Universitat de Barcelona


# NMR Basic training

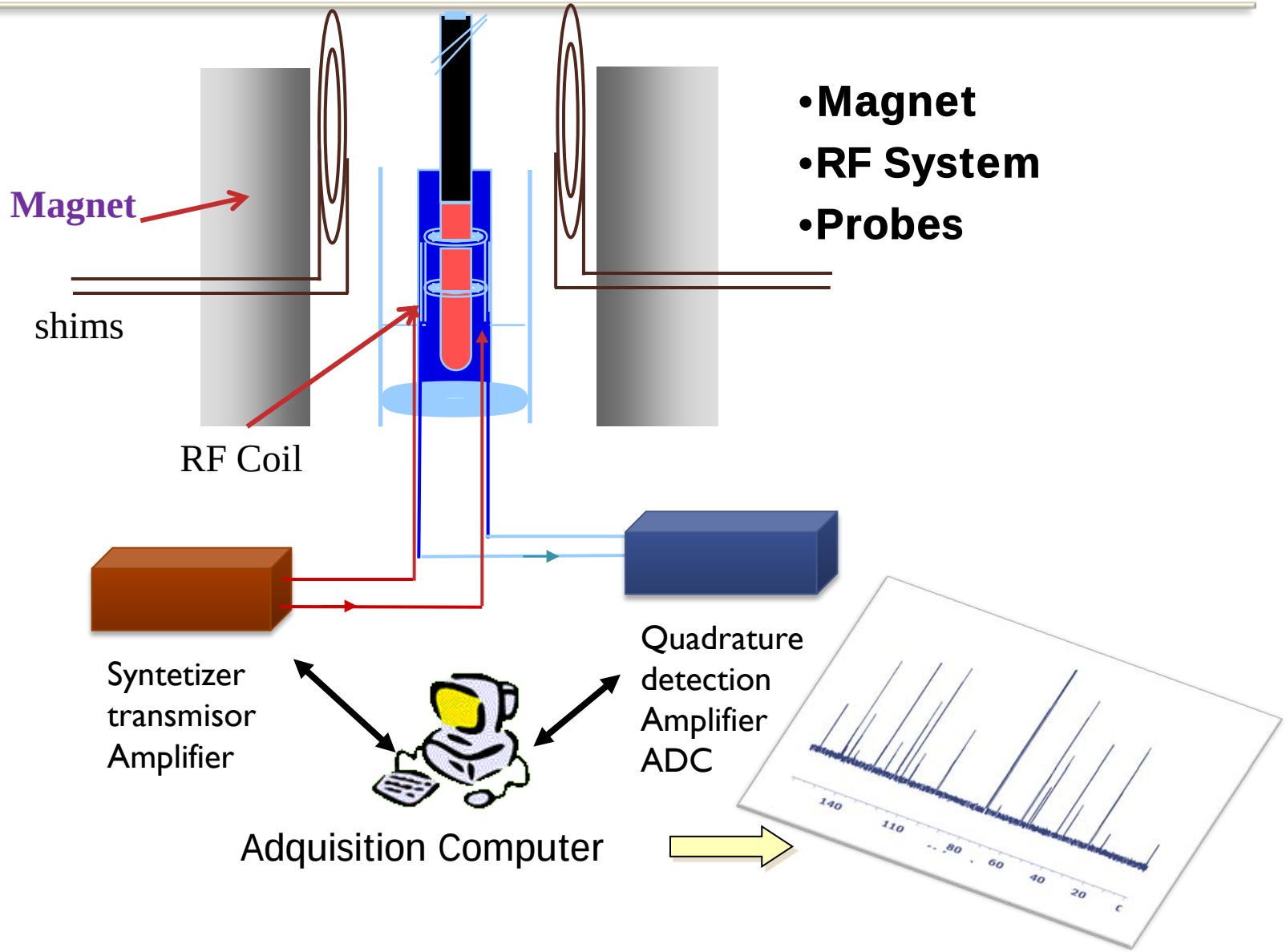
## Part-I

## 2018

# Index

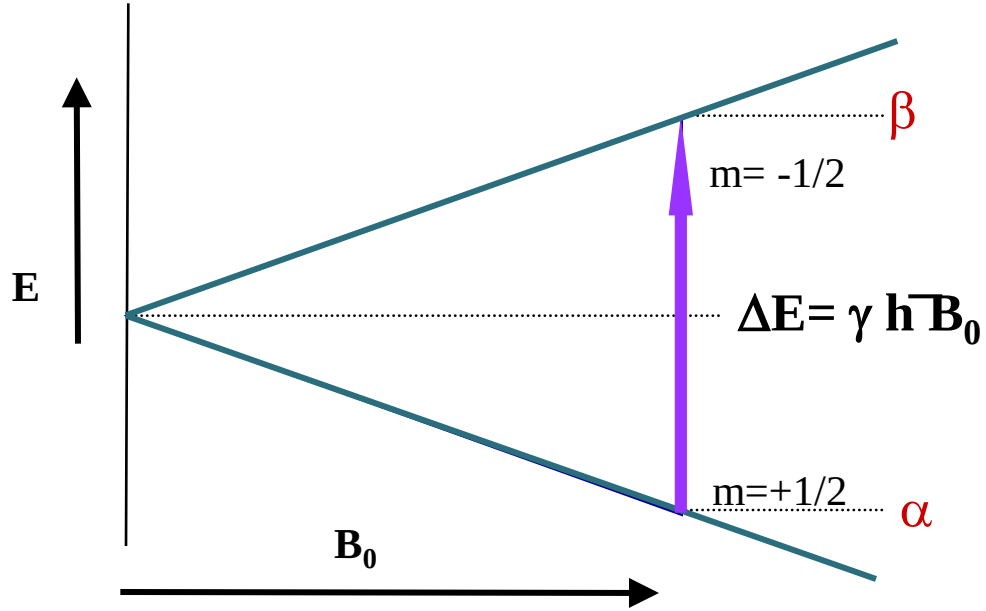
- NMR Spectrometer
  - Description
  - Lock,  shimming
- NMR Sensitivity
- Security: Magnetic Field cautions
- Rules
- Samples
  - Sample preparation
  - NMR tube

# NMR Spectrometer, Block Diagram



# RMN sensitivity

Higher the field strength → higher the sensitivity




$$\frac{N_\beta}{N_\alpha} \approx e^{-(\Delta E/K_B T)}$$

$$\frac{N_\beta}{N_\alpha} \approx 1 - \frac{\gamma \hbar B_0}{K_B T}$$

$K_B = 1.38 \times 10^{-23}$ J/K

$K_B = 1.985$ cal/Kmol

**1H**

$B_0 = 1.41$ T ( 60 MHz) $\quad \frac{N_\beta}{N_\alpha} = 0.9999904$

$B_0 = 7.05$ T ( 300 MHz) $\quad \frac{N_\beta}{N_\alpha} = 0.99995$

Low Sensitivity

Differences ≈ 1-20 ppm

# Magnetic field

## Resolution

## Dispersion

## Sensibility

60MHz
*(≈1965)*

1.000 MHz
*(2009)*

Higher the field strength → higher the sensitivity and spectral dispersion

# Superconducting Magnet

liquid helium temperature (4K ≈ -269 °C),
liquid $N_2$ (75K≈ -198 °C)

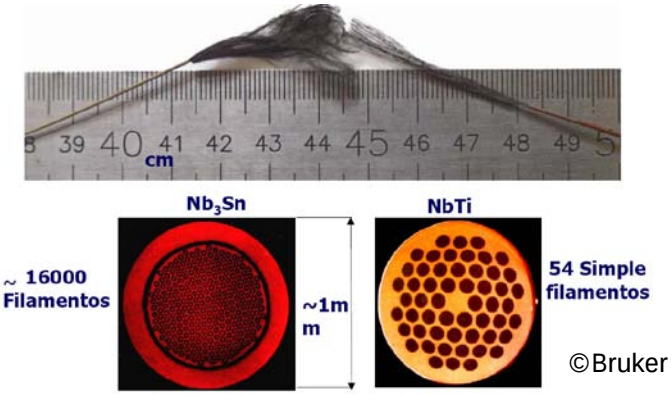
Solenoid coil from superconducting

Nb-Ti Wires

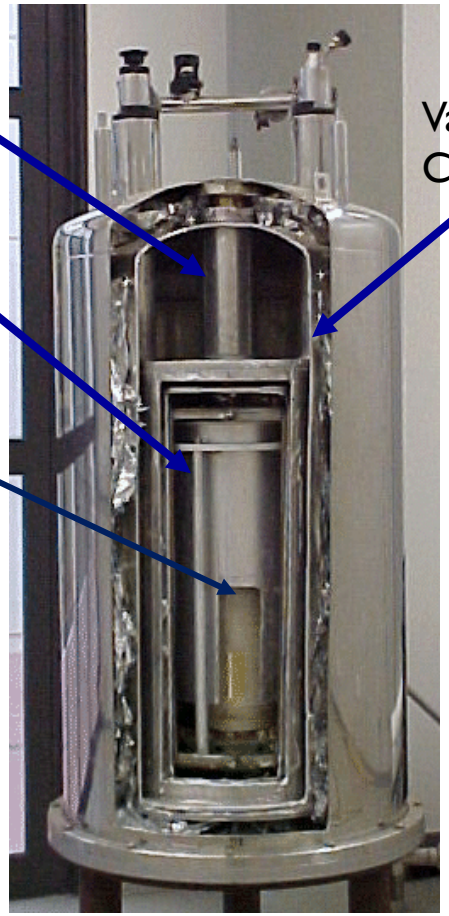
$Nb_3Sn$ y Nb-Ti Wires

$(NbTa)_3Sn$ Wires (magnets at 2ºK)

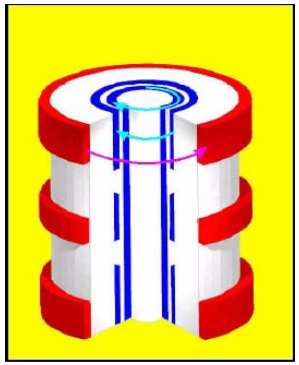
***Use up to 300 Km of wire!***




**Magnet**

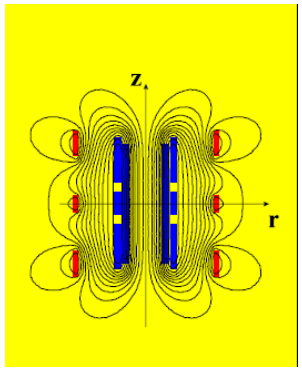


# Shielded Magnets



**Blue Main Coil**
**Red Coils to Shielded**

coil outside of the main coil which cancel out much of the fringe field




©Bruker

New Coils to minimize the stray field

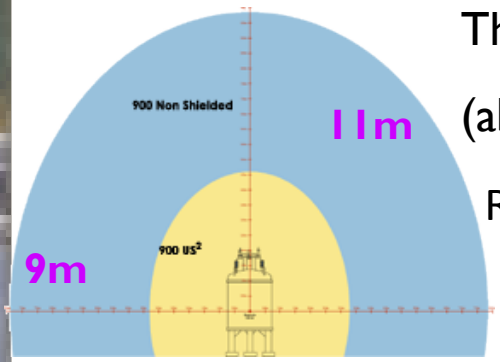
- Excellent homogeneity and stability of Magnetic field
- External field perturbations are efficiently attenuated
- Low external residual magnetic field
- Minimize laboratory space requirements

**Shielded Magnets in NMR Facility**

3 Mercury-400 MHz NMR
1 VNMRS-500 MHz
1 Bruker Avance III 600 MHz
1 Bruker Avance III 400 MHz

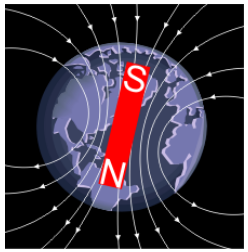
# The Stray field in the NMR spectrometers

NMR magnetic field: Tesla or specifying the $^{1}H$ Larmor frequency for the magnet.




The earth's magnetic field is approximately $10^{-4}$ T

(about 0.5-0.1 Gauss)




Refrigerator magnet 100 -150 Gauss

1 T=10.000 Gauss

The 5 gauss Level

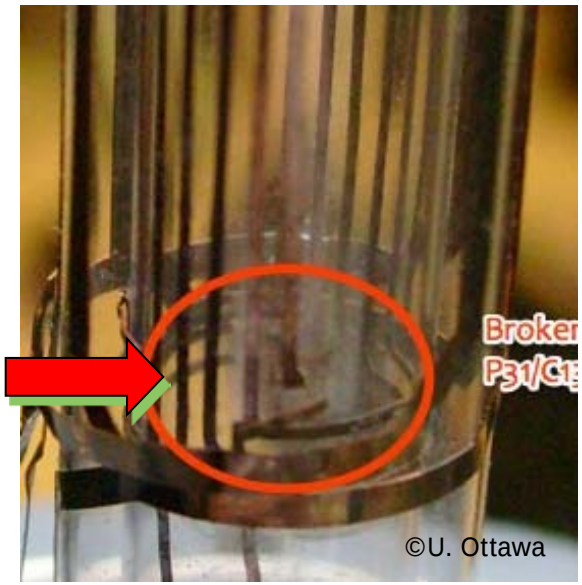
## Magnets in NMR Facility

| Field (Teslas) | $^{1}H$ frecuency (MHz) | Radial Distance | Axial Distance |
|---|---|---|---|
| 18.8 | 800 | 6.3 m | 8.0 m |
| 14.08 | 600 | 3.6 m | 4.0 m |
| 14.08 | 600 | 0.7m | 1.4 m |
| 11.74 | 500 | 2.8 m | 3.6 m |
| 11.74 | 500 | 0.8m | 1.25 m |
| 9.39 | 400 | 0,9 m | 1,5 m |
| | | | |

