not modified.

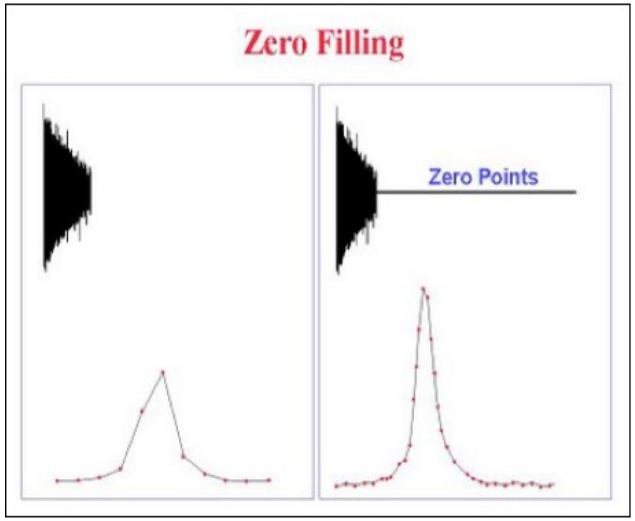
In the data directory, the processed data are stored in a subfolder called « pdata » and the raw data are stored as a « fid » file. You can try whatever processing you want, the fid will not be affected.

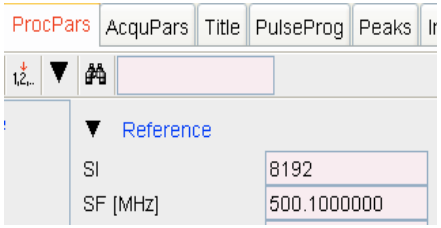


These processing techniques can not make up for everything. Try to optimize your experiment BEFORE the acquisition.

Zero filling/ Linear prediction

Zero filling = adding zeros to the end of the FID to increase the digital resolution

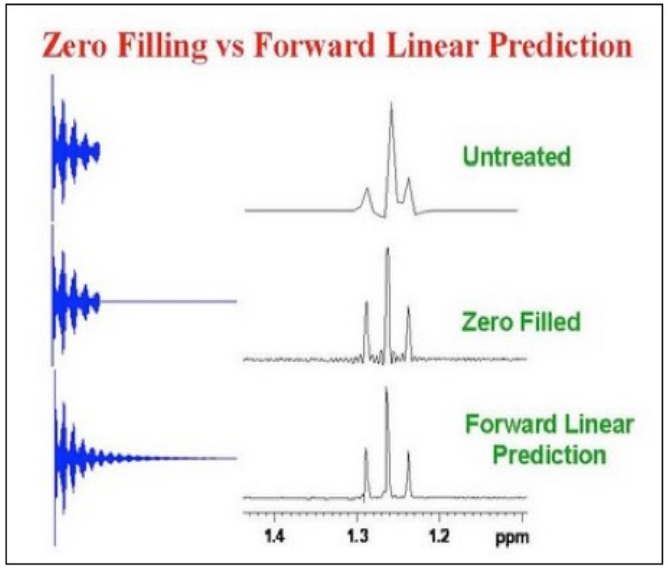




SI = TD/2: normal

SI > TD/2: zero filling

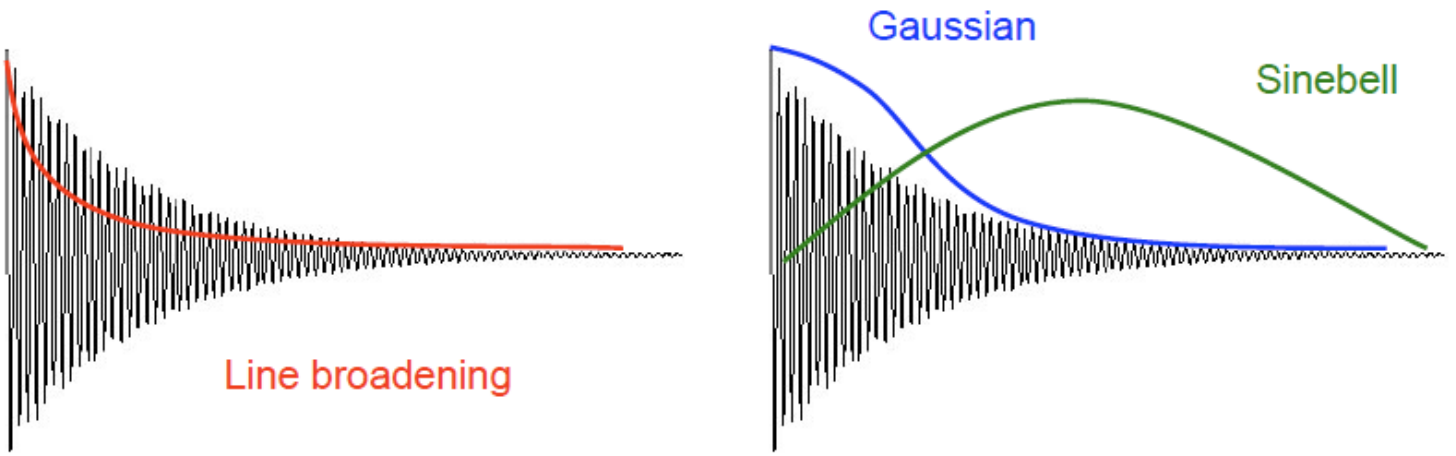
Linear prediction = using the collected data to predict the end of the FID. Really useful for 2nd dimension in 2D experiments (truncated FID).



Apodization

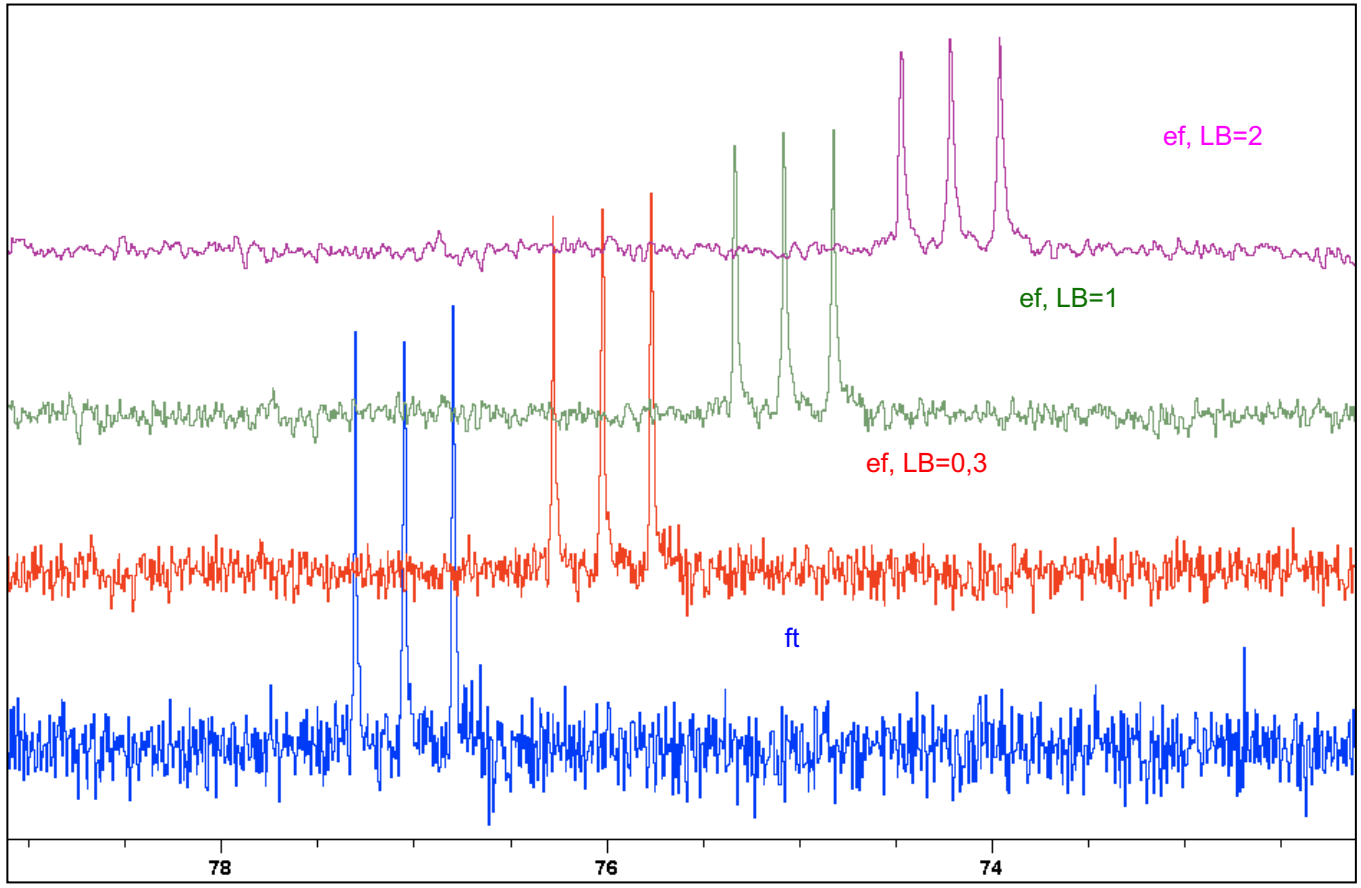
= multiplication of the FID by a function (exponential, sine...) before Fourier Transform

- **exponential function** for better S/N but resolution loss
- **gaussian function** for better resolution but decreased S/N

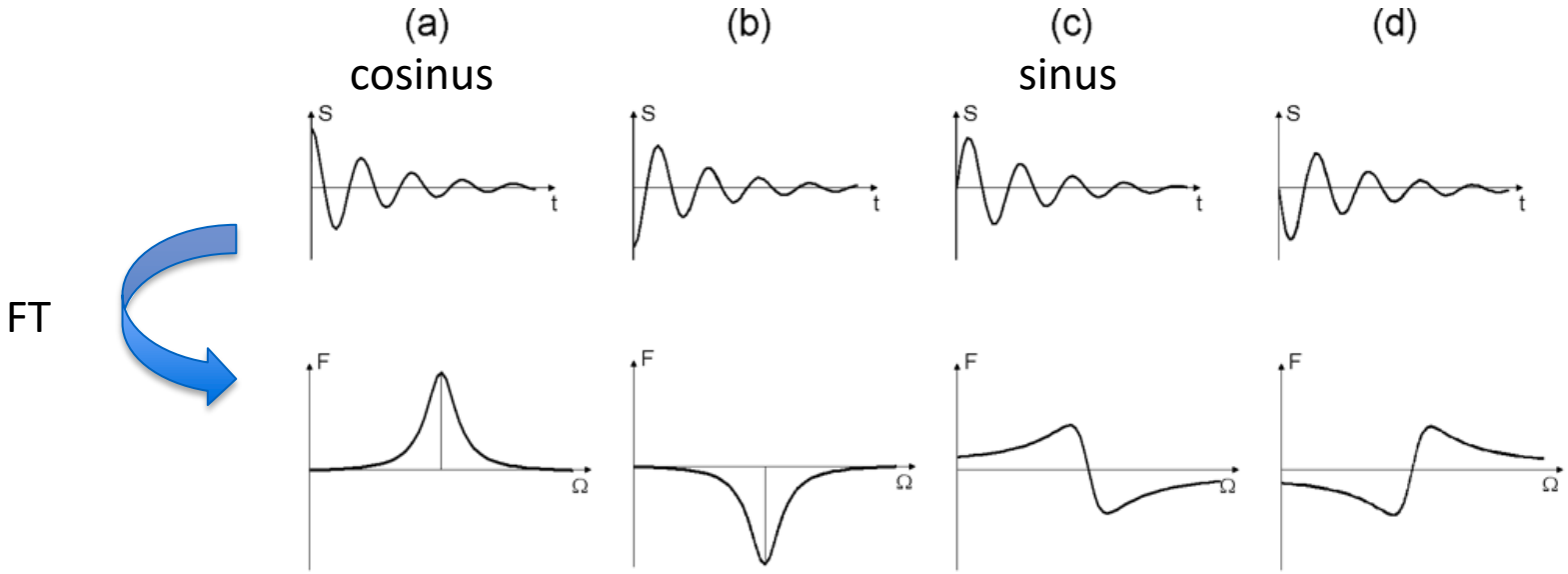


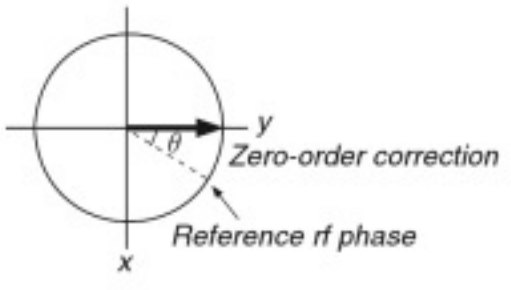
To optimize the line broadening (LB) value for an exponential, measure the half height width of a narrow peak in a spectrum processed with fp instead of efp.
→ Value in Hz = LB to use with efp