# Probe

**Tuning the probe to optimize:**

- **RF pulses**
- **Detecting NMR signal**




**The probe can be affected by:**

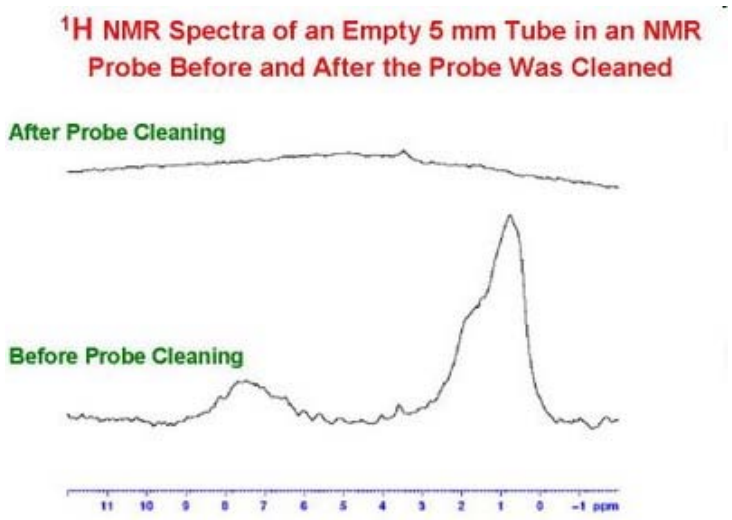
Quality NMR tube

product outer the tube

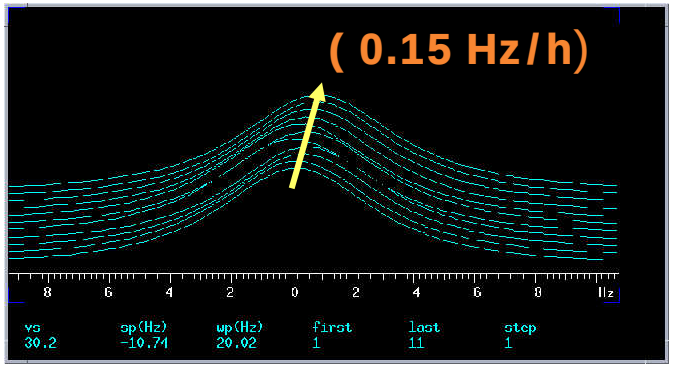
Dirty tubes

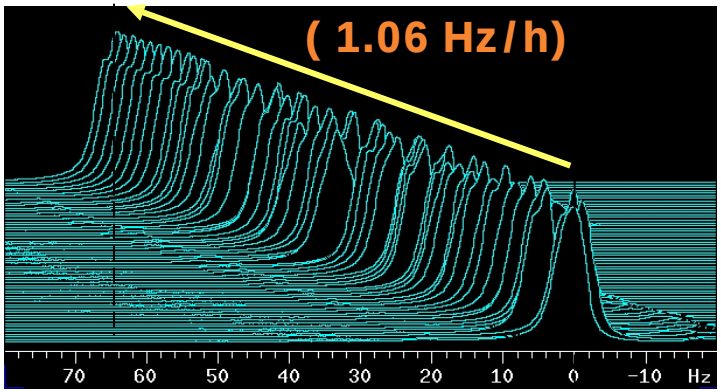
**Probe repair≈10.000 $ (3 months)**

**New probe ≈ 60.000 $**



# Lock System, *Field Drift*



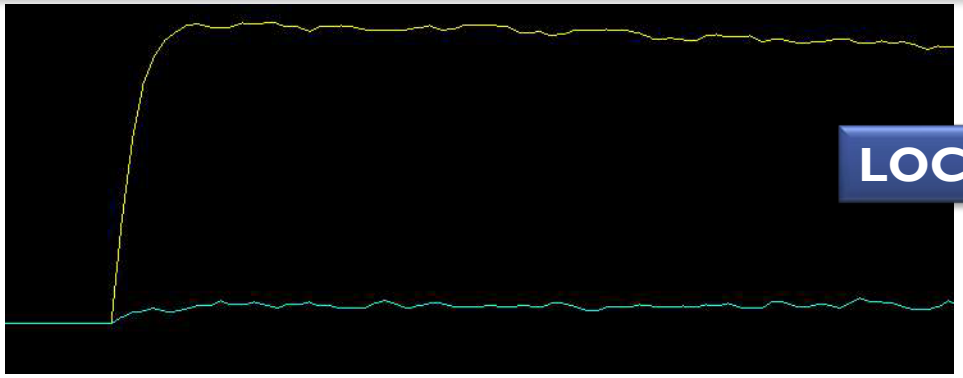




NMR magnetic field slowly drifts with time

The field drift can be affect to the NMR Signals

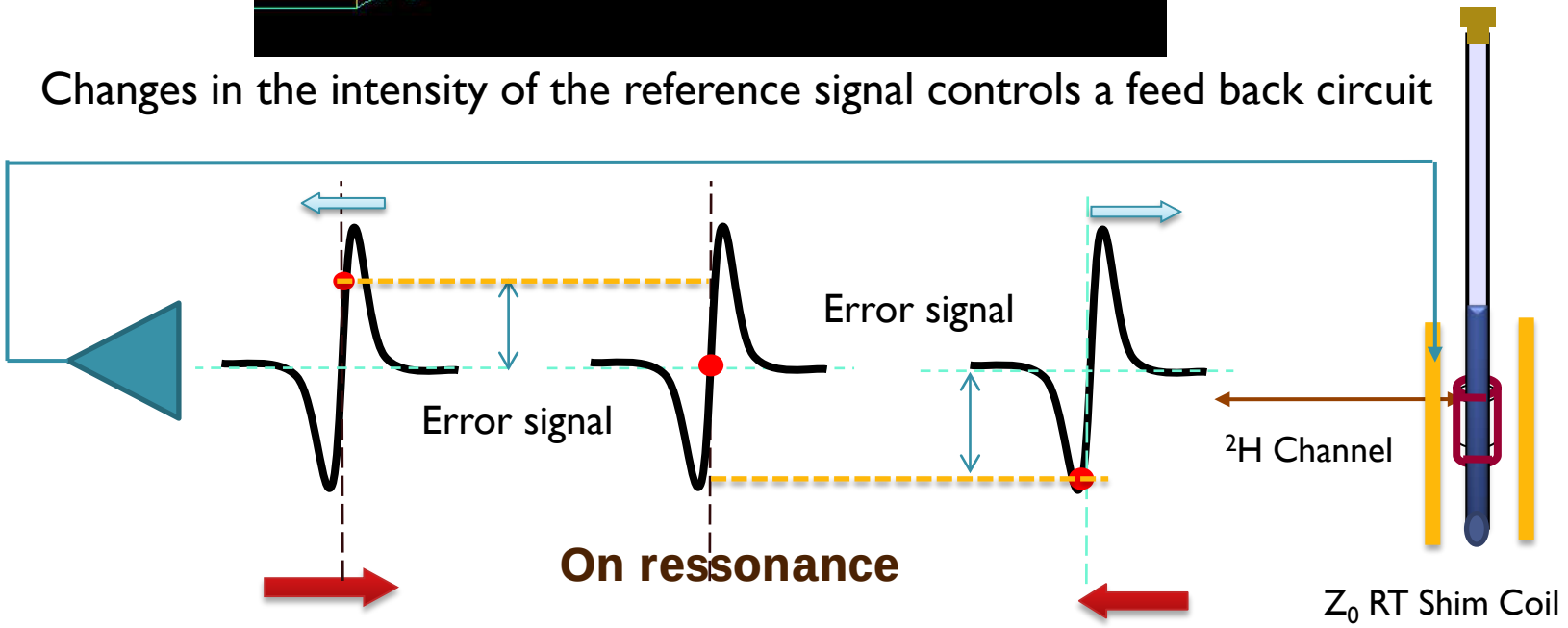
- ❑NMR probes contains an additional transmitter coil tuned to deuterium frequency
- ❑Need to constantly correct for the field drift during data collection
- ❑Deuterium NMR resonance of the solvent is continuously irradiated and monitored to maintain an on-resonance condition

# The feed back del lock System



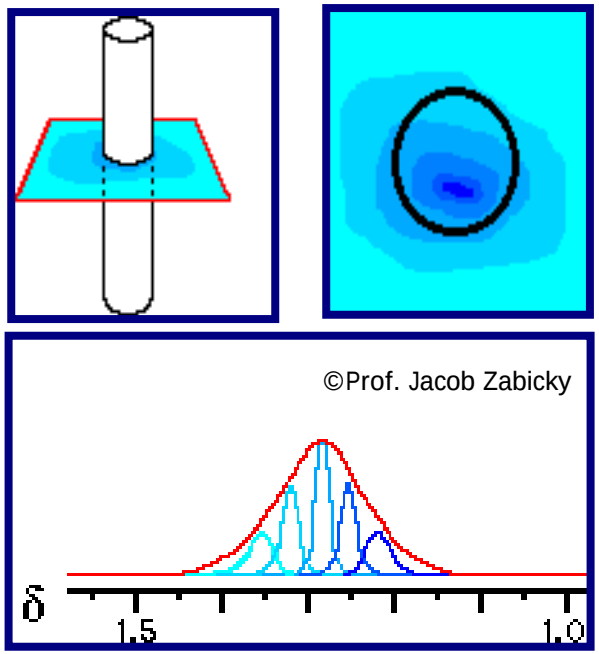
**LOCK on-resonance**

Changes in the intensity of the reference signal controls a feed back circuit




With a shim coil ($Z_0$) creates a small magnetic field to moved the main field to place and the lock-signal back into resonance

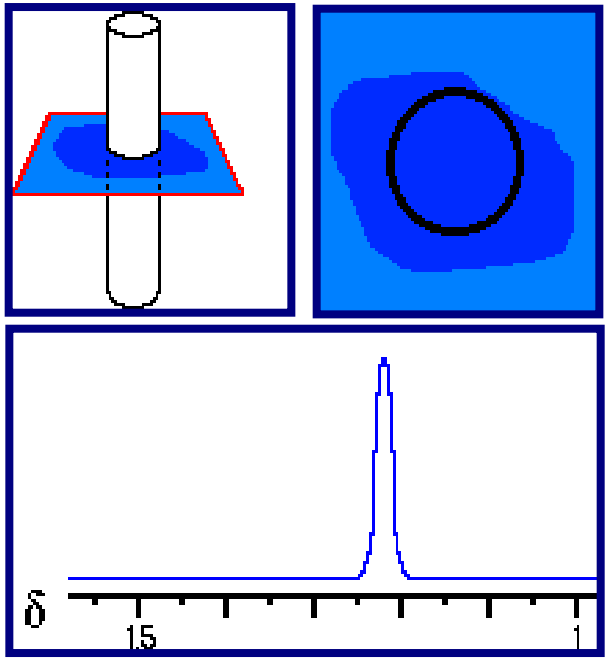
# Homogeneity in the magnetic field




If the magnetic field is heterogeneous across the sample

**broader NMR signals**

The same proton experience different $B_0$ magnetic field

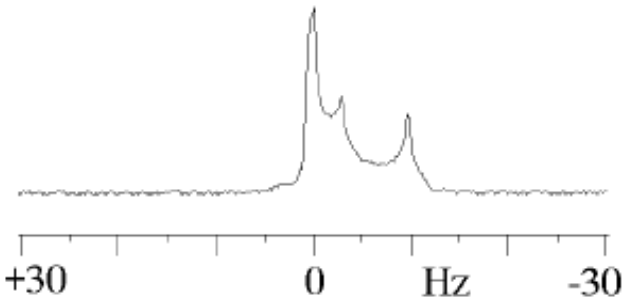



If the magnetic field is the same throughout the volume of active probe

**narrow NMR signals**

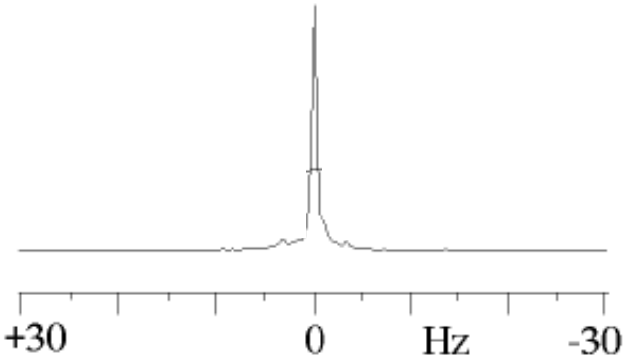
# Magnetic Field Homogeneity

Frequency of absorption: $\nu = \gamma B_o / 2\pi$




Poor Homogeneity → multiple peaks at different effective $B_o$

Resonance depends on *position* in NMR sample




Good Homogeneity → single peak with frequency dependent on $B_o$

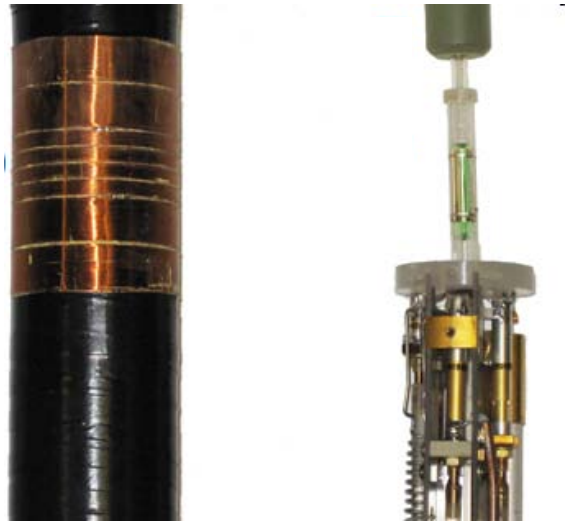
# Adjust the homogenity : Shim Coils

The electric currents in the shim coils create small magnetic fields which compensate the inhomogeneity's in the magnet
The coils have different geometric orientation and function

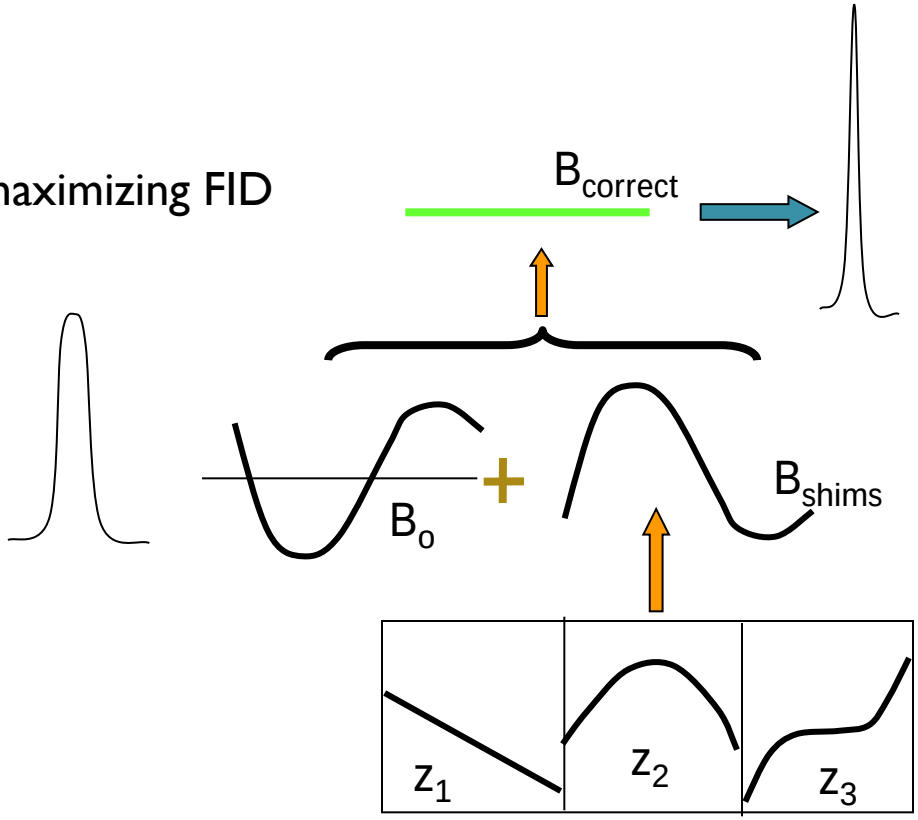
- **Z1, Z2, Z3, Z4,** Z5, Z6, Z7
- X, XZ, XZ2, X2Y2, XY, Y, YZ, YZ2, XZ3, X2Y2Z, YZ3, XYZ, X3, Y3

Optimize shims by:

- minimizing line-width
- maximizing lock signal or maximizing FID

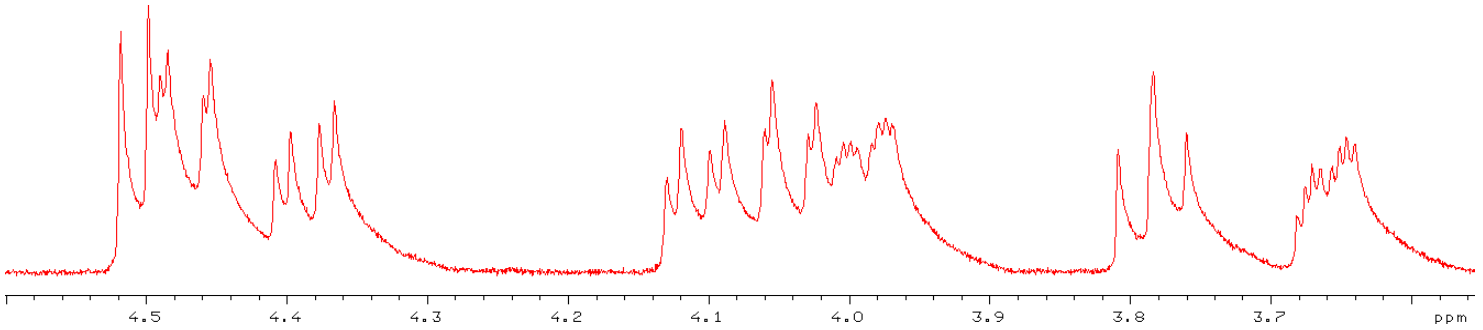






# Gradient Shimming

## Mercury and VNMRS Gradient map ($^2H$ or $^1H$)

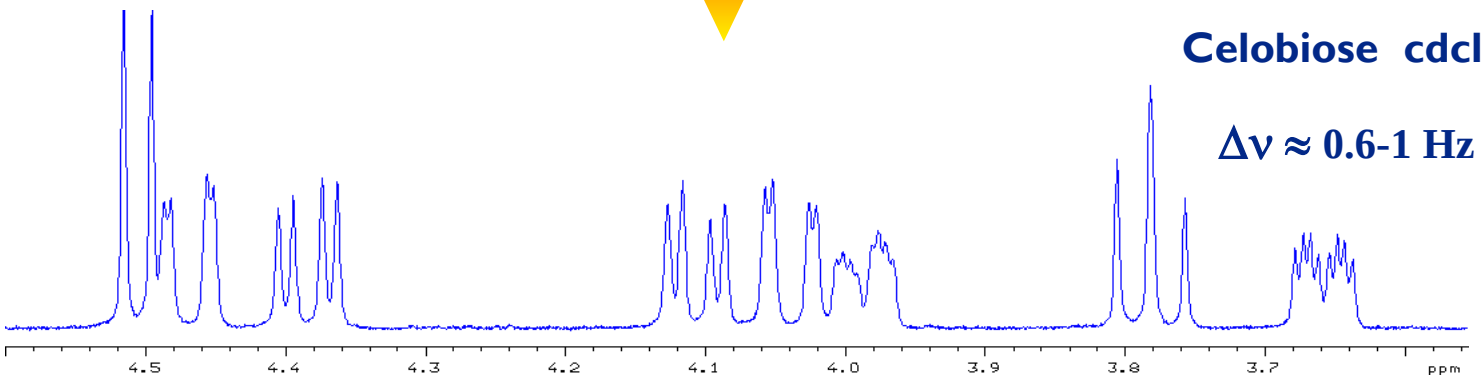



***N°*** **iterations (10 max)**

**gradient shim (Z 1-4)**
**3 iterations ( aprox 1 minute)**

**Celobiose cdcl3**

**$\Delta\nu \approx 0.6\text{-}1$ Hz**




Line widths near of 1Hz

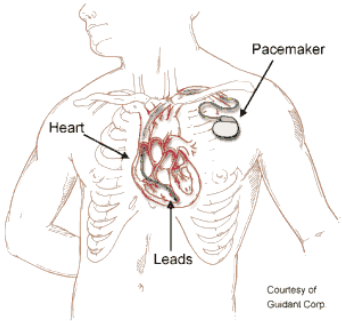
# Safety

# Magnetic forces

**Metal objects must remain outside the 5-gauss perimeter.**

**The greater the mass of the object, the more strongly it is attracted by the magnet.**

**The shorter the distance to the magnet, the stronger the force.**




**10 gauss**

**5 Gauss Line**

# Warnings

**PERILL**

## CAMPS MAGNÈTICS MOLT INTENSOS

**És prohibida l'entrada en aquesta sala de persones que portin estimuladors cardíacs o pròtesis metàl.liques**

## CAMPOS MAGNETICOS MUY INTENSOS

**Se prohibe la entrada a esta sala de personas con marcapasos o prótesis metálicas**

# RESTRICTED ACCES AREA

## Only allowed to:

- Users of Unitat d'RMN
- Unitat d'RMN authorised personnel

- **Forbidden access** to people with cardiac pacemaker or metallic prosthesis
- **Forbidden access** with iron or magnetic objects; strong attractive magnetic fields surrounding the magnet are present
- Keep analogical clocks and all kind of magnetic objects (credit cards, diskettes, transport cards, etc) out of the 5 G line, otherwise they can get useless

# QUENCH

**Quench:** is the sudden loss of supercondutivity in the magnet's main coil that produces a rapid evaporation helium liquid to gas

Quench Video-

## Don´t

- Hit or moving the magnet
- Manipulating security ports
- Incorrect transferring cryogenic liquids.

## In the event of a "magnet quench:

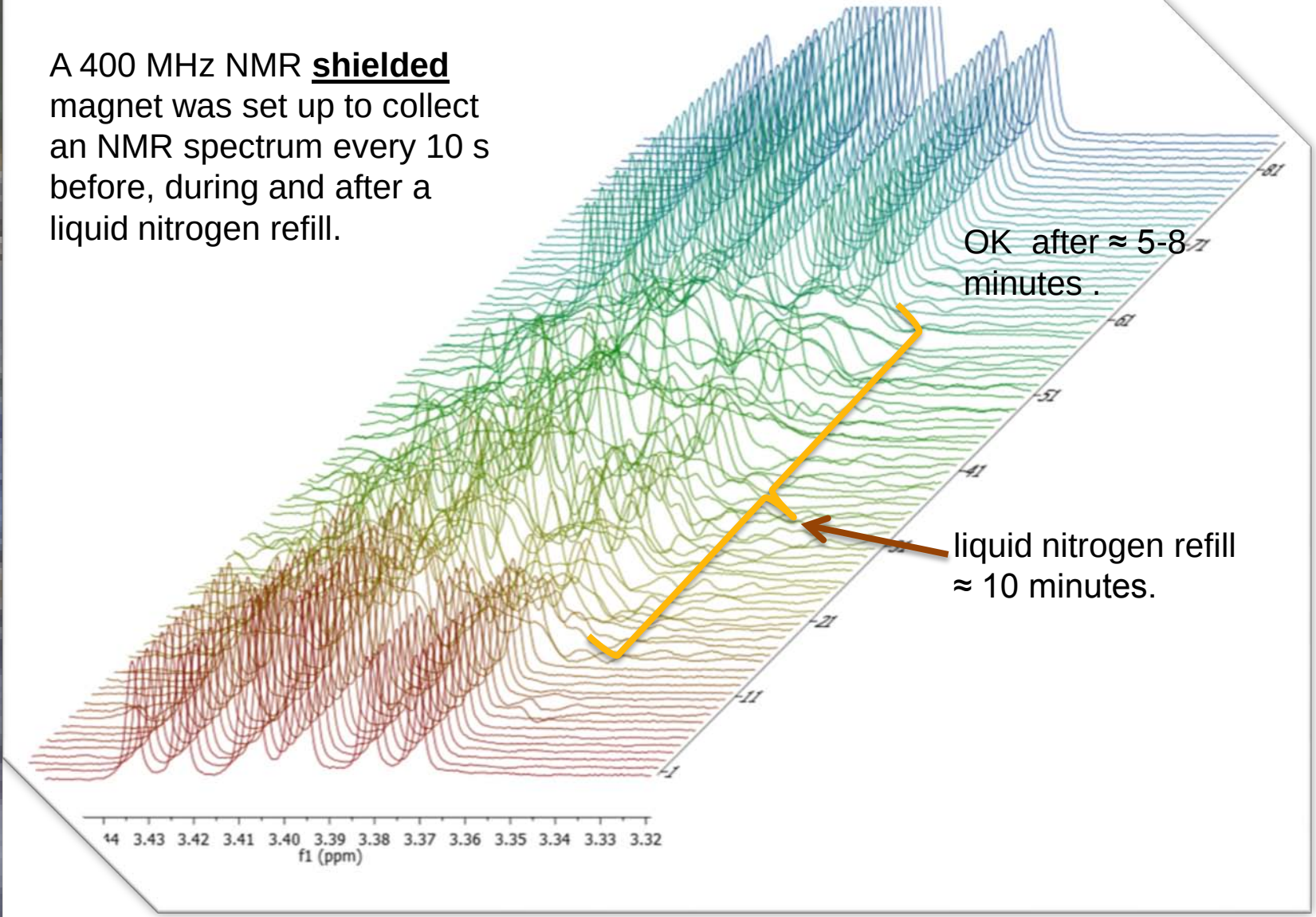
- Leave the room immediately
- Do not re-enter in the room until the oxygen level has returned to normal
- Activate the ventilation system

# Maintenance Operations
# Performance Guarantee

- Refill criogenic liq
  - **N2 y He liq**
- Checking the NMR performance
- Calibrations
- Preventive Maintenance
- Updates and improvements

# Resolution in the Criogenic's Refill

A 400 MHz NMR **shielded** magnet was set up to collect an NMR spectrum every 10 s before, during and after a liquid nitrogen refill.



# NMR Facility Rules

- Report any incidence: Notify the staff immediately if a sample is broken inside or around the magnet.
  - To record incidents in the books of the NMR Spectrometer
- Handle the spinners with care (**are very expensive**)
- Do not exceed the boiling or freezing points of your solvent sample.
- Be very careful with sample tubes as they are fragile and break easily.
- Follow all safety recommendations
- Should avoid all unauthorized access

# What Does it Cost?

- **Hourly Rates:** Approved annually by the UB
  - Are available on the website of the UB http://www.ub.edu/finances/tarifes/tarifes.htm and are summarized in the NMR web
- The cost is based on the usage time and / or reserve time
- Cover only part of the operation of NMR spectrometer
  - Manual: two rates day / night
  - Auto : a single rate
- Three different rates
  - UB Grup
  - Academic Users others Universities
  - Industry

**Included in base price:**
~~**standards NMR solvents,**~~
**plotting of spectrum**

# Sample Preparation

# The Sample

- Adjust the sample concentration to the solubility

  Avoid product precipitation
- Use a single deuterated solvent

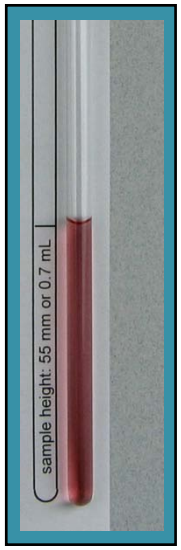
  Reference for lock
- Avoid heterogeneous samples.

  Avoid air bubbles, suspended particles, sample separation
- The low quality NMR tubes → distorts magnetic field homogeneity

  Breaks easily → damage the NMR probe
- Adjust the Properly position NMR sample in the magnet with the NMR gauge

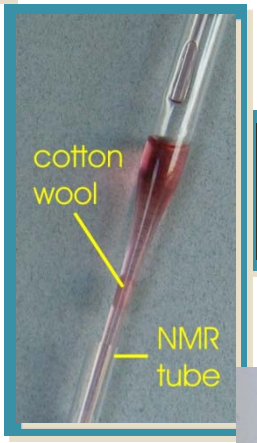
# NMR Sample Preparation

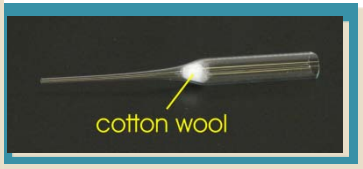
**For 1H Use about 5-25 mg of product**




**1-0.75 ml deuterated solvent**

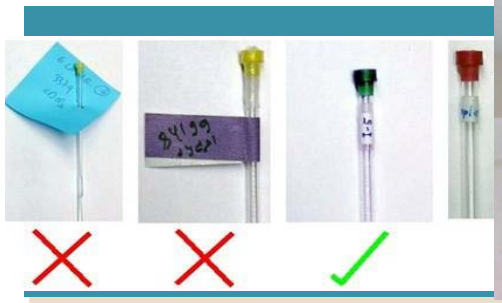
The sample should be about 4.5-5.5 cm of liquid

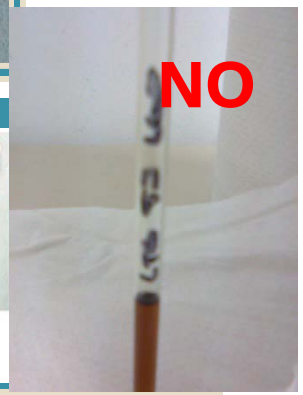



**The sample must be free of suspended material: You can filtering using a cotton wool**

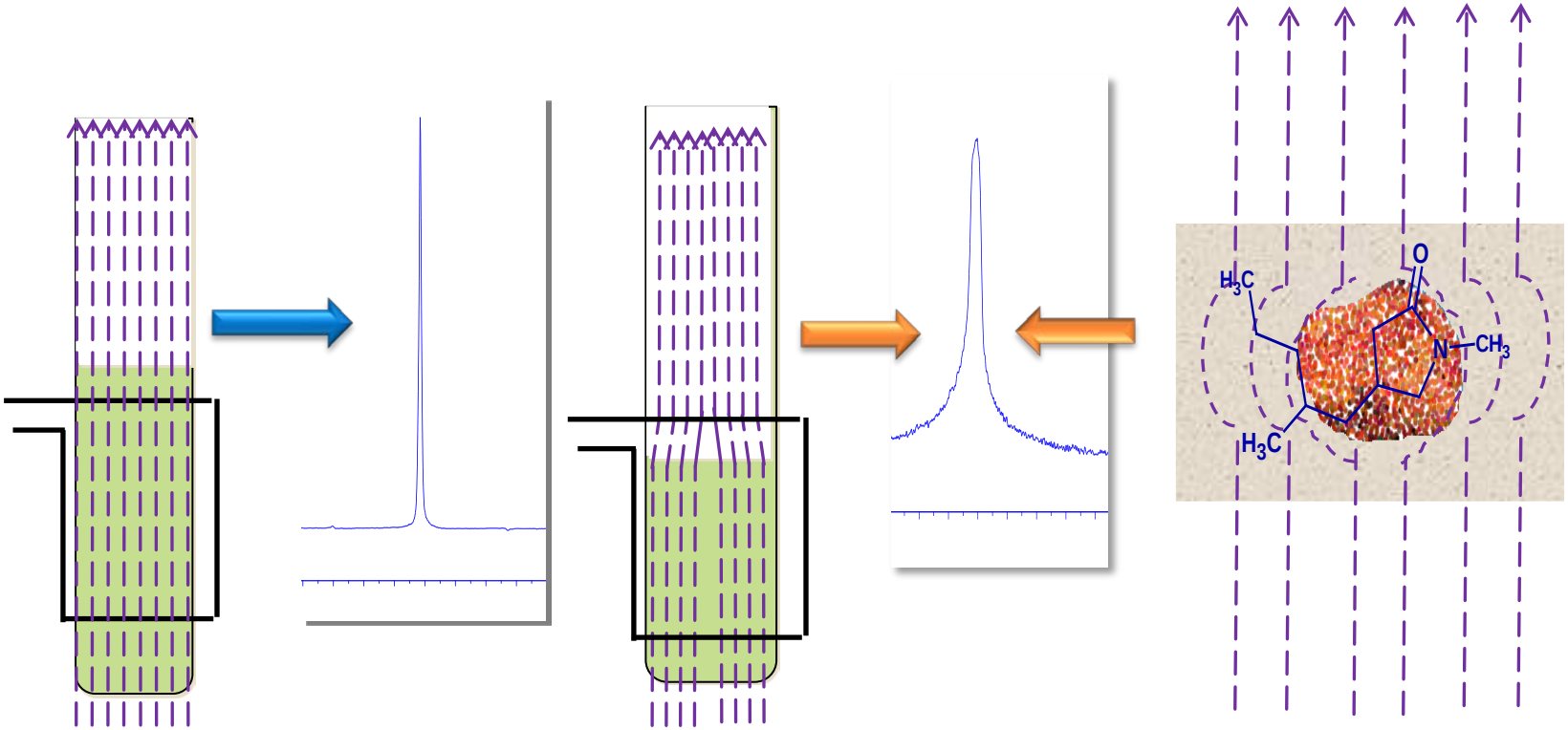
**label your sample**





# Effects due to incorrect sample preparation

An insufficient volume or the presence of precipitate in the solution leads to a distortion of magnetic field lines. The result is a change in the line shape in the signals of the spectrum or a peak splitting

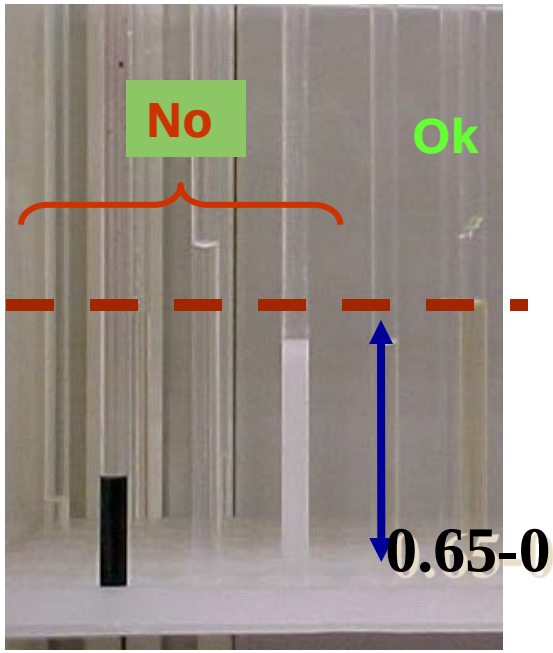



Overfilling the NMR tubes may cause problems due to temperature gradients in the sample

# Before placing the sample in the autosampler

## Check

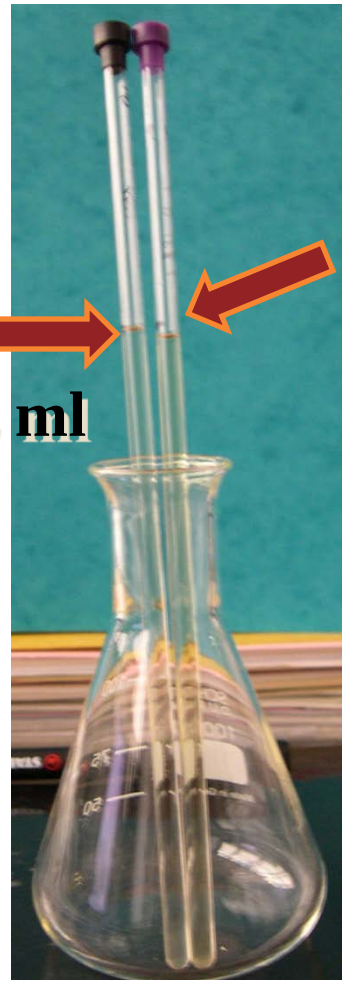
- The NMR tube is clean? (use isopropanol and *kimwipe*)
- The sample is homogeneous?
- The NMR tube is tightly closed?
- The spinner is Ok?
- Are you adjust the position NMR sample with the NMR gauge?