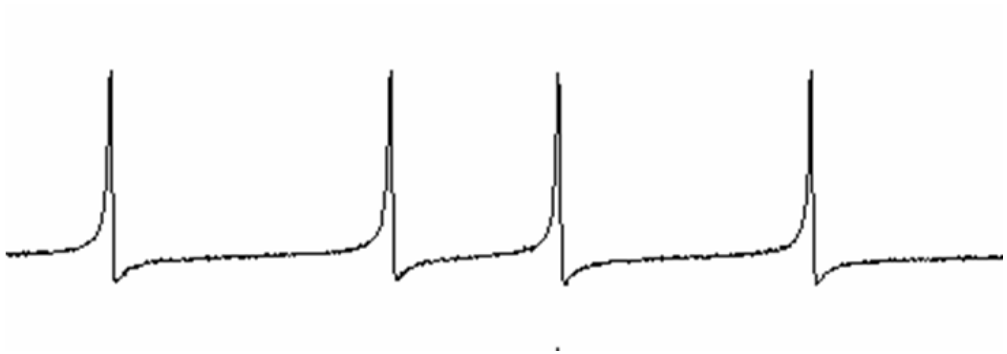
Line broadening factor



Phase correction: 0 order

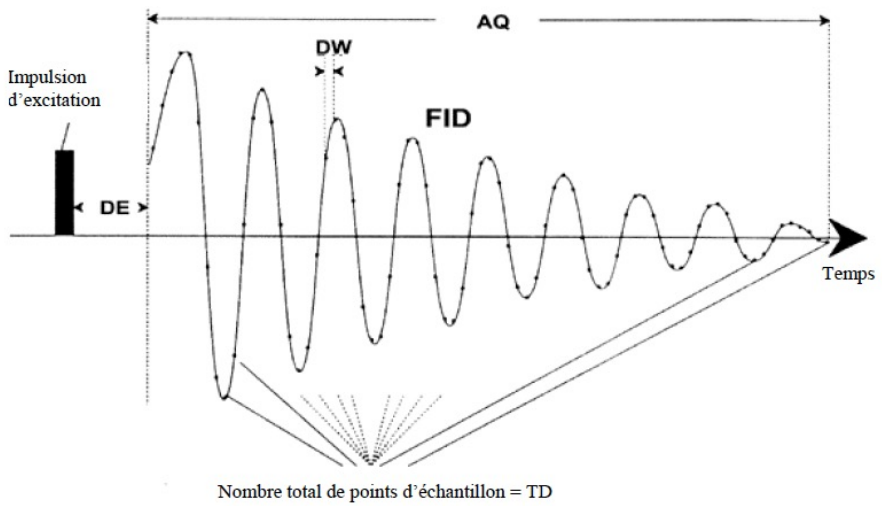


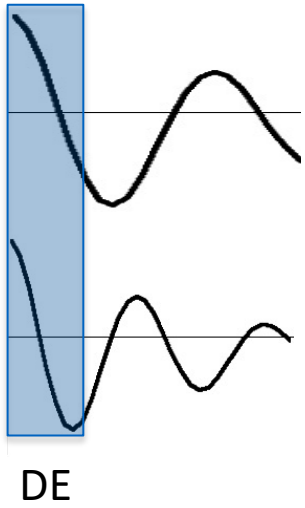




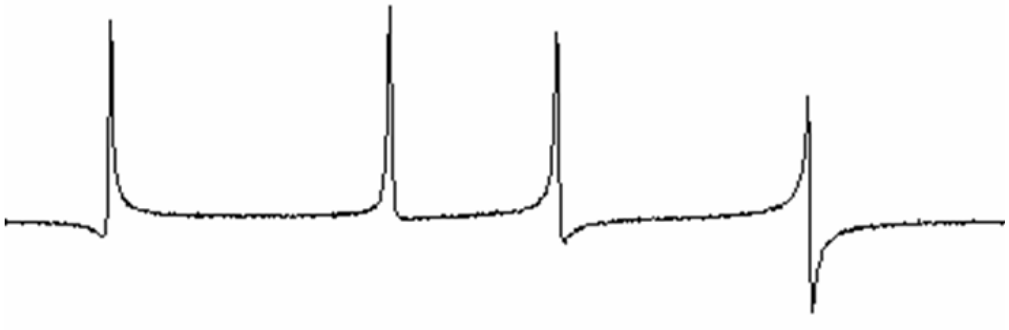
The phase of the detected does not match the phase of the receiver. The error is the same for every signals and is called zero-order phase error.

Phase correction: 1st order





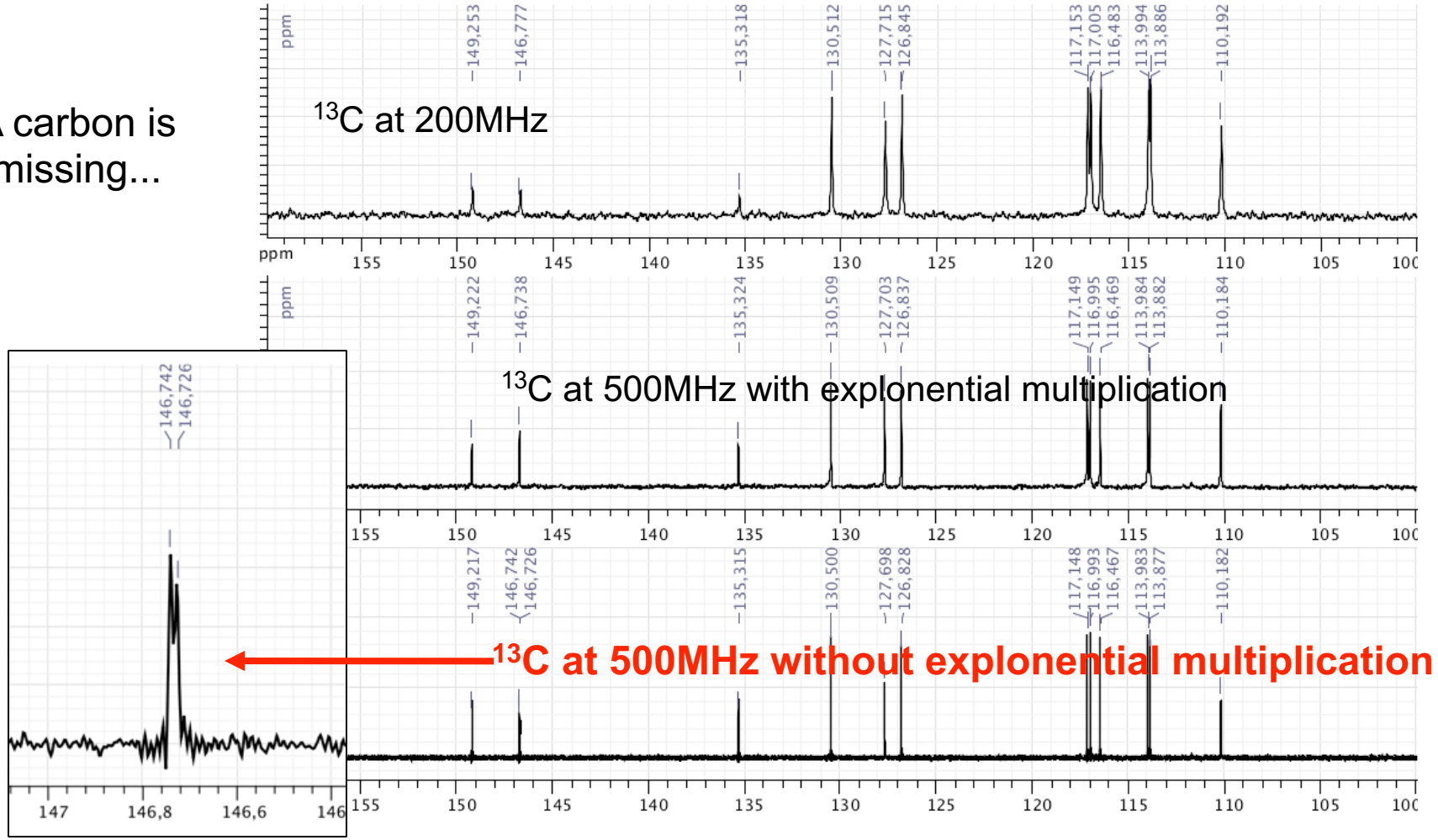
A dead time (DE) is necessary for the coil to switch from excitation to receiving mode. During this delay the magnetization begins to relax but no signal is acquired. Signals with different frequencies develop a phase difference which varies accross the spectrum. It is called first-order phase error.



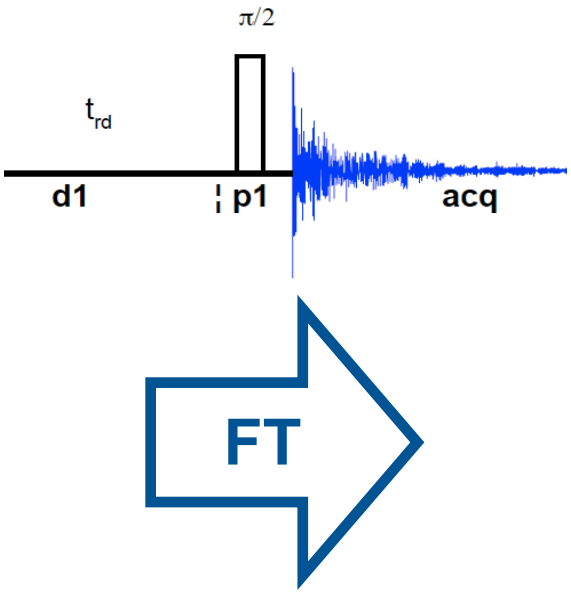
Be careful with processing!