No

> 2 ml



## Tubos de RMN encontrados mal preparados

Tubos excesivamente cortos (ponen en riesgo la sonda)

Volumen excesivo

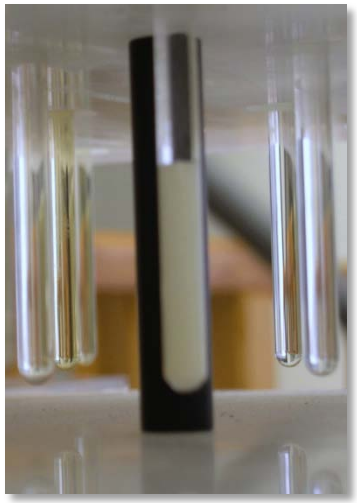
Volumen correcto 0.7-0-9 ml

Tubos con muy poco volumen (perdida de homogeneidad)

Tubo correcto

# The quality of the spectra is not enough?

- **Failure due to sample preparation**
  - Dilute samples do not benefit from a short liquid length. The sample should be about 4.5-5.5 cm of liquid
  - The height of the NMR-tube in the spinner is not correct
  - Contaminations affecting spectral quality. You should: avoid:
    - Paramagnetic Substances
    - High salt concentration
    - Particles "fishes" and not dissolved compounds
    - Mixes of deuterated solvents



**The time required for shimming increases significantly and may be impossible to obtain a quality spectra**

# At the autosampler

- **Cautions about the autosampler**
  - Put the spinner with tube into the SMS changer at the position marked on the monitor screen (check also del logbook).
  - **Never put any spinner-tube in the same assigned position to the sample which is into the magnet**
  - Do not put the spinner (or anything else) under the robot fingers!
  - Never put an empty spinner in the autosampler
  - The robot arm may move quickly and unexpectedly– watch out when samples are being changed.
  - Do not remove spinners from the lab or move them between the spectrometers.

Placed at PCB, Chemistry and Pharmacy

Placed only at Pharmacy

# NMR Sample Tube Care of tube and cleaning

- Do not heat the NMR tube in an oven, the tube will warp and may cause probe damage. The bent tubes may cause severe probe damage.
- Clean the NMR tube with a suitable solvent and dry with acetone and then nitrogen or air
- Tube caps are very cheap and disposable, dirty caps or old caps can contaminate your sample
- Use only EIGHT-INCH tubes on the SMS sample changer!
- The price of NMR tubes range from <2-3€ a piece to >50€. For high-quality NOE-based spectra at higher **fields use high-quality tubes**

**Recommended for 400-500 MHz:**
**Wilmad ref PP 507 or PP 528 (7-14 $) Length: 8 inches**

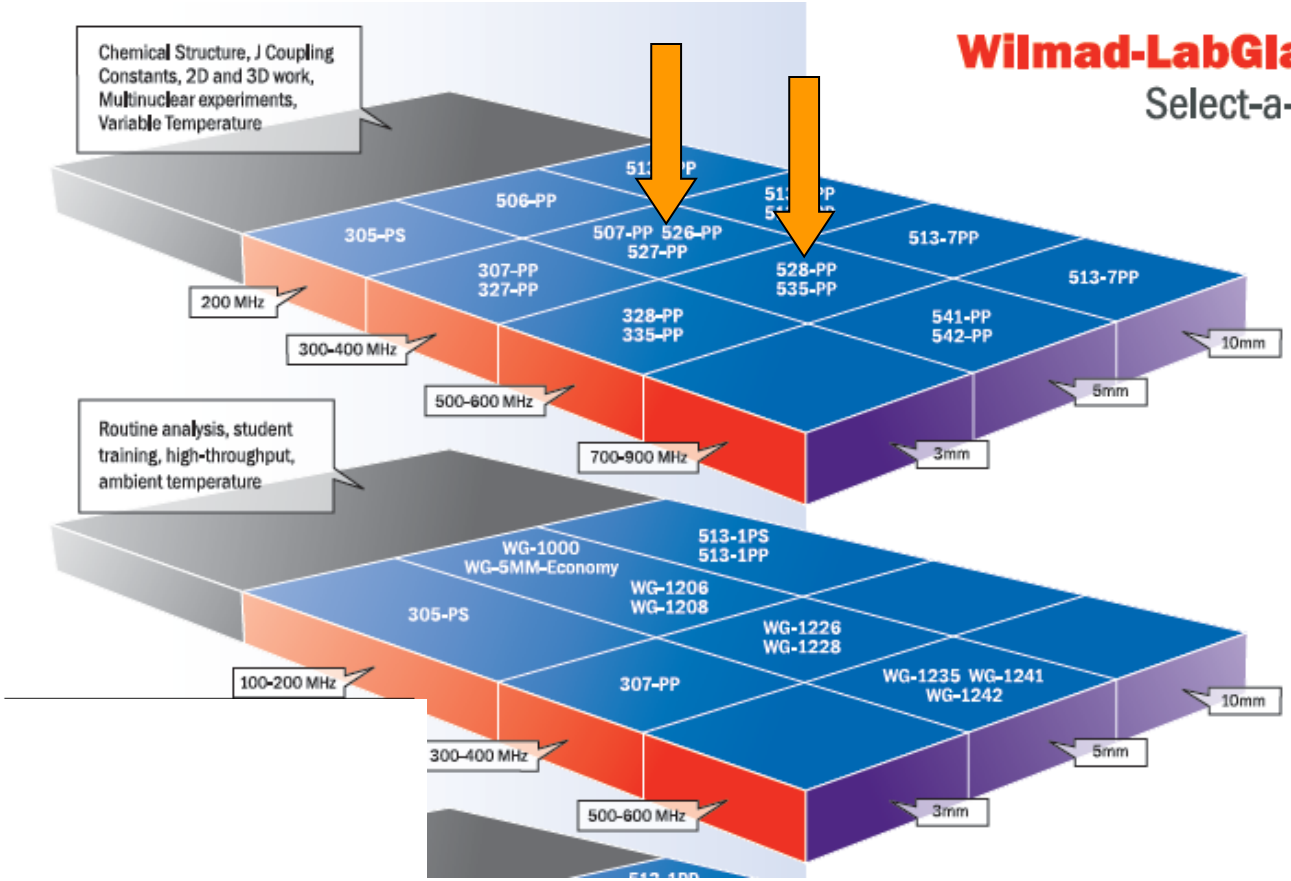
http://www.wilmad-labglass.com/services/technical_NMR_EPR.jsp

http://www.cortecnet.com

http://www.newera-spectro.com/

http://www.nmrtubes.com/shop-online.php?c=nmrtubes

# NMR sample Tubes



Universitat de Barcelona


# NMR Training part II 2018

# Operating Modes

9 samples

4 automatics spectrometers placed at PCB, Fac. Chemistry, Fac. Pharmacy equipped with autosampler

50 samples

50 Samples (3) or 9 samples (1)

- Autosampler
- Preset conditions
- Easy and flexible use
- Operation in continuous mode
  - *24h a day every day of the year*

# Operative system

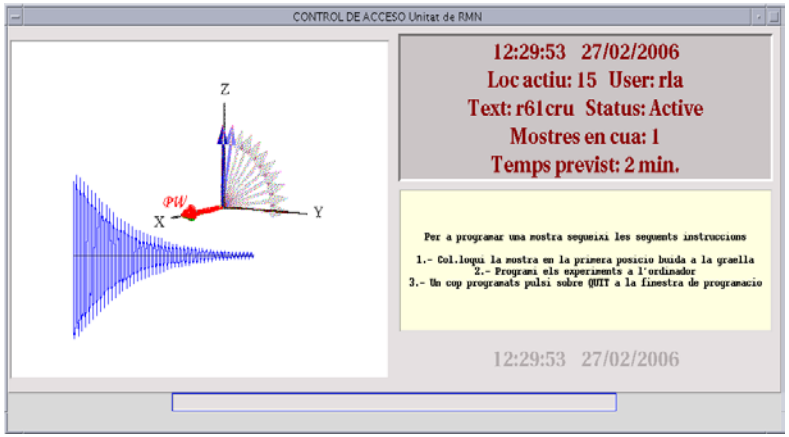
- Two systems: the same way

  VNMR 6.1C (old System G300 )

  Evolution to Java

  VNMRJ (2.2 C/D)

  (M400 )





- The experiments and basic parameters are the same

- **But some differences in the graphic display**

# Which Instrument Can I Use ?

- *VNMRS-400 (Fac Pharmacy)*
  - Autosampler 9 Samples
  - Experiments: 1H, 13C, 19F, 31P y exp 2D
- Mercury 400 ( Fac Pharmacy)
  - Autosampler SMS 50 Samples
  - Experiments: 1H , 13C, 19F y exp 2D
- Mercury 400 (PCB)
  - Autosampler SMS 50 Samples
  - Experiments: 1H, 13C, 19F y exp 2D
- Mercury 400 (Fac Chemistry)
  - Autosampler SMS 50 Samples
  - Experiments: 1H, 13C, 19F y exp 2D

**B400 MHz NMR Fac. Chemistry**

↑

**Not access in self-service but it can work (e.g. 31P-NMR) programmed by NMR facilities**

↓

**500 MHz NMR spectrometers PCB**

# Detection & Sensitivity

Signal-to-noise of an NMR measurement depends:

$$\frac{S}{N} \propto NAT_s^{-1} B_0^{3/2} \gamma^{5/2} T_2^* (\mathrm{NS})^{1/2}$$

γ $^{1}$H = 26,753 rad/G

γ $^{13}$C = 6,728 rad/G

Ratio (γ $^{1}$H/ γ $^{13}$C)$^{3}$ ≈ 64

If we consider the term A (Natural abundance) $^{1}$H ≈ 100% ; $^{13}$C ≈ 1%

**$^{1}$H is 6400 times more sensible than 13C**

γ $^{15}$N -2,71rad/G, A≈ 0,27% **$^{1}$H is ≈ 27*10$^{5}$ times more sensible than $^{15}$N**

## Alternatives to Increase SN

1-Increase the concentration in the active volume.

2- Increase the Magnetic Field (e.g. 500 MHz or B400 MHz cryoprobe)

# Which spectrometer Can I use for $^{13}C$ ?

# Basic Capabilities in Automatic Systems

- Instrument Capabilities
  - 4 x 400 MHz spectrometers shielded magnet , Gradient in Z
    - 2 x ATB probe (1H/19F/13C) Mercury 400 (PCB / Chemistry),

      1 x Optimized for 1H/19F y 13C high tolerance salt and solvent changes (Pharmacy)
    - 1 x 1H/19F/13C/31P probe VNMRS400 (Pharmacy)

| Núcleo | VNMR500 | Mercury-400 | B400Q |
|---|---|---|---|
| 1H | 730:1 | 220:1 | 1050:1 |
| 13C | 240:1 | 158:1 | 450:1 |
| 31P | 135:1 | 183:1 | 350:1 |
| 19F | 650:1 | 175:1 | - |
| | | | |

only in self-service

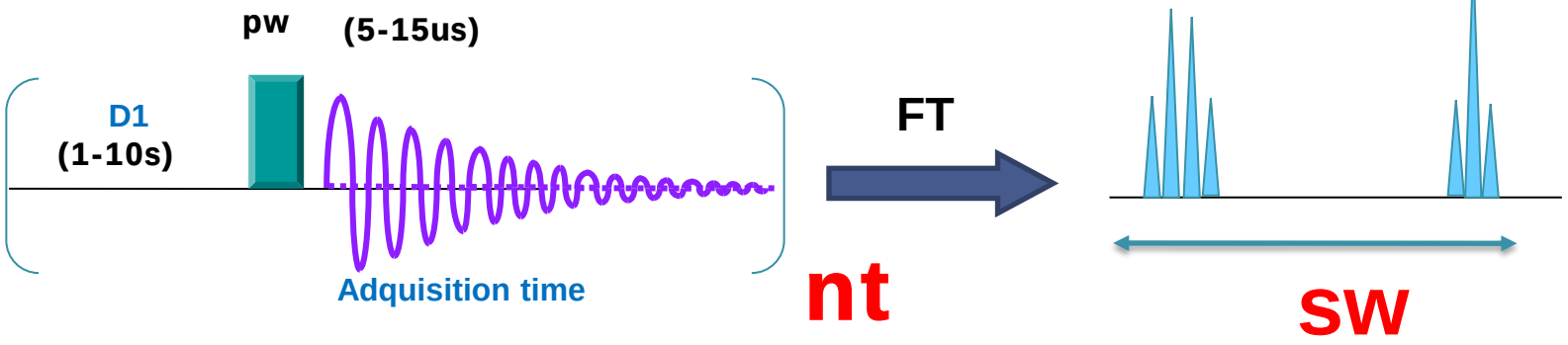
# Some useful information shoud be required before programming the NMR experiments

- Chemical characteristics of the compound
  - Molecular weight
  - Functional groups
  - Symmetry
- Solubility in mg/ml ?
- The product contains a transition metal ? (e.g. organometallic compound such as Pt, Pd)
- Is a mixture or a single compound ?
- The spectrum is needed for
  - Check the reaction ??
  - Identification ??
  - Structure determination ?
  - Quantitative ??