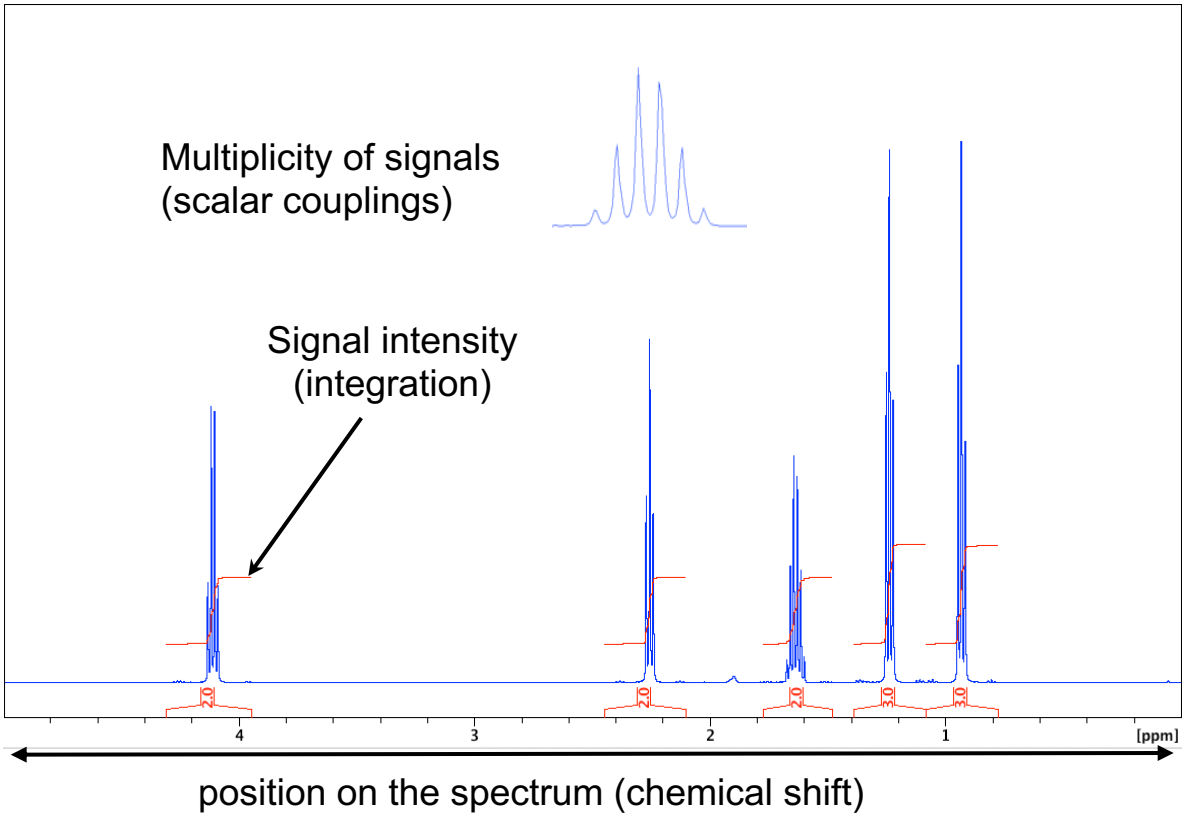
Don’t hesitate to try different processing strategies on your data.
A process that works well with a spectrum may not be ok for a another dataset!

A carbon is missing...



NMR spectrum





Fourier Transform is used to obtain the frequency domain spectrum from the time domain signal (FID). By default the spectrum is displayed in ppm, not in Hz, for an easier comparison of chemical shifts that are field independent when expressed in ppm.

Chemical shift

The local field experienced by a nucleus i depends on its environnement:

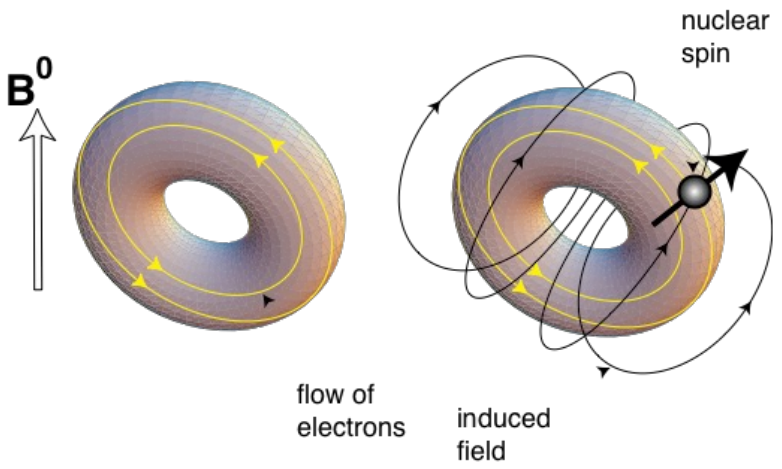
$B_i^{loc} = B_0 + B_i^{induced}$

with $B_i^{induced} = \sigma_i B_0$ (σ_i)

$B_i^{loc} = (1 - \sigma_i) B_0$

The chemical shift is normalized with TMS as a reference

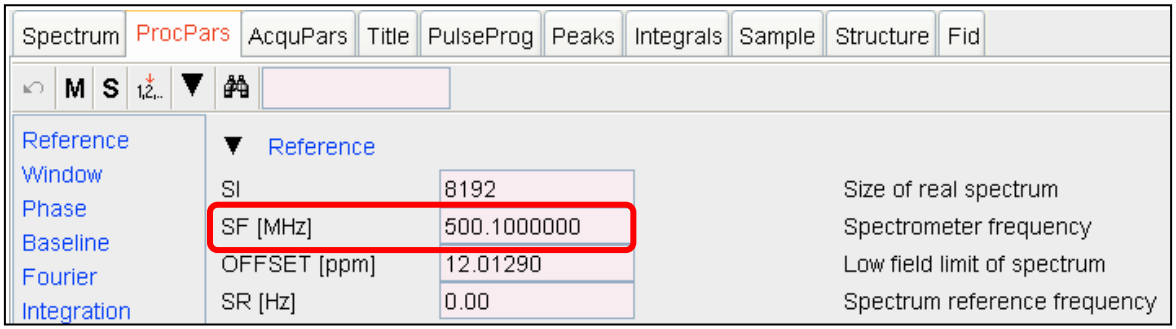
$$\delta_i = \frac{\nu_i - \nu_{ref}}{\nu_{ref}} \times 10^6$$



Thus the chemical is field independent and given in ppm (parts per million)

From ppm to Hz:
- 200 MHz spectrometer (4,7T): 1ppm ⇔ 200Hz for proton
- 500 MHz spctrometer (11,7T): 1ppm ⇔ 500Hz for proton

X spectrum calibration

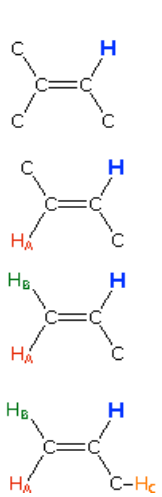


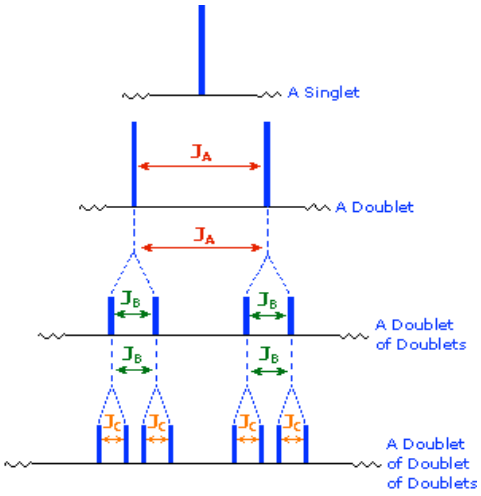
You can calibrate your X spectrum (X = ^{13}C, ^{19}F...) without adding a reference, based on the proton signal from your solvent. You need to multiply the proton SF value by the frequency ratio of the X nucleus. This is described in *Pure Appl. Chem., Vol. 73, No. 11, pp. 1795–1818, 2001.*

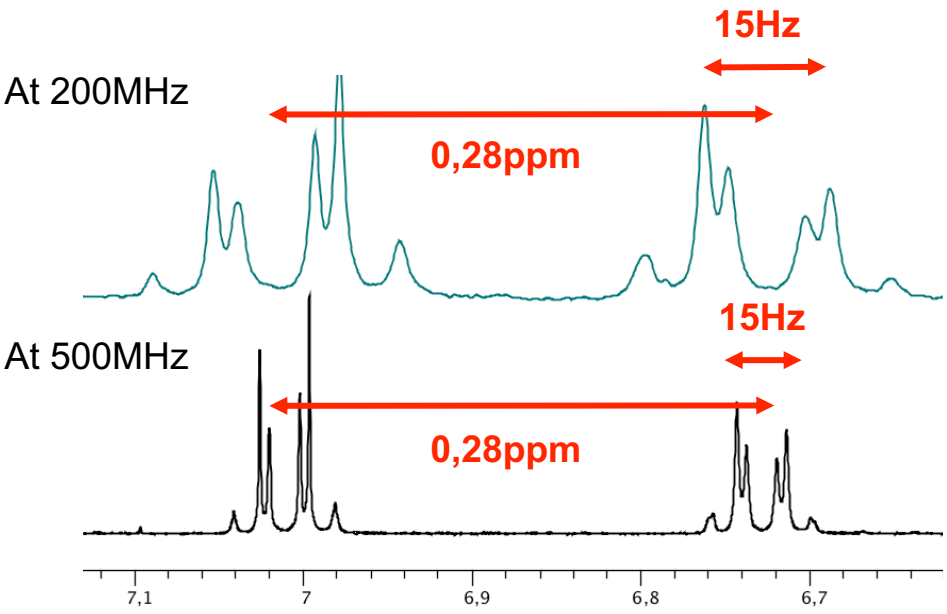
Nucleus	Frequency ratio Ξ	Ref molecule
^{13}C	0.25145020	TMS
^{19}F	0.94094011	CCl_3F
^{29}Si	0.19867187	TMS
^{31}P	0.40480742	H_3PO_4

Scalar couplings

Scalar couplings may be homo- or heteronuclear (J_{CH}, J_{CF}…)
The multiplicity depends on the number of couplings.
A nucleus can experience several couplings, leading to a more complex multiplet..





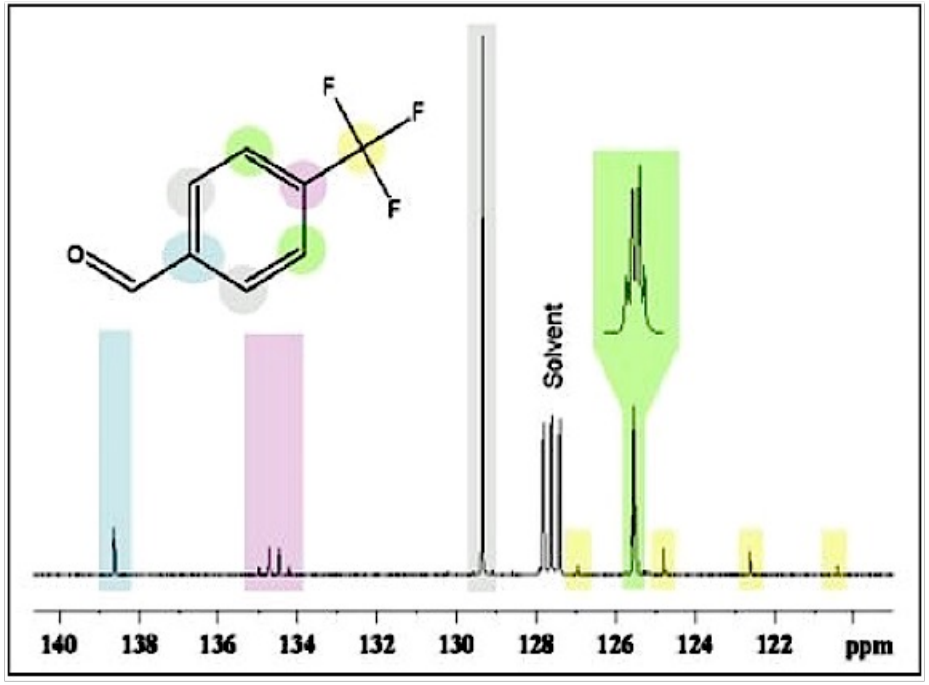


Chemical shifts in ppm: field independent
Scalar couplings in Hz: field dependent

Scalar couplings

Some heteronuclear couplings might complicate the spectrum.