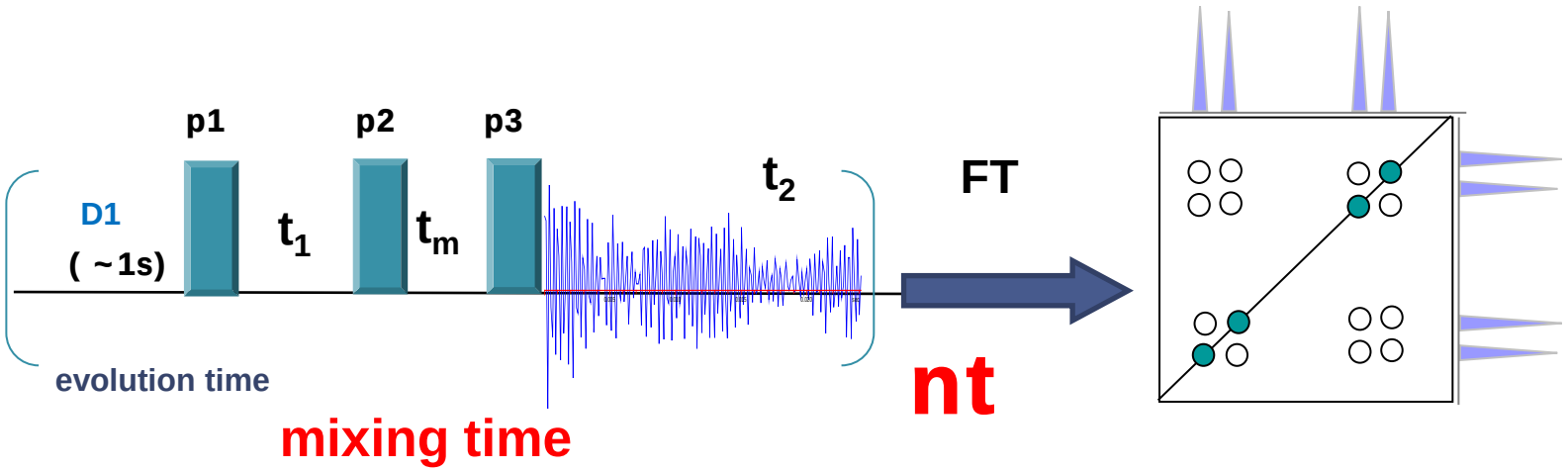
**When you know this answers then select the spectrometer, experiment and parameters**

# Data acquisition parameters: "the key"

- ***Basic 1D NMR sequence***

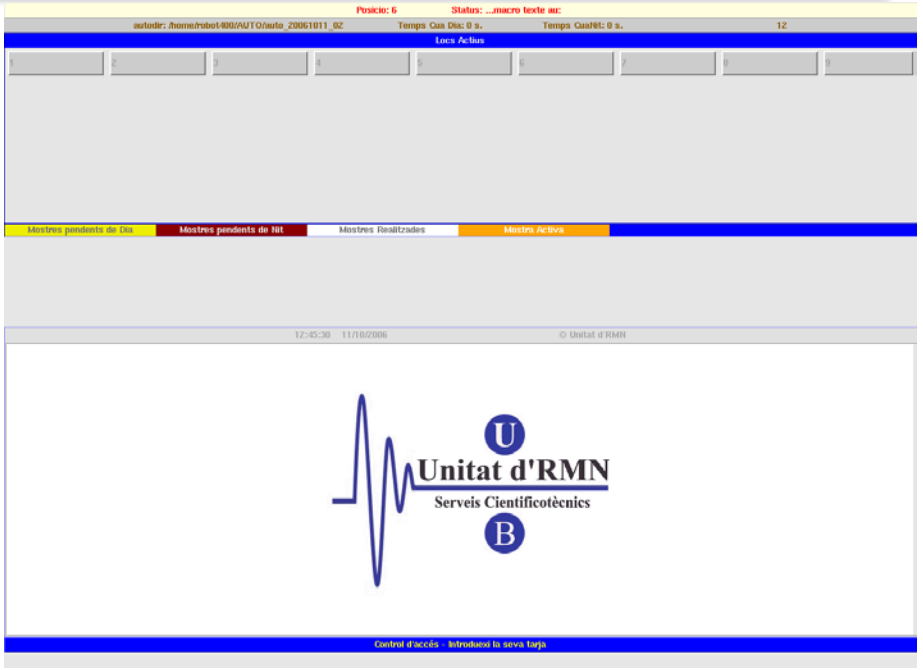



- ***2D .NMR sequence ( NOESY)***



# Automatic System: User Interface

Acess Card



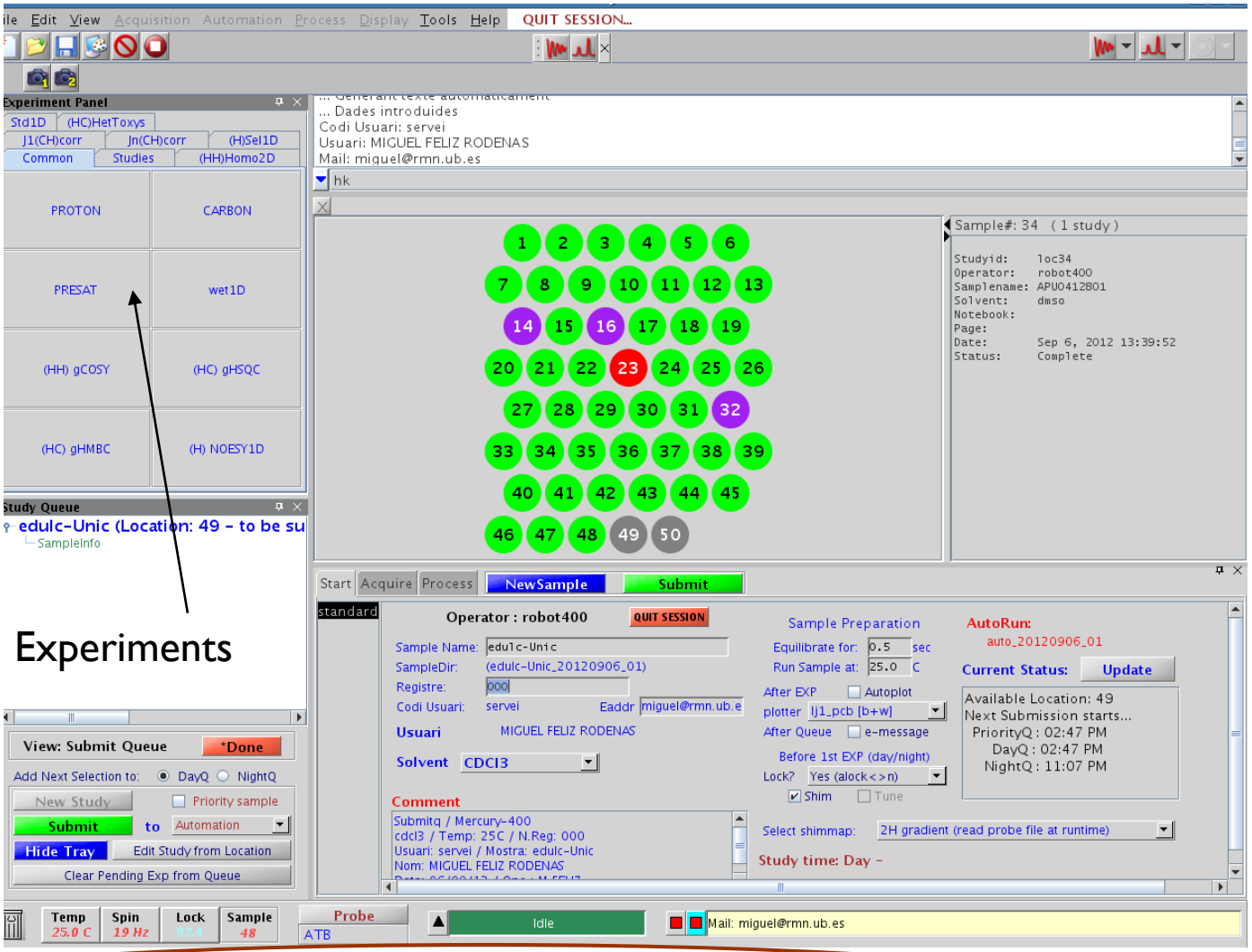

User Identification
(group, e-mail, name )

## ¿What experiment I can do it now?

### Scheduled Activity

- 8-15 minutes (e.g. 1H, 19F, 31P)
- if More than 15 min it must be programmed in the night way (e.g. $^{13}C$, NOESY, HSQC, etc.)
- unlimited time experiment (only in the weekend)

# Automation Interface Appearance




**information about the current acquisition**

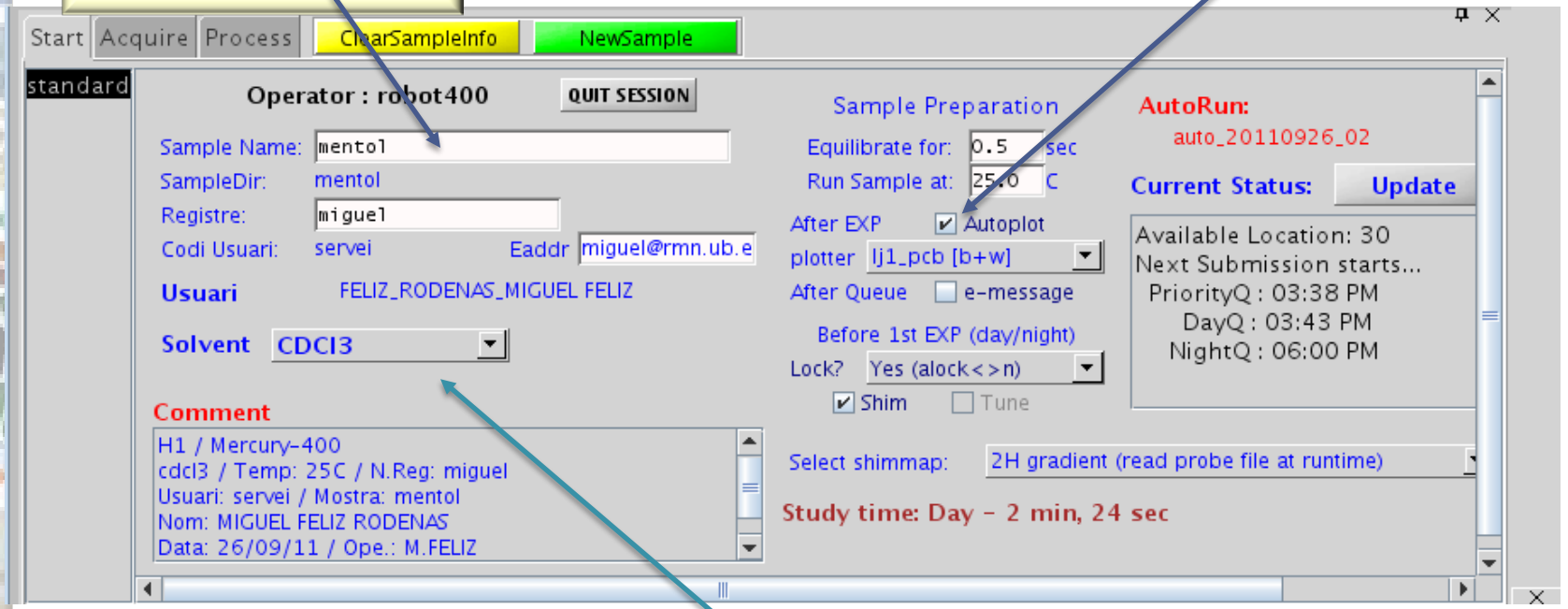
# How to program my sample ?

## First step: introduce sample's name and solvent

**Sample Name**
**max 20 characters**
***return***

**No |@/&$***

**Select plotter yes or not**



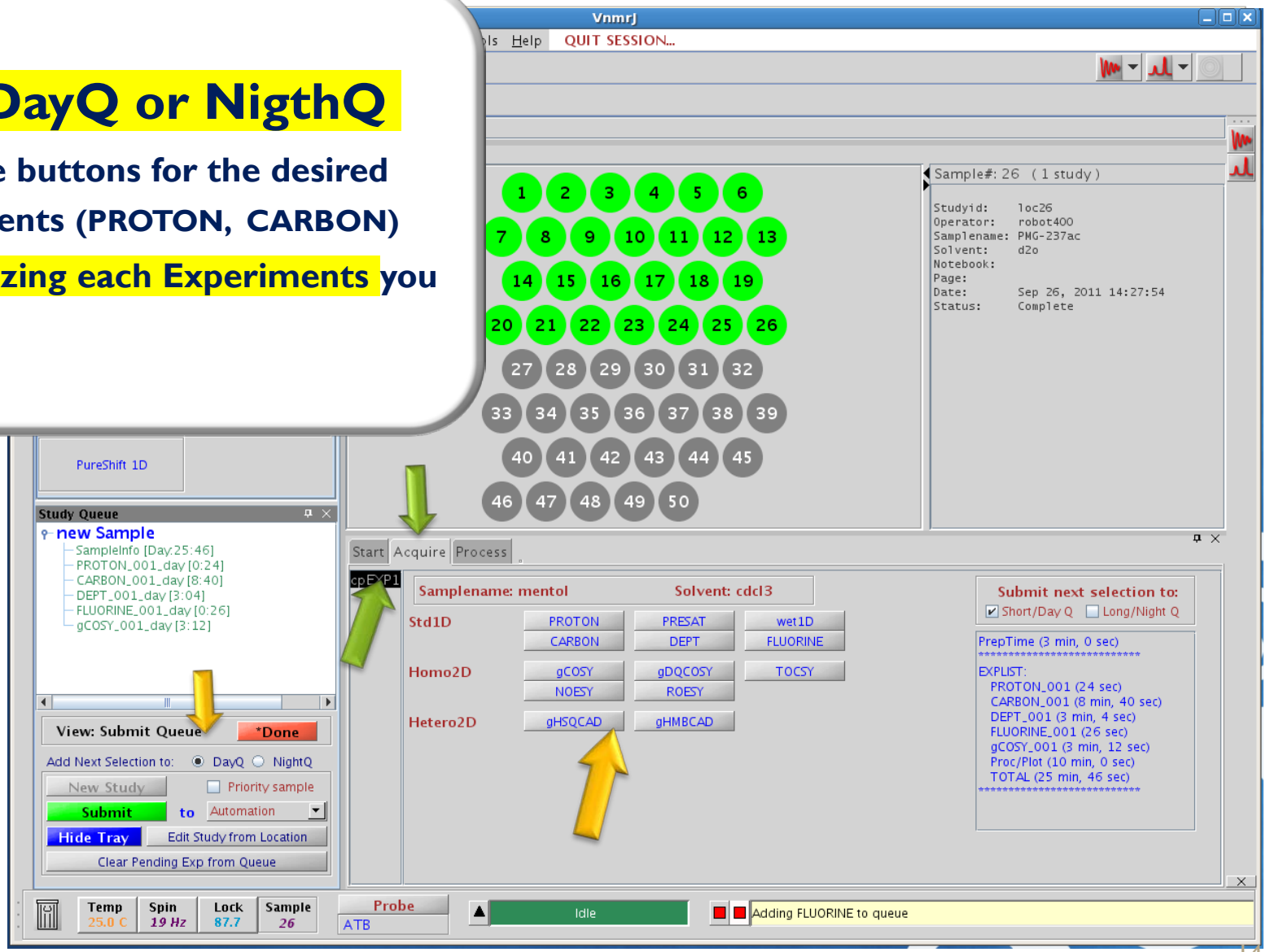

**VERY IMPORTANT**
**Select the solvent at the beginning**

Write sample's name and choose solvent. The name will be the same for all experiments from this sample

# Second step: select your experiment

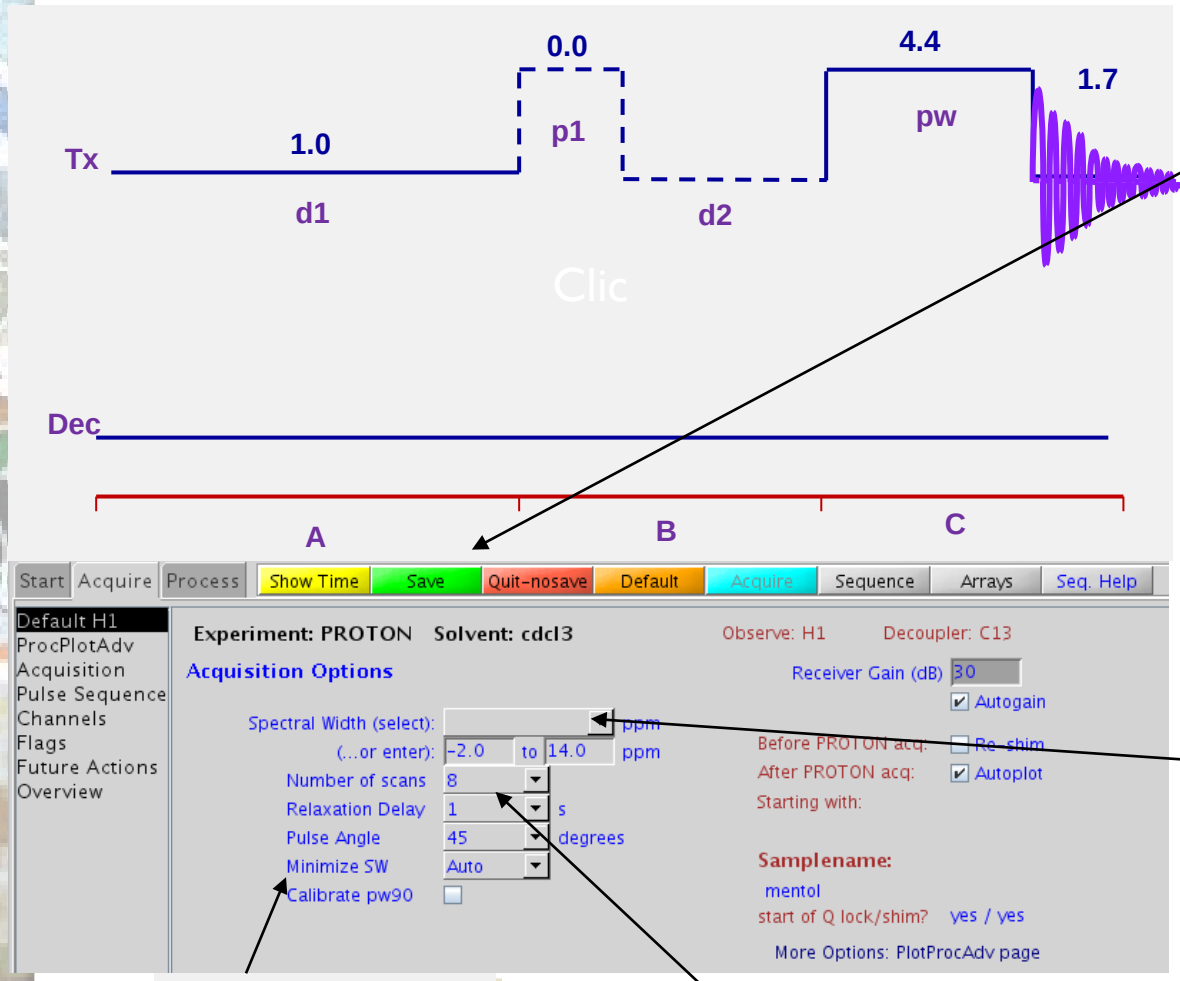
## Acquire

- Select **DayQ or NigthQ**
- Click the buttons for the desired experiments (PROTON, CARBON)
- **Customizing each Experiments** you selected



# Third step customize each experiment

## for example, modifying a PROTON acquisition parameters



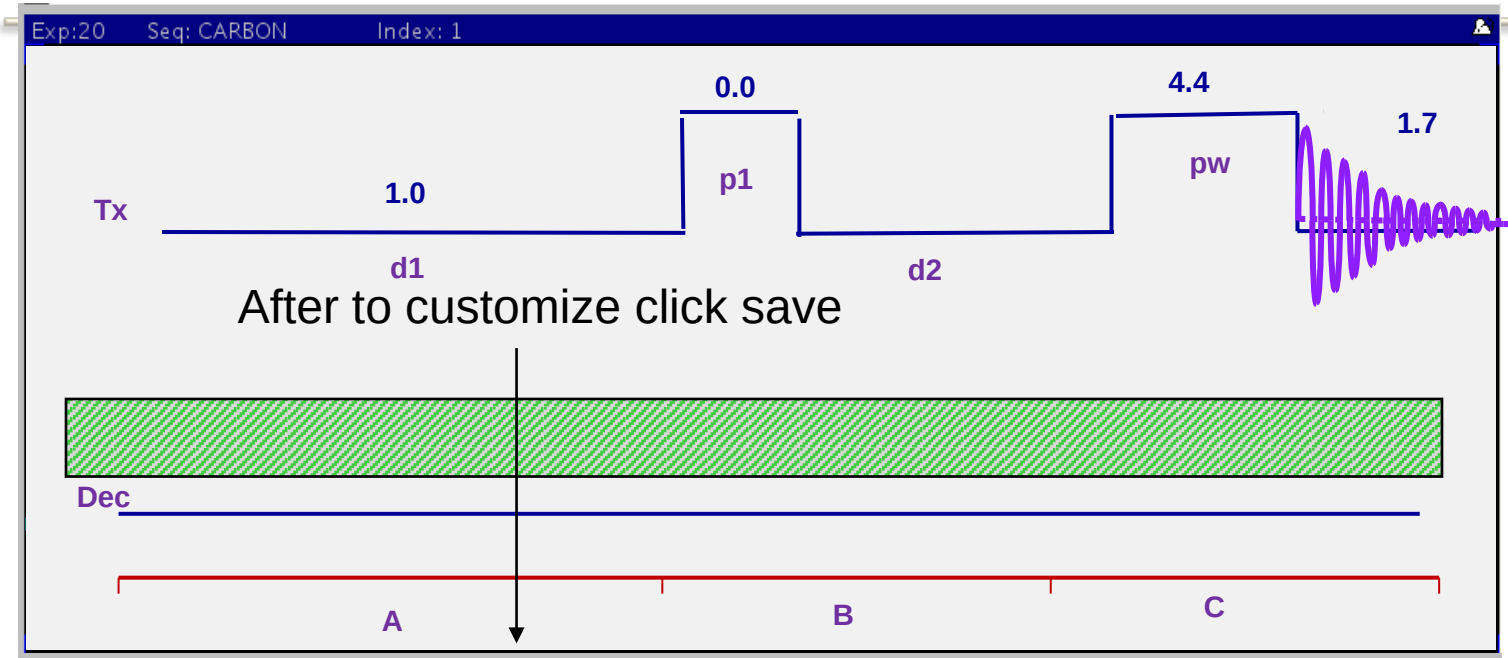

Save the customized parameters and continue the programming if you need it

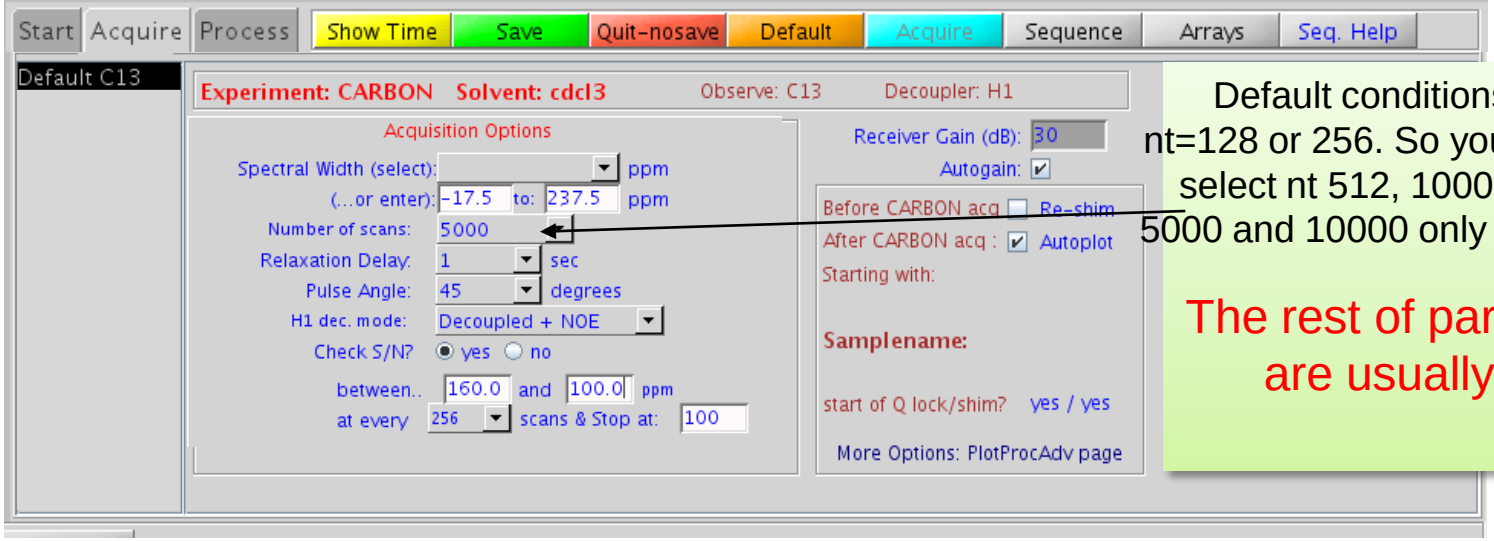
Select spectral window

Minimize SW is very important in 2D exp
Auto

Select Scans 4 to 1024 (time limit)

# $^{13}C$ Customizing parameters



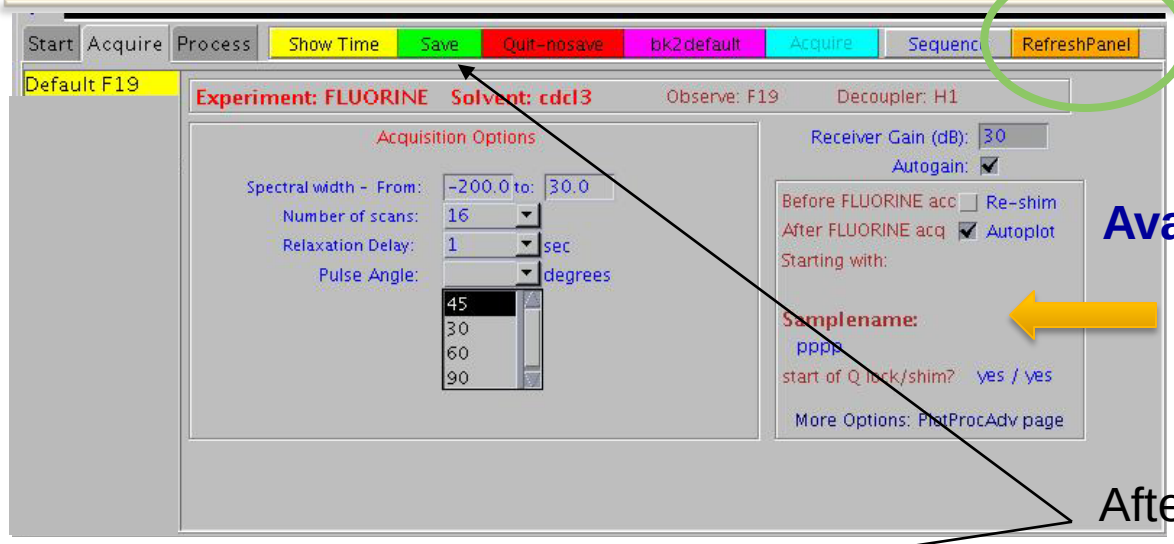




$^{13}C$

Default conditions are nt=128 or 256. So you have to select nt 512, 1000, 2000. 5000 and 10000 only weekend