Ex 1: ^{19}F-^{13}C coupling



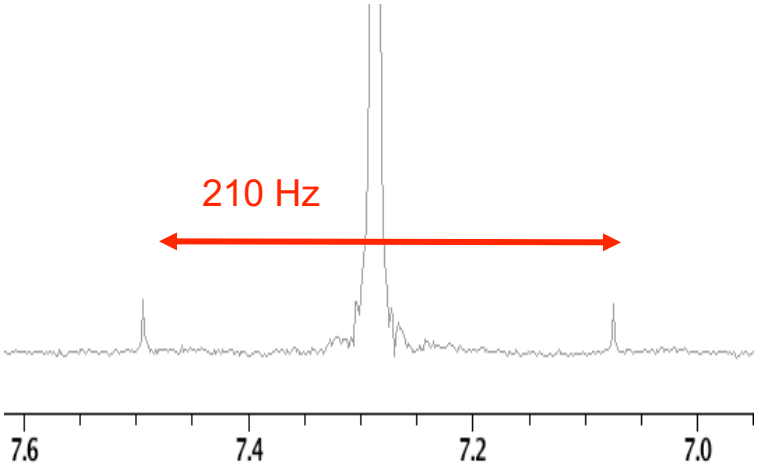
Also ^{31}P-^{13}C couplings …

Ex 2: ^{1}H-^{13}C coupling

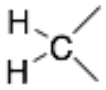
Intense signals are from protons on ^{12}C. But the 1,1% of ^{13}C are coupling with protons and ^{13}C satellites may be observed

➔distance between 2 satellites = $^{1}J_{CH}$

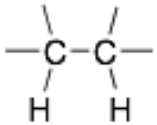
➔intensity = 0,55% of main signal



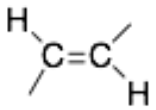
Some couplings



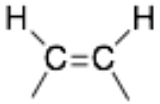
−20 - +6 Hz



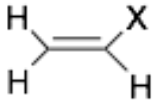
5,5 - 7,5 Hz



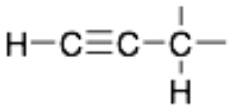
12 - 19 Hz



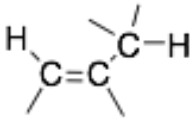
7 - 10 Hz



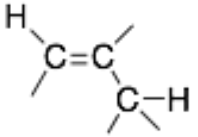
X	$^2J_{HH}$
Li	+7,1
H	+2,5
Cl	−1,4
OCH_3	−2,0
F	−3,2



1,5 - 3 Hz

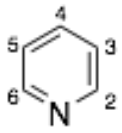


0 - 0,3 Hz

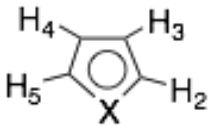


0,5 - 2,5 Hz

o 6 - 9 Hz
m 0,5 - 4 Hz
p 0 - 2,5 Hz

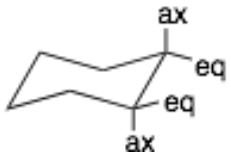


J_{23} 5 - 6 Hz
J_{24} 1,5 - 2,5 Hz
J_{25} 0,5 - 1,2 Hz
J_{26} 0 - 0,5 Hz
J_{34} 6,5 - 8,5 Hz
J_{35} 1 - 2 Hz



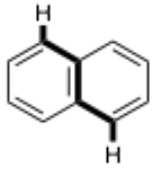
J_{23} 1 - 2,5 Hz
J_{24} 0,5 - 1,5 Hz
J_{25} 2,5 - 4 Hz

X = S, O ,NR

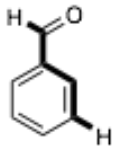


$J_{ax,ax}$ 9 - 14 Hz
$J_{ax,eq}$ 2 - 4 Hz
$J_{eq,eq}$ 2,5 - 4 Hz

Long distance couplings:



0,8 Hz



0,4 Hz

7 Hz

3 - 4 Hz

1,1 Hz

^{13}C NMR

→ «standard» spectrum: decoupled carbon (zgpg)

Each signal is a singulet: better sensitivity, less complexity

→ spectrum without decoupling(zg) to observe JCH couplings

Sensitivity can be an issue, multiplets may overlap!

→ **jmod**: edited spectrum where Cq and CH2 give positive signals whereas CH and CH3 give negative signals

→ **dept135**: edited spectrum as jmod but more sensitive because of the polarization transfer from ^{1}H to ^{13}C. No signals for Cq. USE HSQC INSTEAD

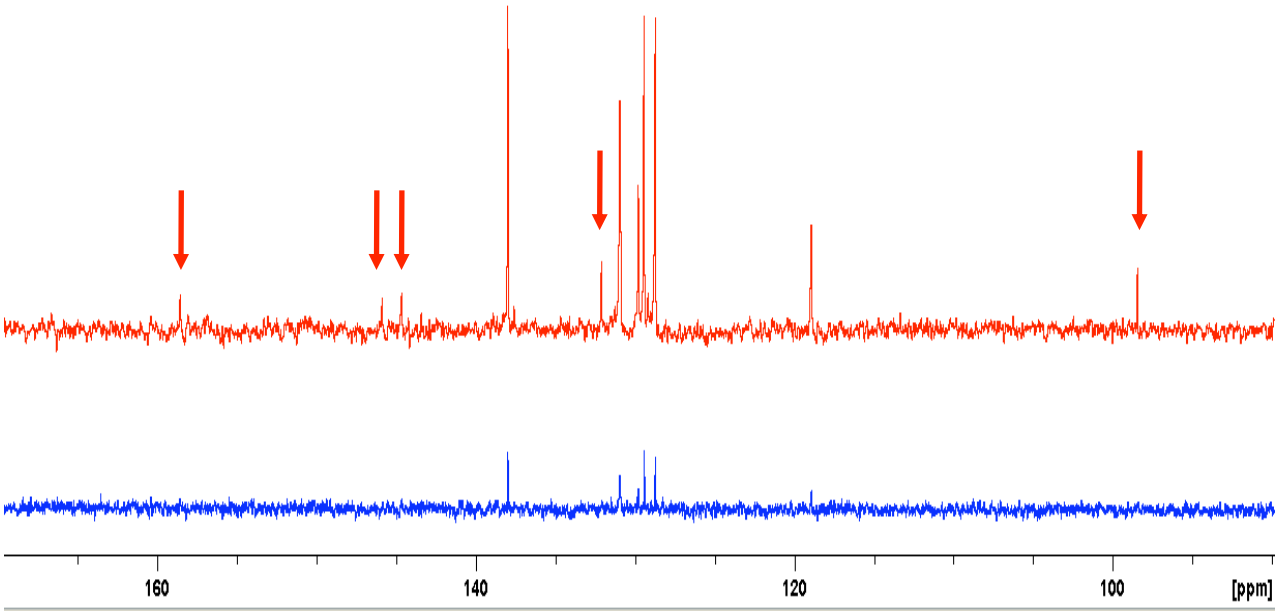
→ **udeft** for quaternary carbons giving weak signals

Always run a proton experiment before a carbon spectrum to check that everything is fine with your sample, with the shims... Remember to tune before starting your carbon experiment, otherwise you might acquire only noise!

^{13}C NMR

udeft

- d1 = 4s
- duration ≈ 4H



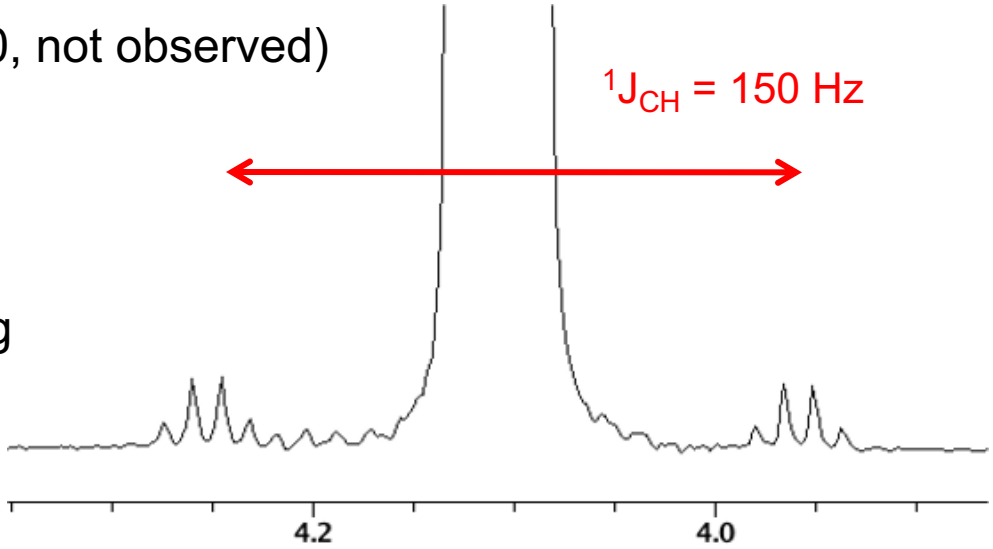
decoupled ^{13}C

- d1 = 20s
- duration ≈ 10H
- even with a long d1, no signals for quaternary carbons

udeft sequence starts with a 200s delay during which nothing happens on the screen, don't try to stop the experiment, there is nothing wrong!

Think about what you are observing

Natural abundance: 98,9% ^{12}C (spin=0, not observed)



Proton spectrum

→Main signals are ^{1}H-^{12}C

→1,1% ^{13}C (spin= ½) ^{1}H-^{13}C coupling observed (^{13}C satellites)

Carbon spectrum

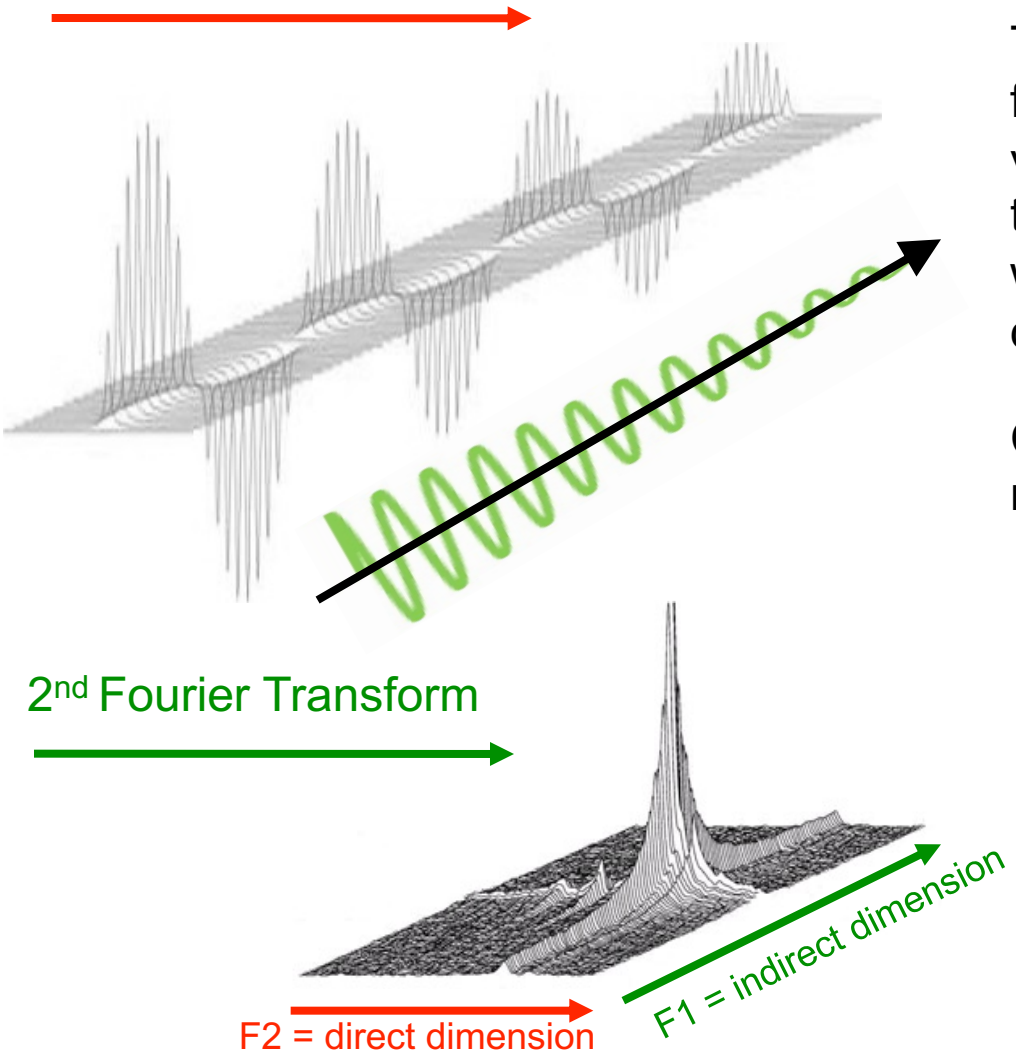
→ Main signals are ^{13}C: only 1,1% of the carbons are observed!

→ These ^{13}C are coupling with ^{1}H: hence the use of decoupled carbon experiment

→ The experiment is PROTON DECOUPLED: you might observe other couplings with ^{19}F or ^{31}P for example

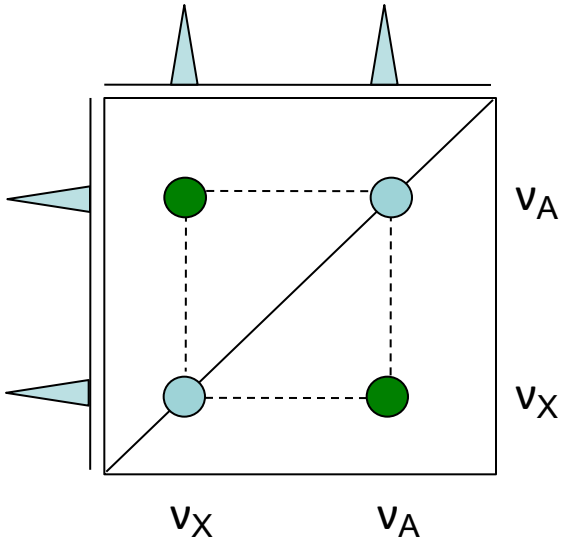
The 2nd dimension

1st Fourier Transform (for each different evolution time)



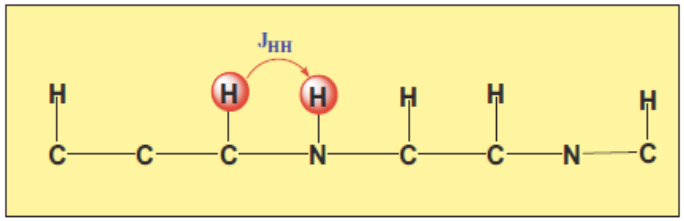
The signals intensity varies as a function of the evolution time. This variation represents an indirect FID that may be Fourier Transformed as well, generating a 2nd frequency dimension.

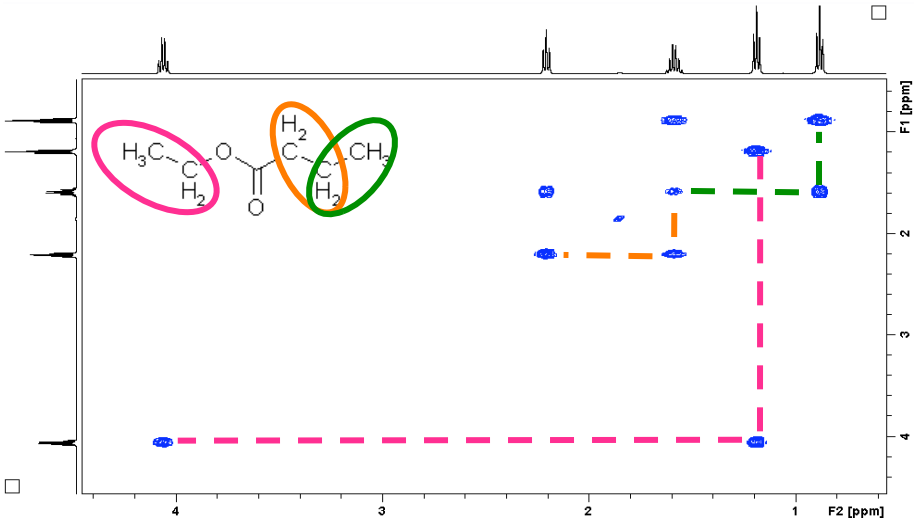
Contour plots are prefered for representation of the 2D data.



COSY

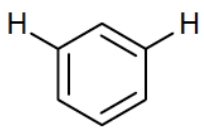
= **CO**rrelation **S**pectroscop**Y,** for scalar couplings



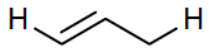


Some long range scalar couplings are large enough to be observed in a COSY spectrum. Coupling across π- systems are the most frequent ^4J couplings.

Another favorable alignement is called « W-coupling » where the C-C bonds are almost coplanar.

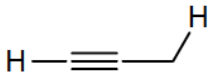


^4J = 2 to 3 Hz



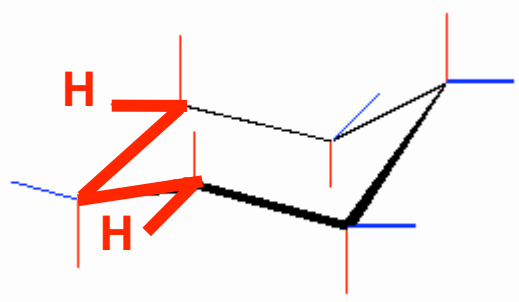
Allylic

^4J = -3 to +3 Hz



Propargylic

^4J = 2 to 4 Hz



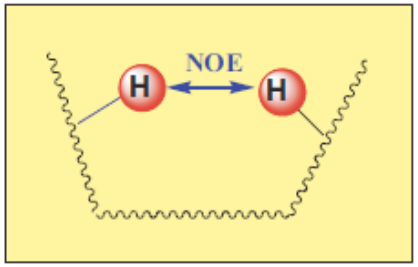
J_{ee} = 0-3 Hz

NOESY

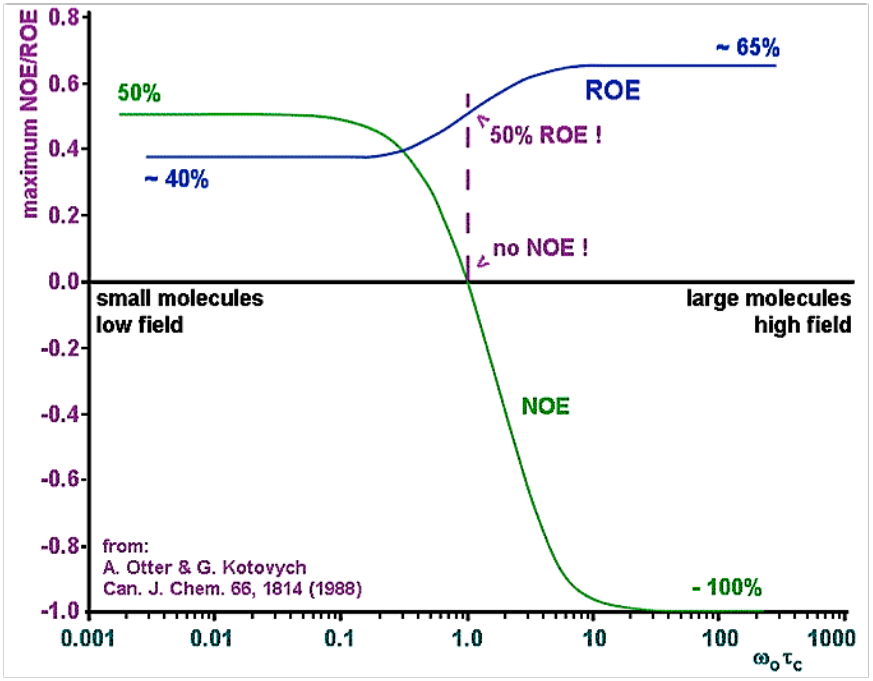
= **N**uclear **O**verhauser **E**ffect **S**pectroscop**y**

The Noe induces a change in the signal intensity when there is a dipolar interaction between the nuclei involved.

NOe is inversely related to the distance between the 2 nuclei (max. distance ≈ 5Å).



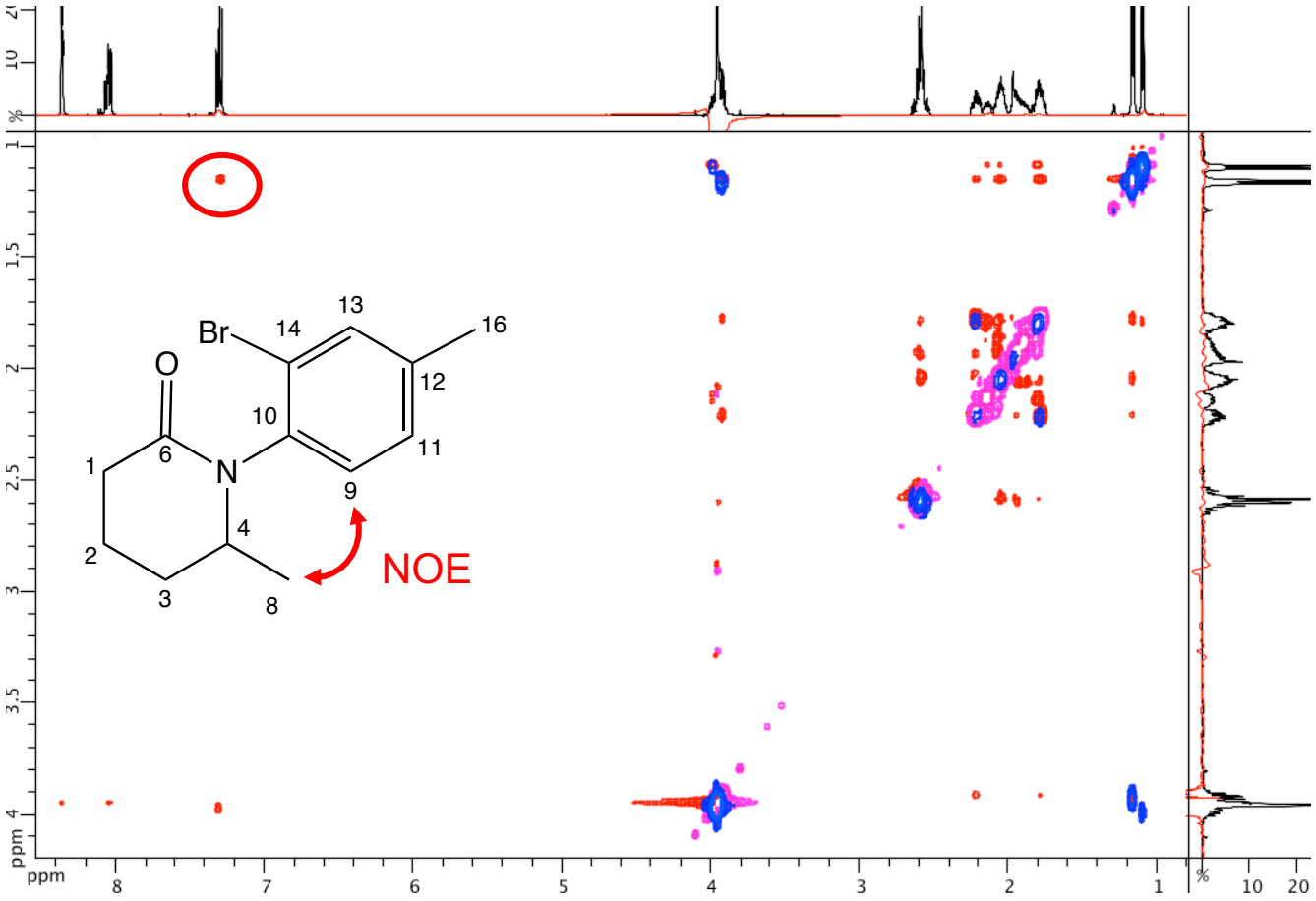
NOE $\sim \tau_c / r^6$



NOe depends may be zero in somes conditions. For middle size organic molecules (600<MW<1500 g.mol^{-1} depending on the solvent viscosity) NOESY may not be conclusive so another experiment called ROESY may be helpful..

the absence of a signal doesn't prove anything !!!