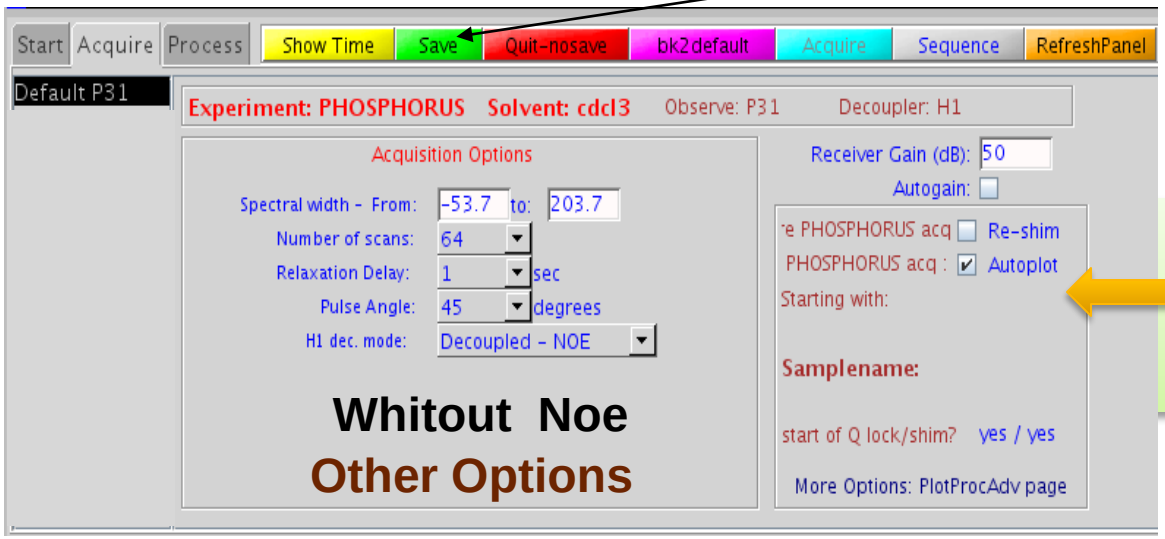
The rest of parameters are usually OK

# $^{19}F$ and $^{31}P$ customizing parameters




**Available in all 400 MHz Spectrometers**

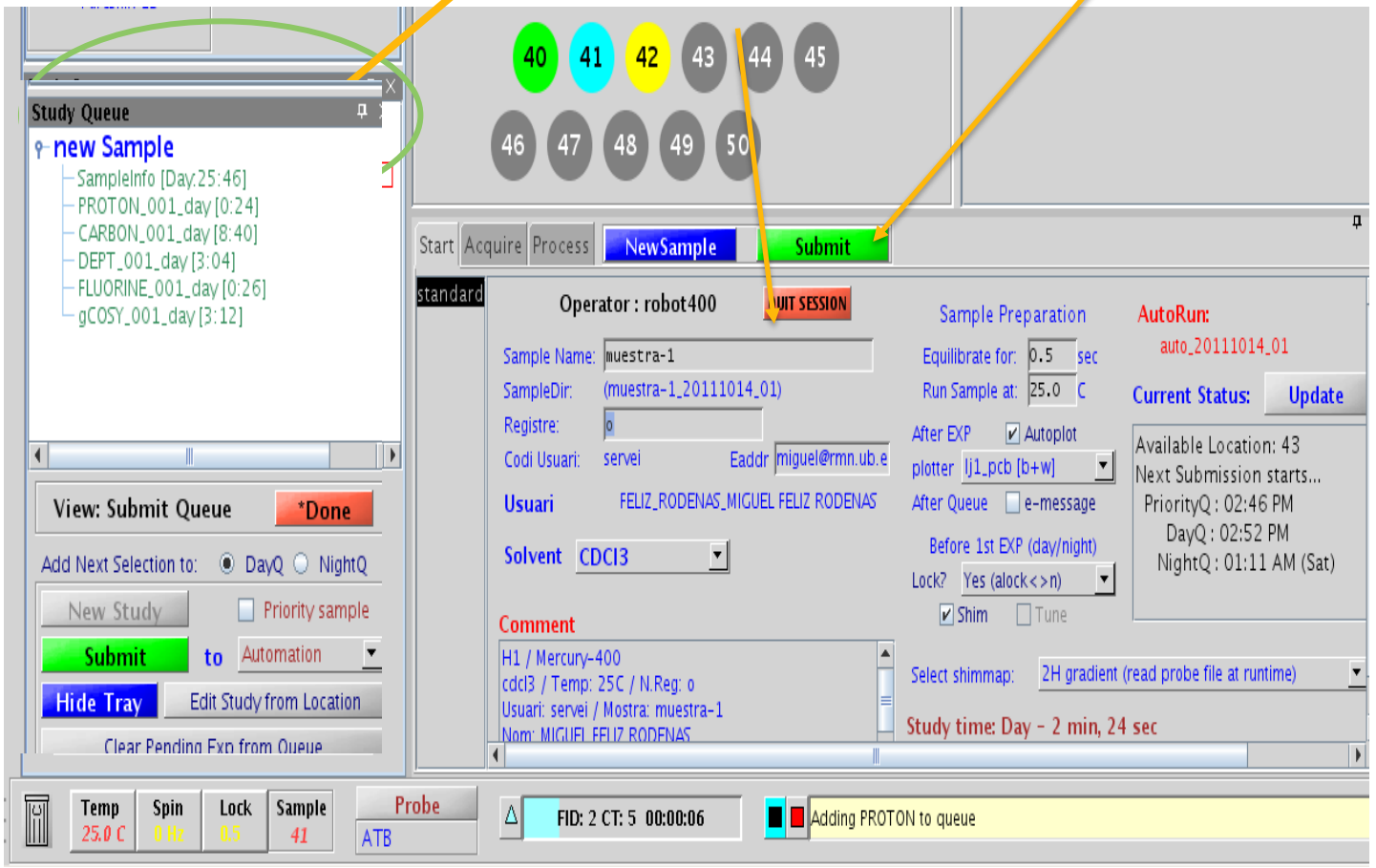
After customizing click save




**Available only in VNMRS-400 Pharmacy**

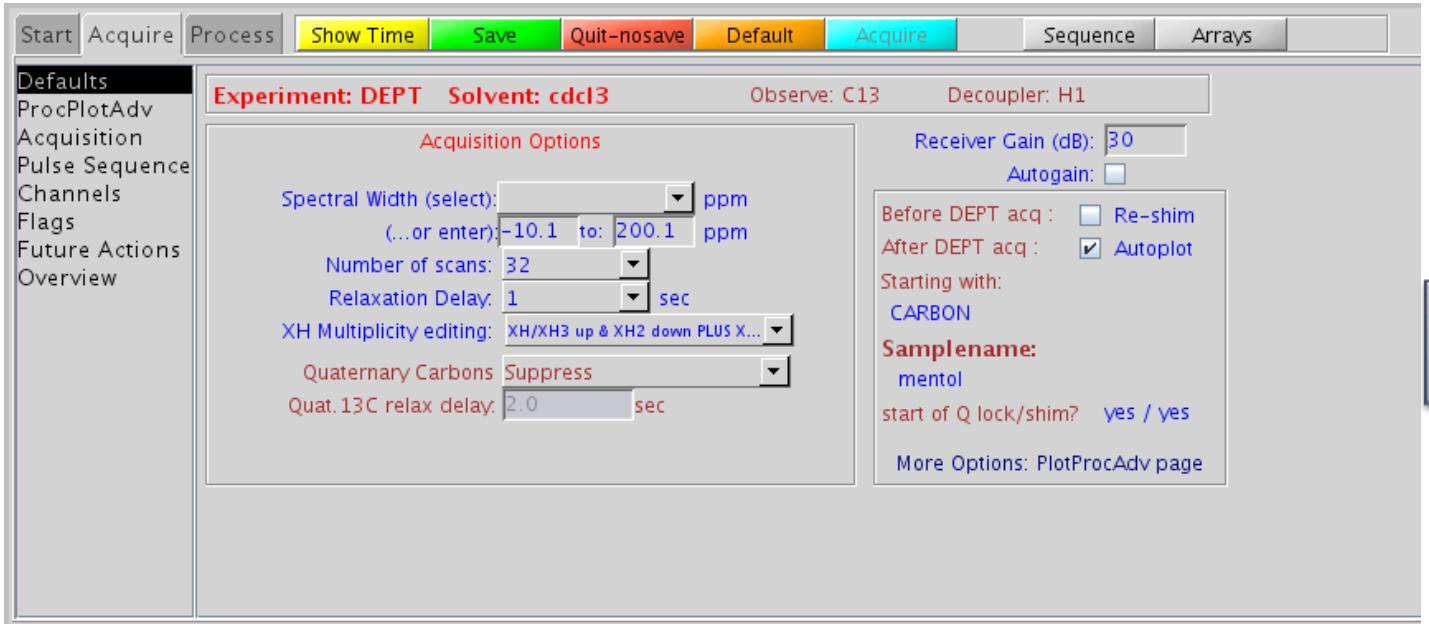
# WE COULD CONTINUE PROGRAMMING MORE EXPERIMENT BUT MAYBE IS TIME TO SUMMIT AND LOGOUT

**Once all experiments has been customized is time to submit them and logout**



# DEPT

The DEPT requires a previous $^{13}C$ ⟶ Adds $^{13}C$ if not scheduled




DEPT

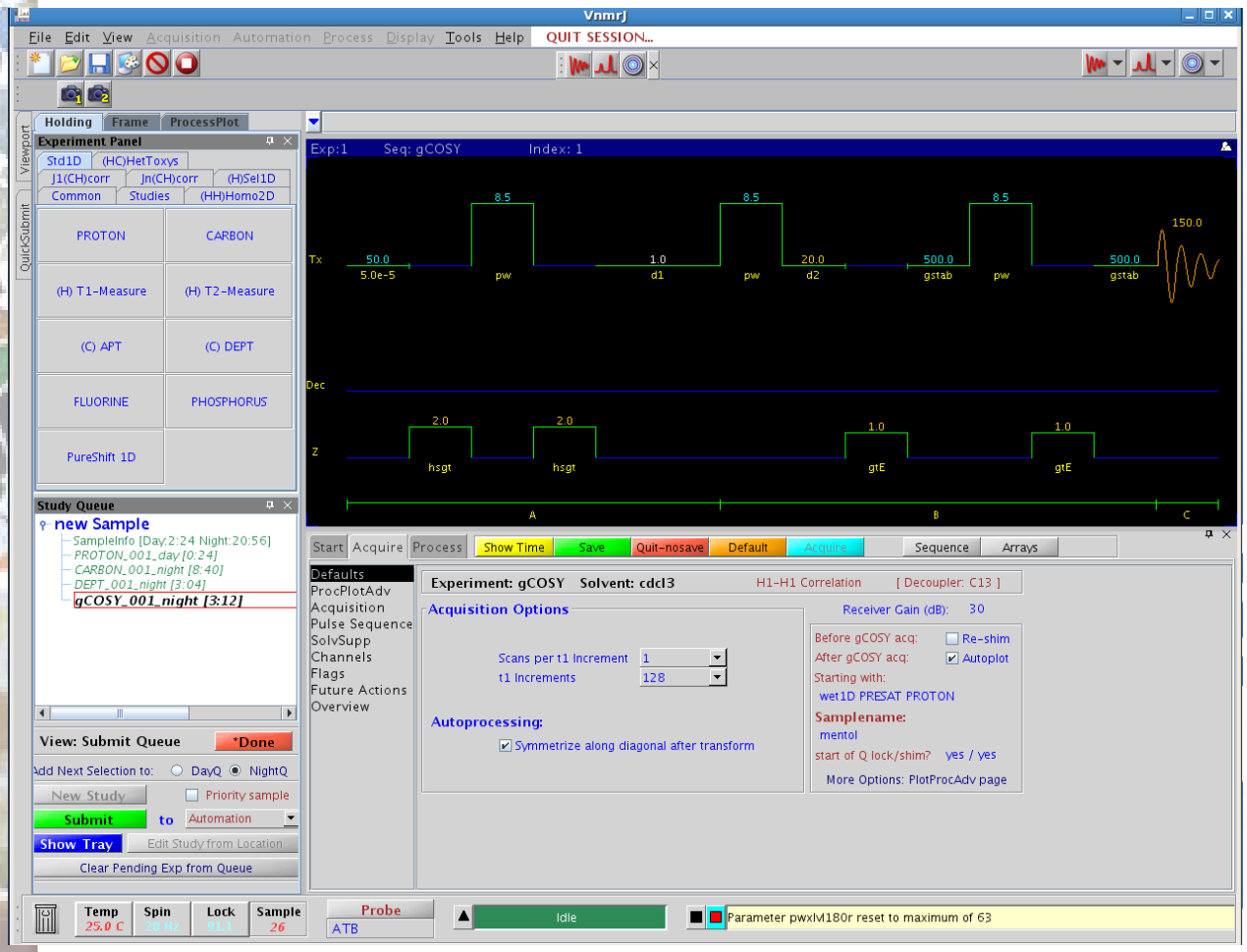
**Scans must be 1/9 or 1/10 of scans required for your carbon spectrum but min 32**

**The HSQC/gHSQC experiment replace the 1D DEPT for routine analysis**

# gCOSY 1H-1H correlation experiment

## The 2D experiments need 1H spectrum before

If not exist in the study Proton was automatically added for gCosy




**Very easy to run** :

Only ***nt*** and ***ni*** need to be adjusted

Solvent suppression can be incorporated PRESAT or wet (selected signals in the 1D exp)

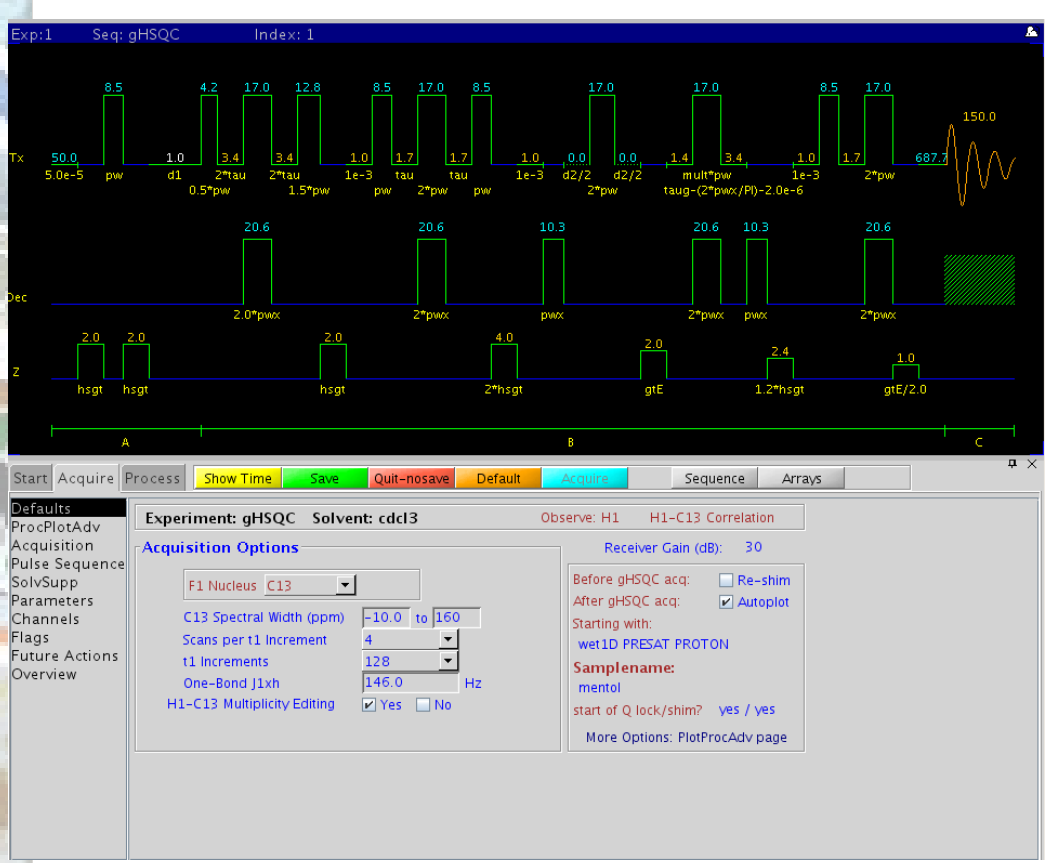
gCOSY

**gCOSY (nt=2 ni=256) time ≈ 11 min**

# gHSQC

## Most important parameter

**One Bond $J_{xH}$ aprox 146Hz**




The average value of the one bond coupling constant can be optimized if necessary (C=C, furane, etc )

Phase sensitive
Apodization function: Gaussian or sq-sinebell
Linear prediction in F1 ni*2- 4

Cross peaks in gHSQC are multiplicity edited by default:
CH and CH3 are + and CH2 are -
so you get the equivalent information of a DEPT

gHSQC (nt=4 ni=128-256) **Time ≈ 20- 60 minutes**

gHSQC

# TOCSY (Total Correlation Spectroscopy).


**TOCSY (nt=2 ni=256) time ≈ 46 min**

## Most important parameter

**Mixing time (mix=70-120 ms)**

- **Correlates a proton to all other protons in a spin system.**

  Peptides, oligosaccharides

- **Useful for resolving ambiguities in the COSY**

Phase sensitive;

Apodization funtion:

gaussian

Linear prediction in F1

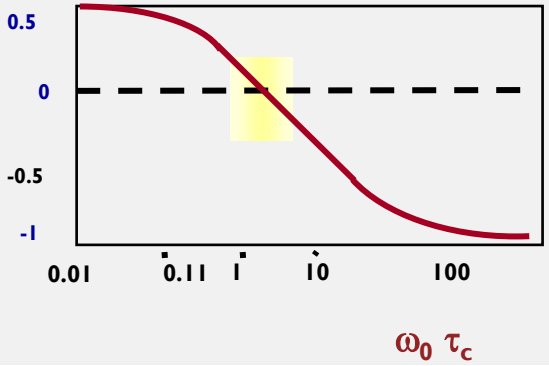
ni*2- 4

**Tocsy**

# Noesy

## Most important parameter

**Mixing time (mix=100-800 ms)**

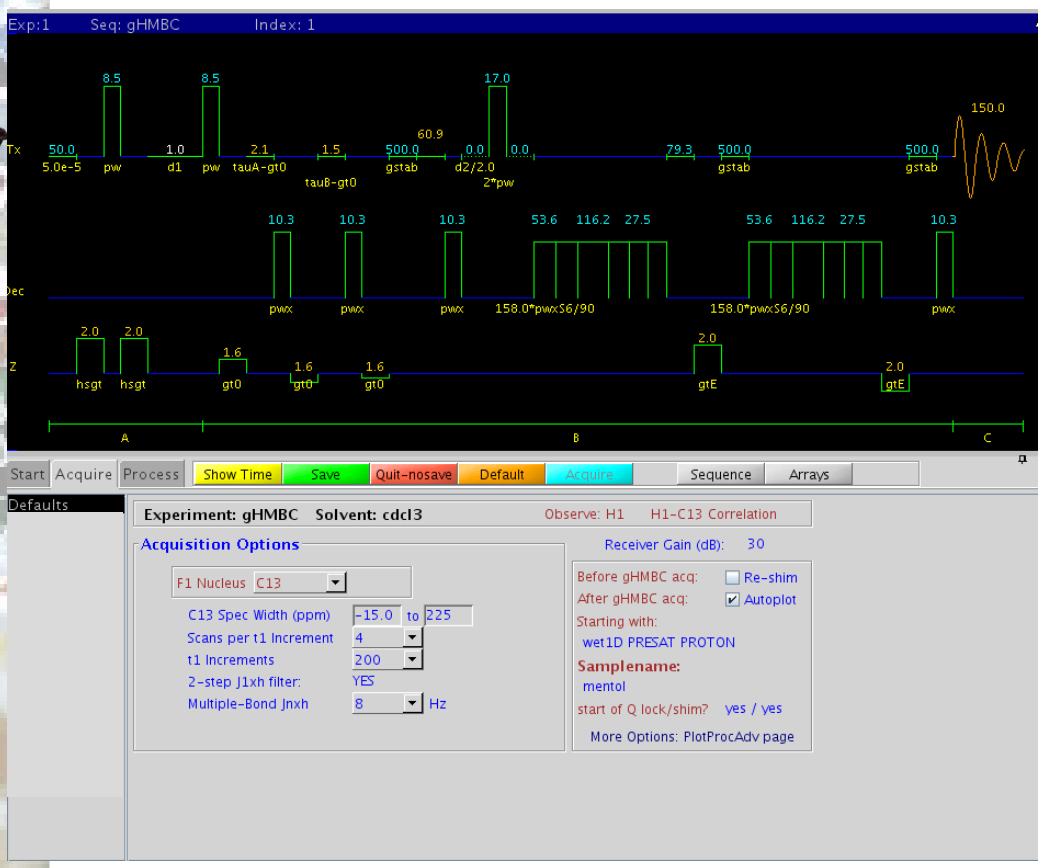

Diagonal peaks are negative, positive cross peaks are positive NOEs, negative cross peaks are negative NOEs or chemical exchange correlations.

Phase sensitive;
Apodization function: Gaussian
Linear prediction in F1
ni*2- 4

NOESY (**nt=16 ni=256** mix=0.5) time ≈ 4h

**Noesy**

# gHMBC




gHMBC (nt=16 ni=256) time ≈1-1.5h

## Most important parameter

**2-3 Bond $Jn_{xH} \approx 3\text{-}8$ Hz**

The average value of the 2,3 bond coupling constant can be optimized.