NOESY

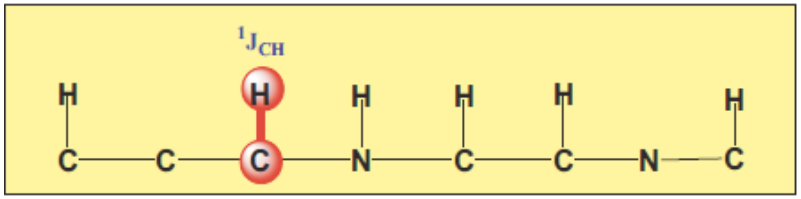


Displaying NOESY (red) over COSY (blue) helps to discard signals that don't give new informations.

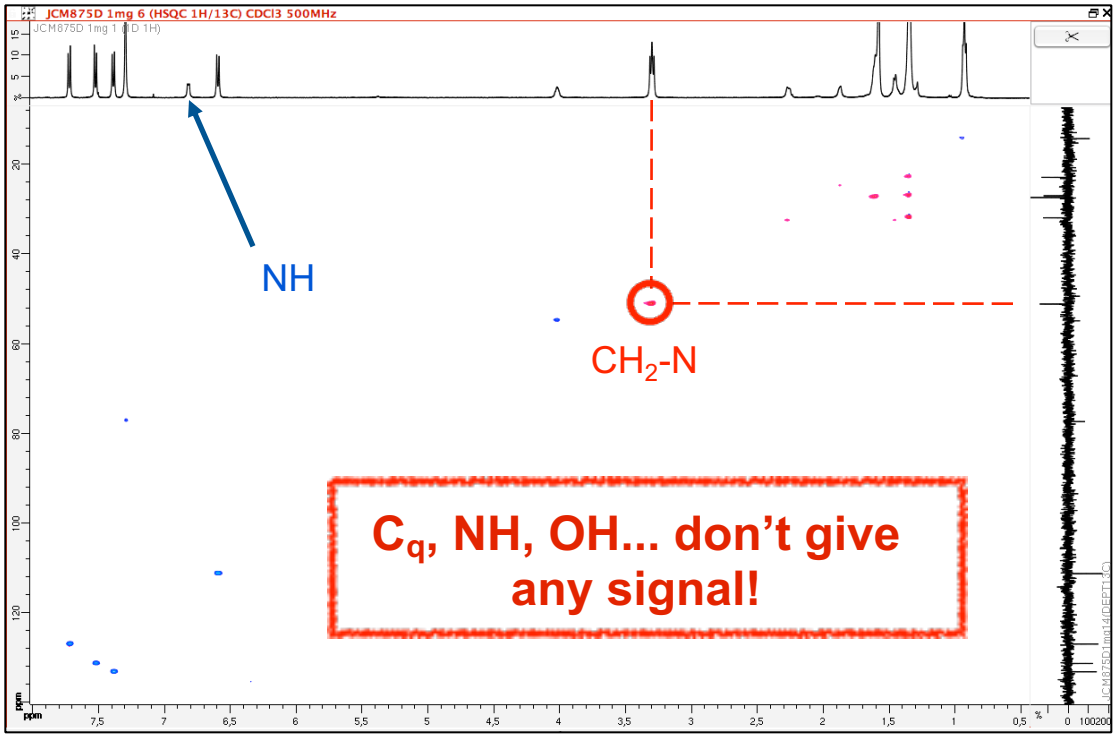
	$\omega_0\tau_c < 1$	$\omega_0\tau_c \approx 1$	$\omega_0\tau_c > 1$
Diagonal signal	-	-	-
NOESY correlation	+	0	-
ROESY correlation	+	+	+
Exchange signal	-	-	-

HSQC

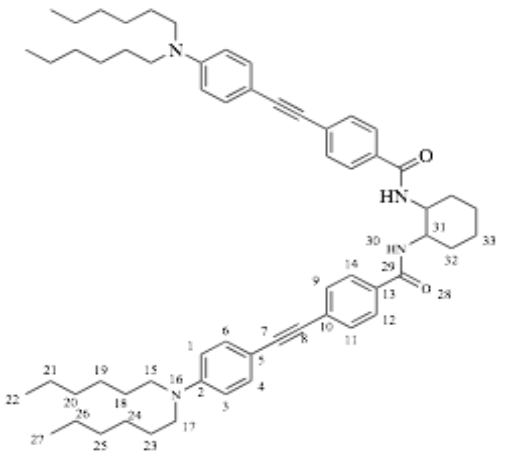
= **H**eteronuclear **S**ingle **Q**uantum **C**orrelation, shows correlations for $^1J_{CH}$



HSQC is an heteronuclear experiment based on a polarization transfer from proton and a proton detection (inverse detection). Thus it is a very sensitive experiment. There are some edited versions of HSQC giving the same informations as 1D DEPT.

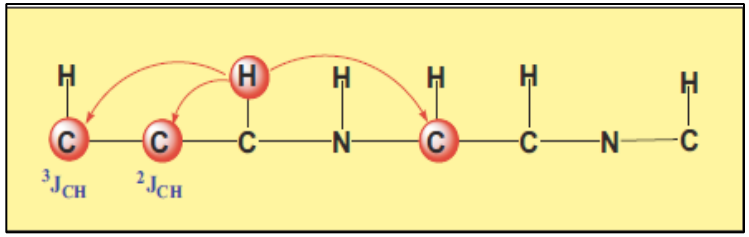


HSQC is faster than DEPT: a 10min experiment is OK for a concentrated sample.

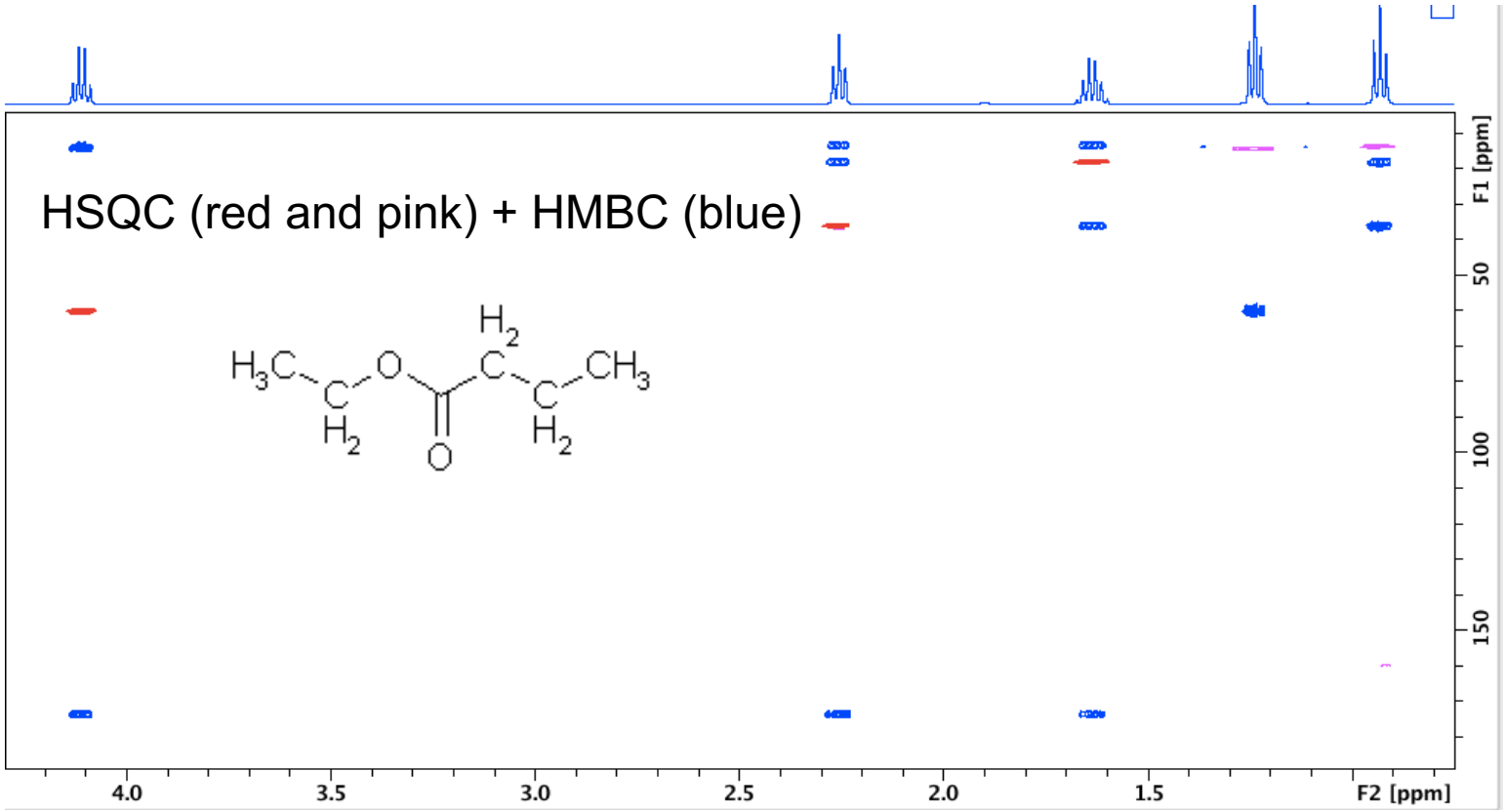


HMBC

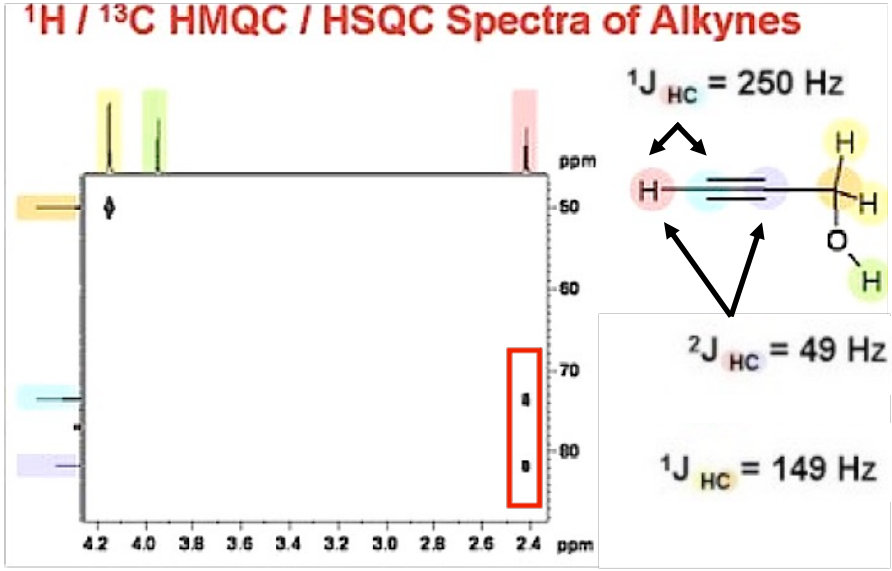
= **H**eteronuclear **M**ultiple **B**ond **C**orrelation, shows correlations for $^{2}J_{CH}$ and $^{3}J_{CH}$.
The experiment is optimized for observation of 8Hz couplings.



HMBC is very useful for structural elucidation and especially quaternary carbons.
Be careful with the interpretation of signal intensities.

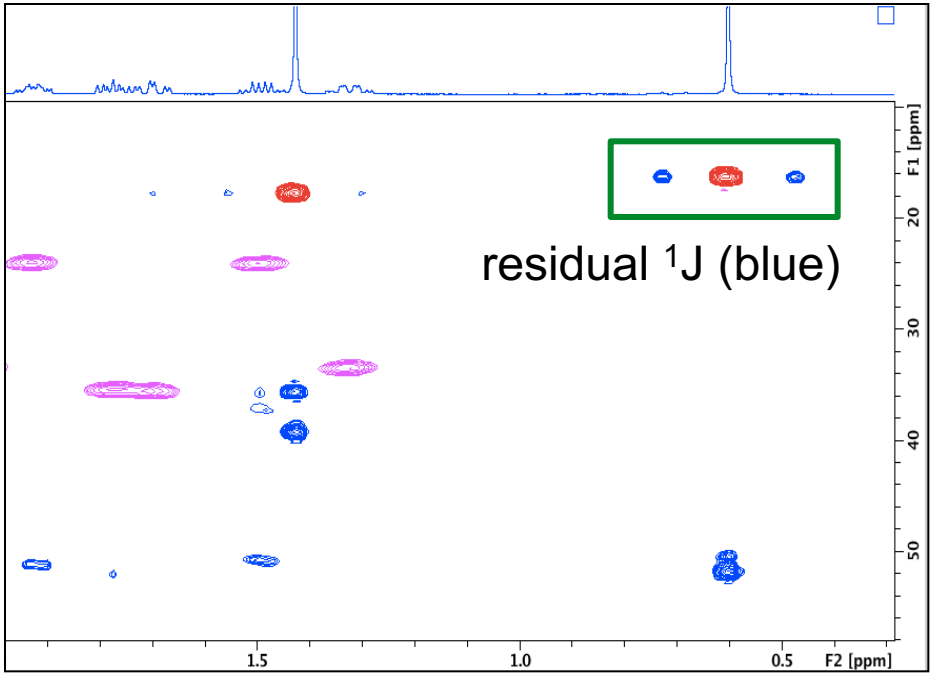


HSQC and HMBC artefacts



HSQC is optimized for observation of $^{1}J_{CH}$ couplings around 145 Hz.

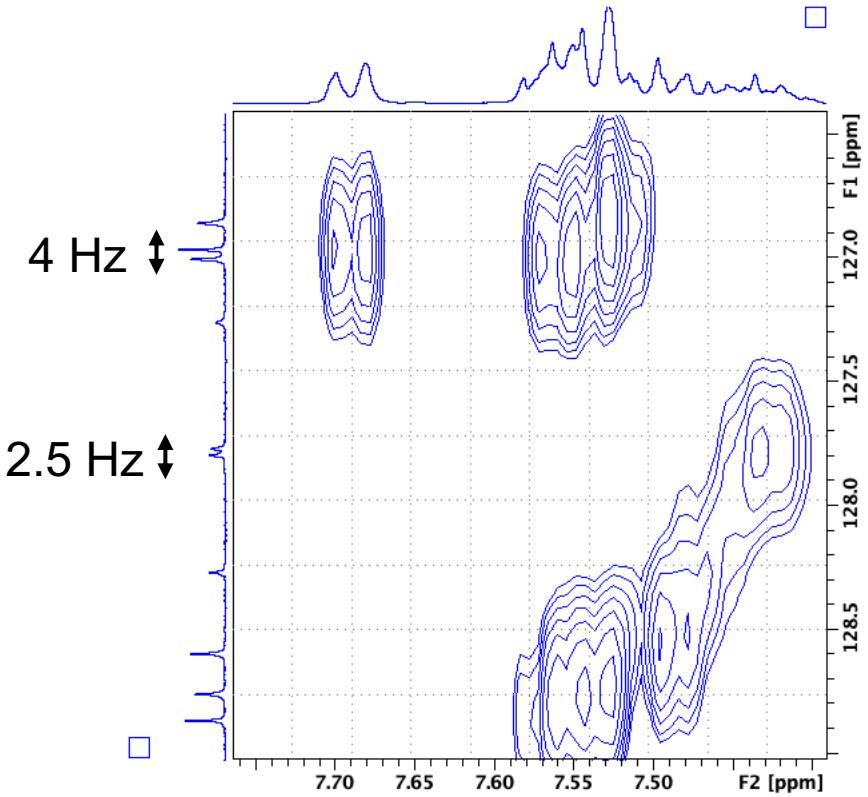
Some residual $^{1}J_{CH}$ couplings may be observed on HMBC in case of intense signals (methyl, solvent…). They are easily identified because they are not decoupled. Superimposition of HSQC over HMBC spectrum helps with their assignement.



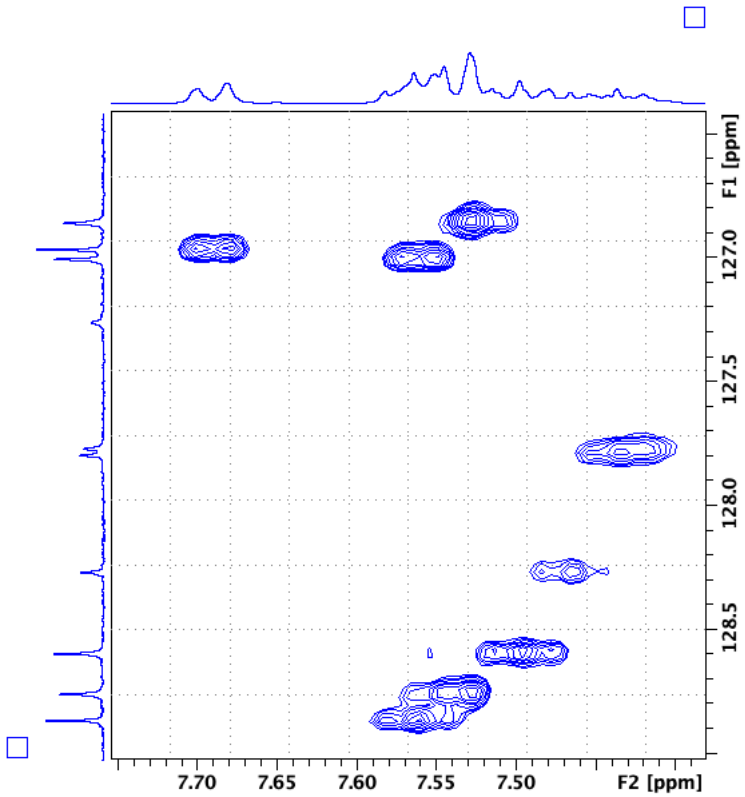
HSQC (red and pink) + HMBC (blue)

High resolution HSQC and HMBC

In routine spectra resolution in ^{13}C dimension is ≈ 70-100Hz and that may be a problem in case of strong overlapping. Non Uniform Sampling (NUS) allows to increase the resolution within the same experimental time.



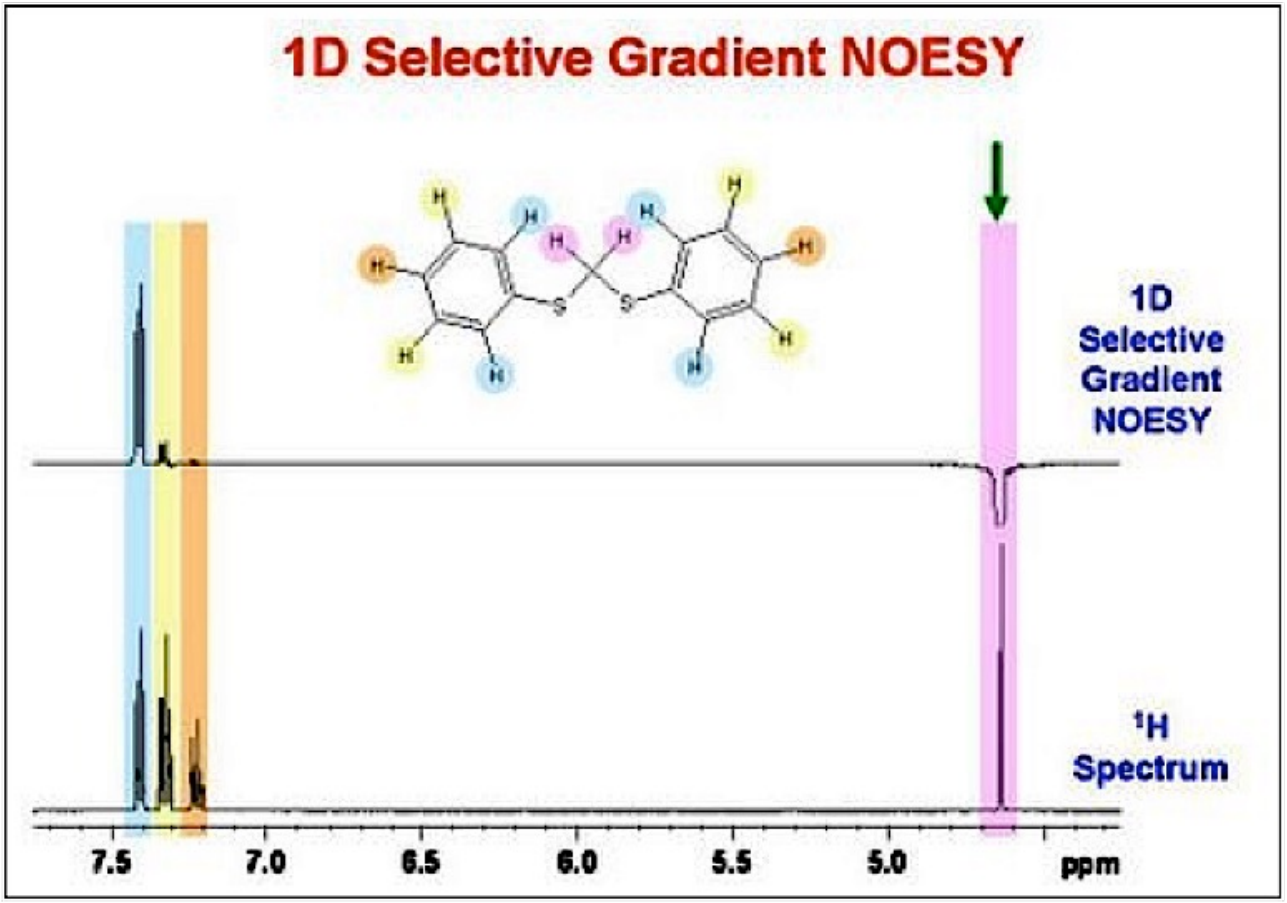
standard, TD1 = 300 pts



NUS 20%, TD1 = 2000 pts

Selective 1D experiments

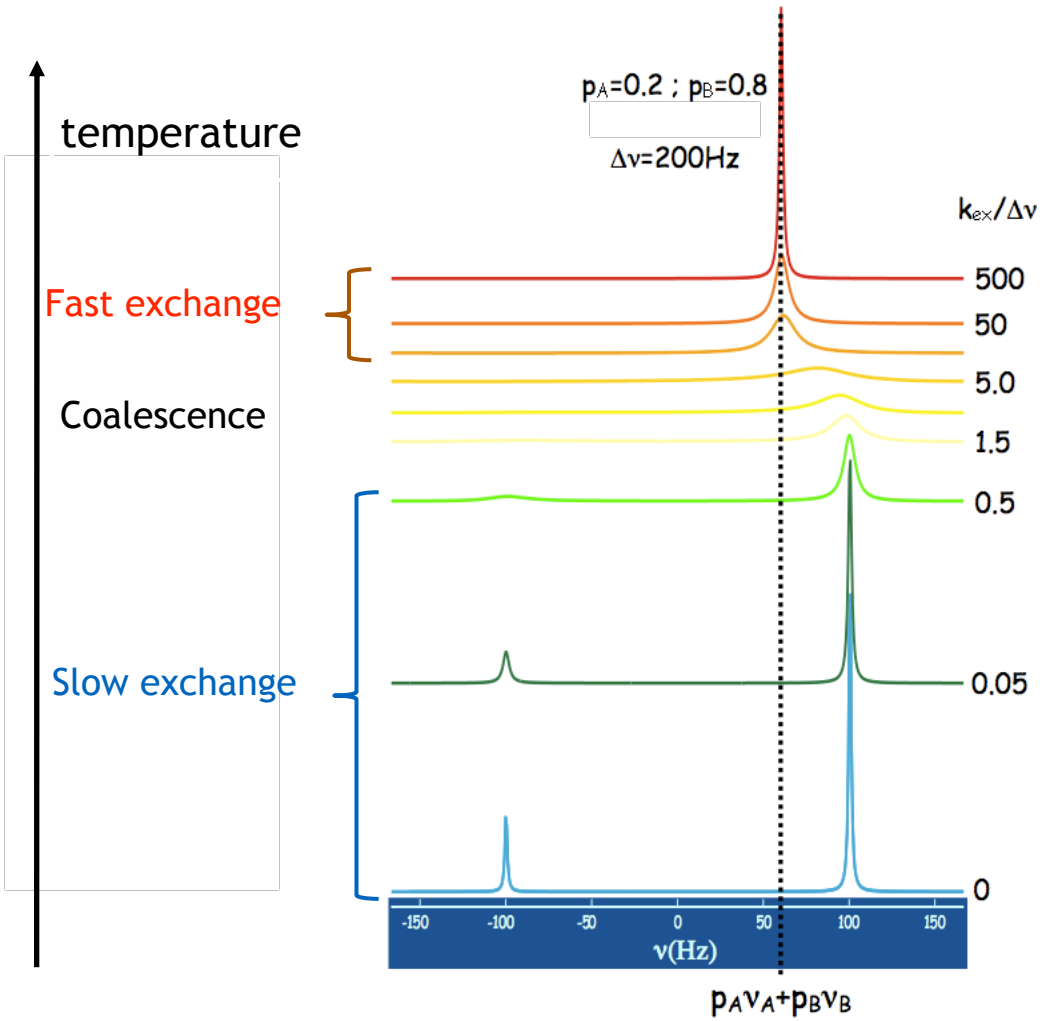
Very useful experiments to observe interactions from one particular ^{1}H. For example a selective 10 min 1D NOESY may give the expected information without needing a 2h 2D NOESY. Main limitation: the signal of interest must be isolated.



Exchange

Ex: system in exchange between 2 non symetric states : the exchange rate depends on $k_{ex}/\Delta v$. Also k_{ex} depends on temperature

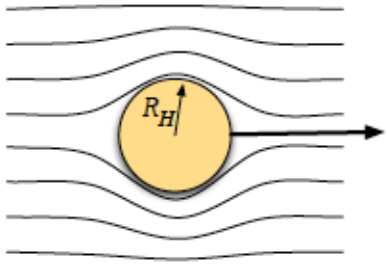
- $k_{ex} >> \Delta v$: fast exchange
- $k_{ex} \approx \Delta v$: coalescence
- $k_{ex} << \Delta v$: slow exchange



Diffusion coefficient

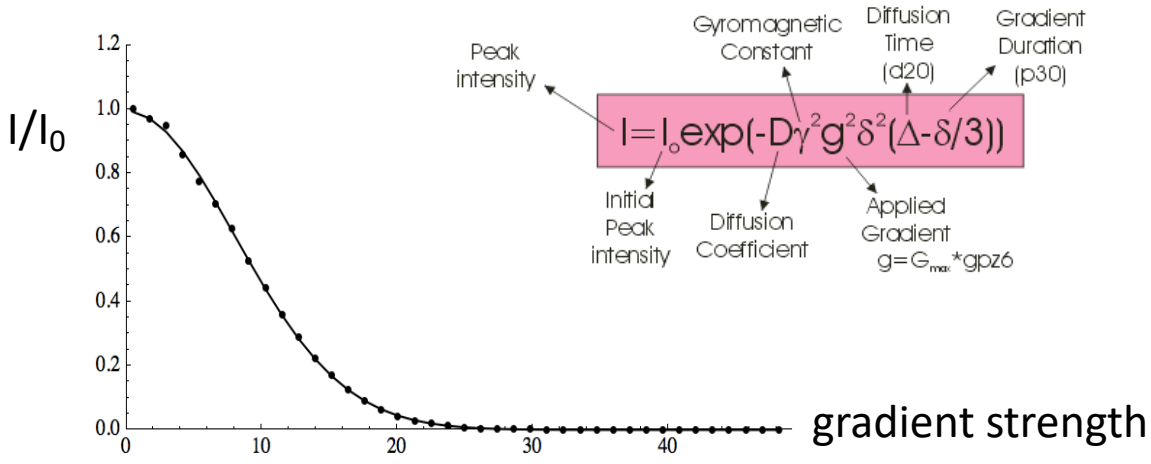
The diffusion coefficient D is a measure of the mobility of a molecule in solution.

Stokes-Einstein model (approximation): hypothesis of spherical diluted molecules diffusing in a continuous media



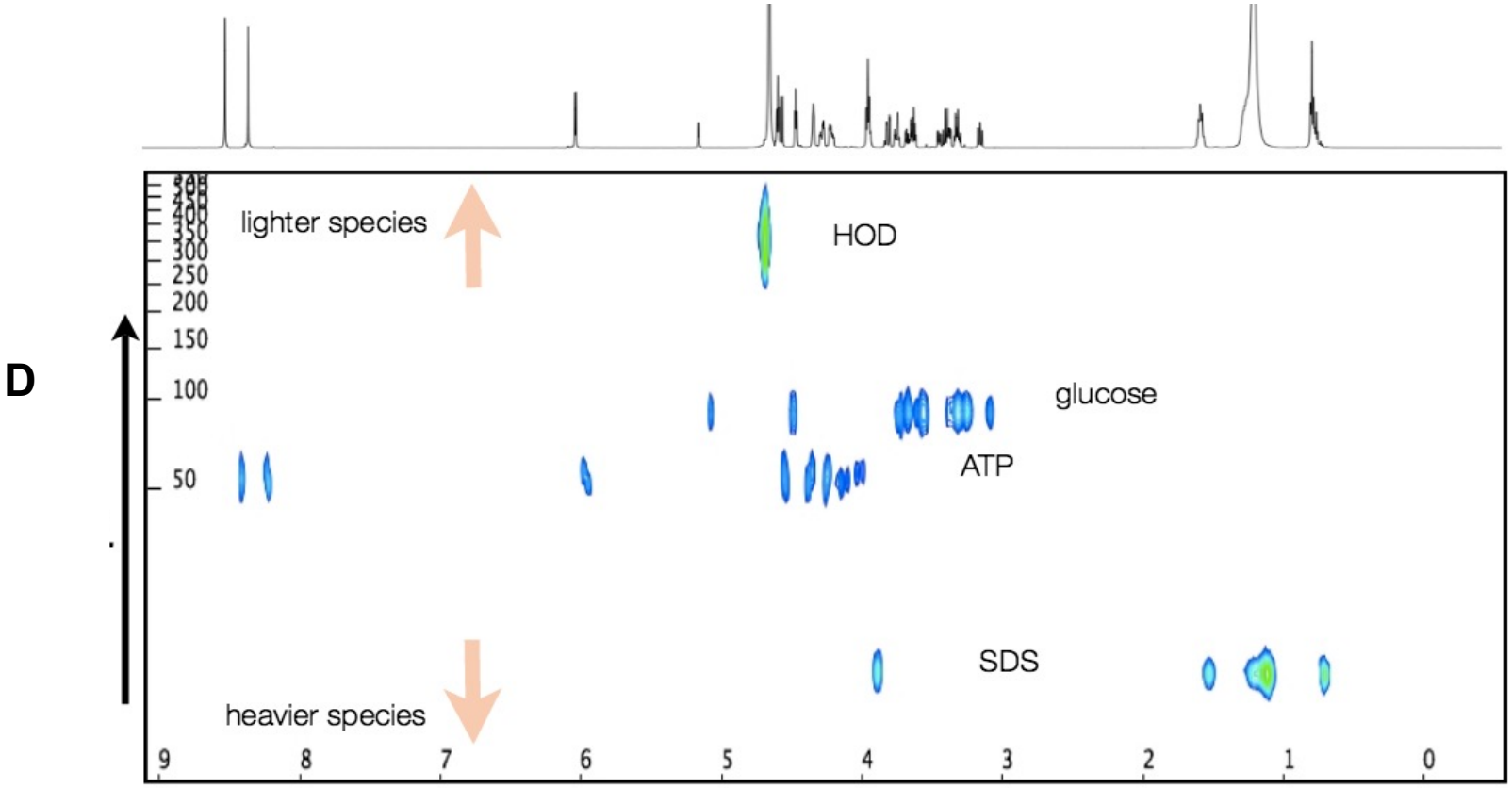
- depends on size and shape of the molecule
- depends on temperature T
- depends on viscosity η (related to temperature)
- measured by NMR

$$D = \frac{kT}{6\pi\eta R}$$



DOSY

= **D**iffusion **O**rdered **S**pectroscop**Y**, separates on a pseudo-2D spectrum the molecules in a complex mixture according to their diffusion coefficient. DOSY is used for impurities identification, molecular mass estimation, polymers polydispersity index determination...



M.A. Delsuc, « DOSY and diffusion measurement in modern NMR » Central European NMR Meeting; 23rd NMR Valtice; April 20th - 23rd, 2008

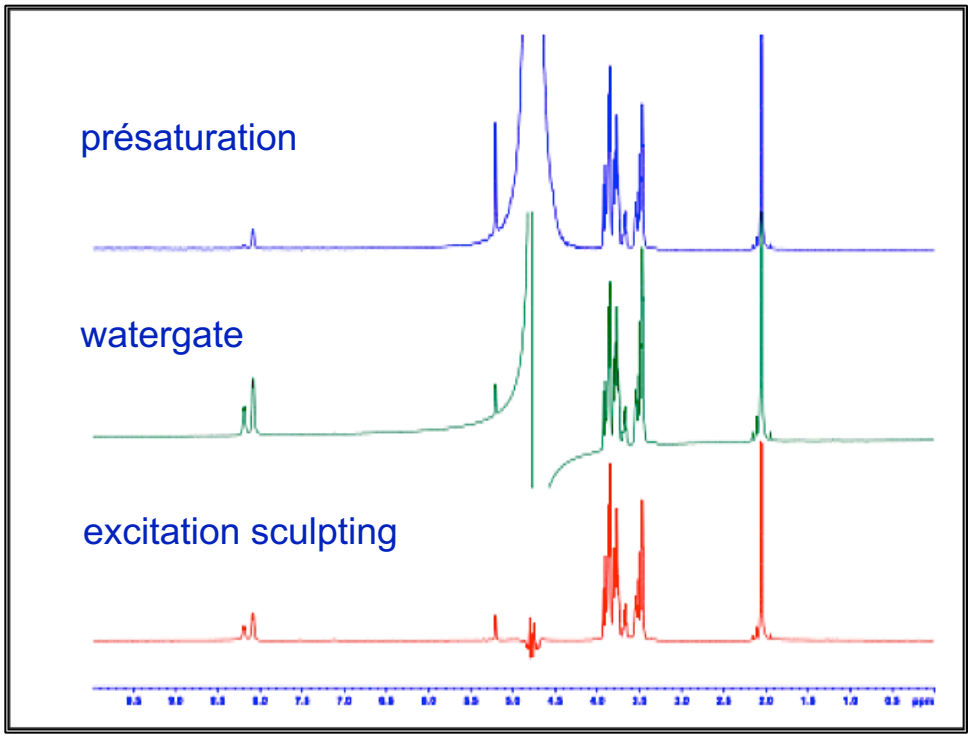
Solvent signal suppression

Different techniques exist for suppression of intense residual solvent signals.

• **Presaturation** consists in irradiating the frequency of the solvent signal during the relaxation delay D1. Be careful as signals from protons in exchange with the solvent are also attenuated.

• **Watergate and excitation sculpting**: this time all signals are irradiated except the solvent signal. These techniques are more efficient but frequencies close to the solvent signal are attenuated as well.

These techniques are mandatory with biological samples diluted in H_2O/D_2O buffers.



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