Can't discriminate between correlations due to 2 bonds and those due to 3 bonds

gHMBCAD: Phase sensitive in F1 and AV in F2 Apodization function: sq-sinebell in F1 and sq-cosine in F2

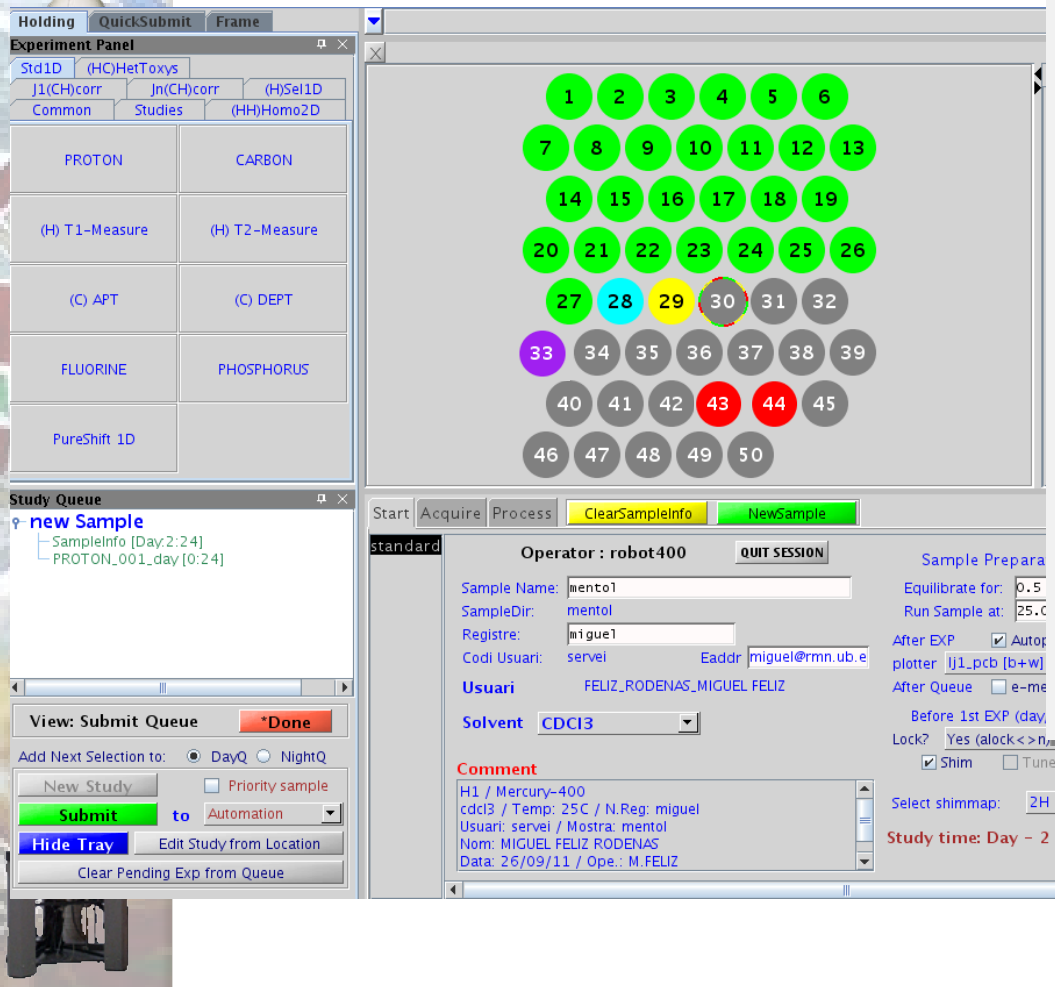
gHMBC experiment AV

Apodization function sinebell in F1 and F2

Linear prediction in F1 ni*2- 4

**gHMBC**

# The last but not least




## Check

- Select Experiments
- Time for performed the experiments.
- If the experiment is send to Day Q or NightQ
- Information about the sample (Name, solvent, ……) in the Start panel
- ¿The location in the autosampler is Ok ?

**System will indicate you the place available. It's not optional is mandatory**

## Submit

- ¿More samples?

## Quit Session

completion

- Status
- Mail
- Info web

# Time experiment guidelines
# Quinine sample (5mg in 0.8 ml $CDCL_3$)

| | nt | ni | Parameters to adjust | Time at 400 MHz |
|---|---|---|---|---|
| 1H 1D | 16 | --- | | 2 min |
| 1D 13C | 4000-7000 | ---- | | 3h -4h |
| gCOSY | 1 | 256 | | 7 min |
| TOCSY | 2 | 200 | Mixing time 30 - 120 ms | 25 min |
| NOESY | 16 | 256 | mix = 0.1 s to 0.3 s for large molecules mix = 0.4 s to 0.8s for small molecules | 3h -4h |
| gHSQC | 4 | 128 | J=140 | 26 min |
| gHMBC | *16-8* | *400* | *J=8* | *1-2h 20min* |

# Today we will work with the colors

Complete Sample (correct)
YOU CAN TAKE IT

Active Sample

Queued Sample DON'T TAKE IT NEVER

In Night-Queue DON'T TAKE IT NEVER

Error it can be reprogrammed again

# More frequent user failures

- **Inappropriate characters in the text.**

  numbers, special characters (*$/&%! .....)

- **Place the tub in a same position that sample in the magnet.**

  The Acqstat or notebook was not check.

- **Small tubes with a length less than 19 cm.**

  The tube can be broken.

- **Mislabeled samples.**

  Incorrect label Labeled on the bottom of the tube.

- **Precipitate.**

  The solution was collapsed by the solid.

- **Shut down computer.**

  Log out or close the program.

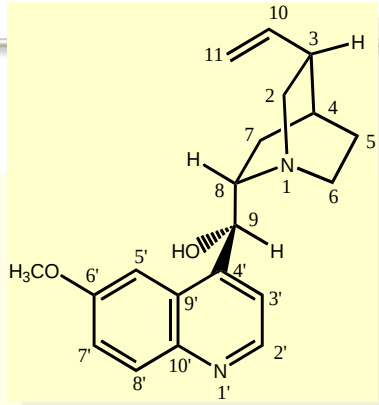
# More frequent spectrometer failures

| | |
|---|---|
| **Spining sample** | Tube/spinner/failure in air supply |
| **Dont found the Lock** | Concentration/ product precipitation / Poor Homogeneity |
| **Receiver Overflow** | very high concentration of sample |
| **Splitted or broad peaks** | Concentration/ product precipitation / Poor Homogeneity |

# WHAT KIND OF INFORMATION GIVE US THE EXPERIMENTS ?

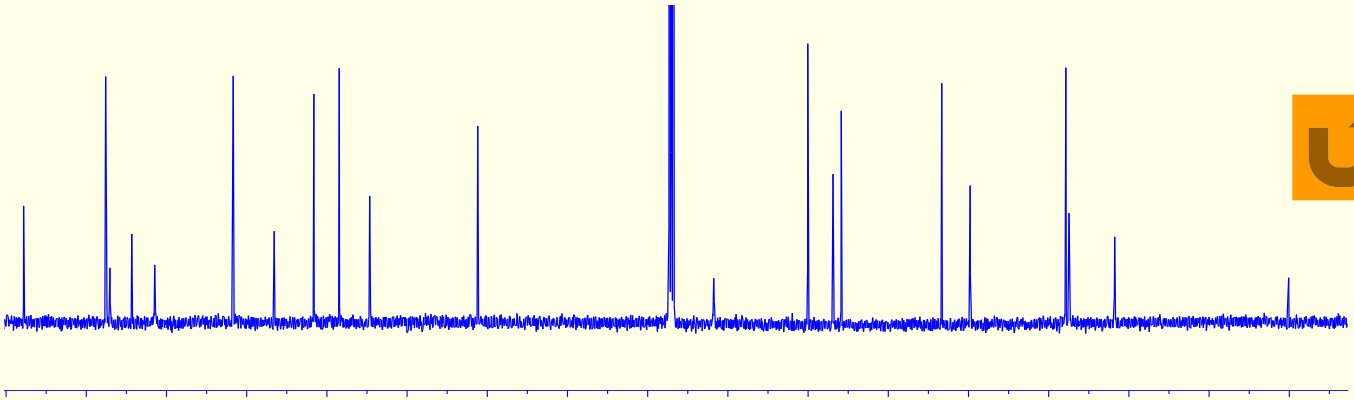
# $^{1}H$ and $^{13}C$

**Quinine 5mg/0.7 ml**







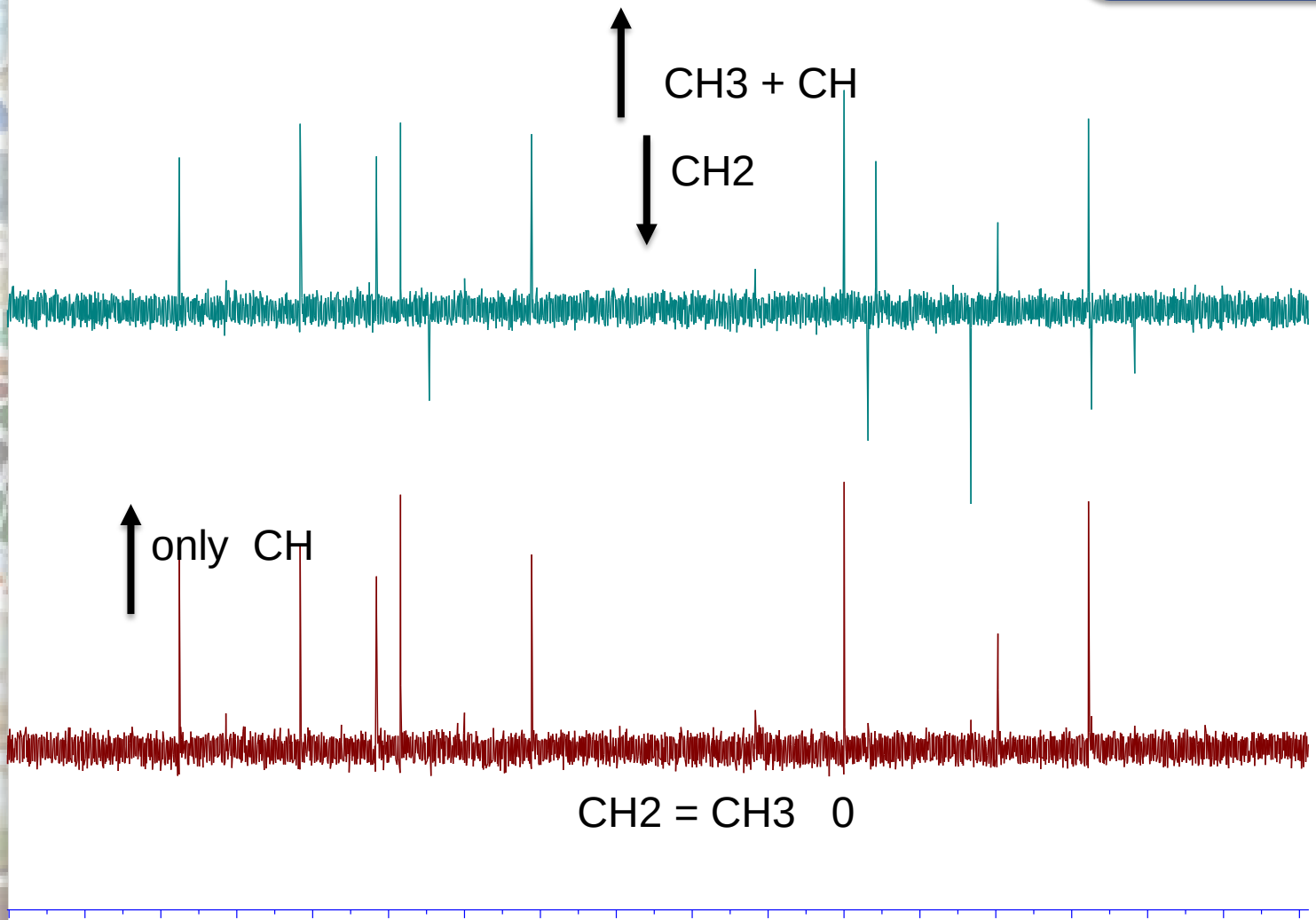


# Dept

Quinine 5mg/0.7 ml

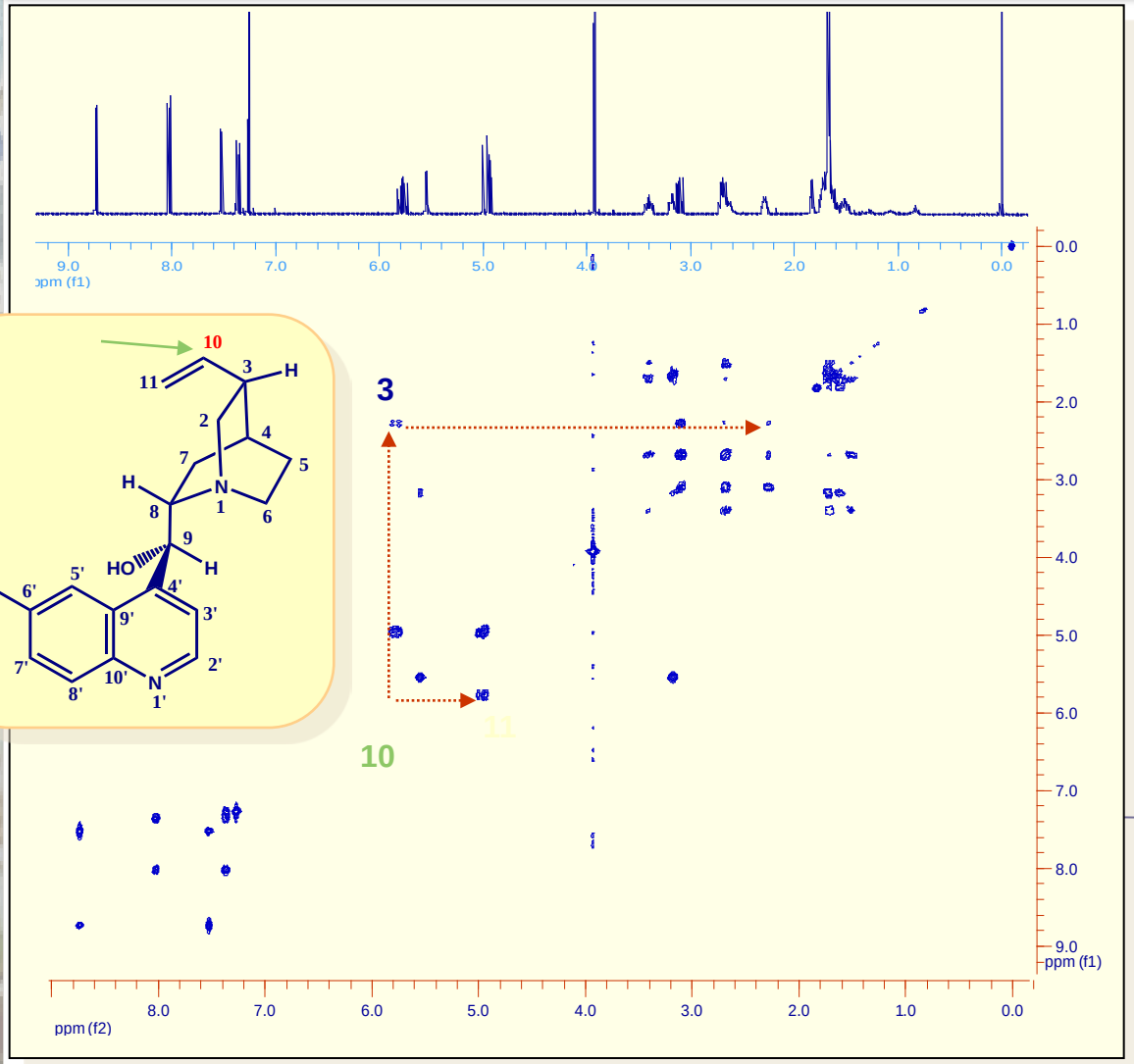
DEPT nt=256

20 minutes



# COSY

1H-1H Homonuclear correlation
direct couplings

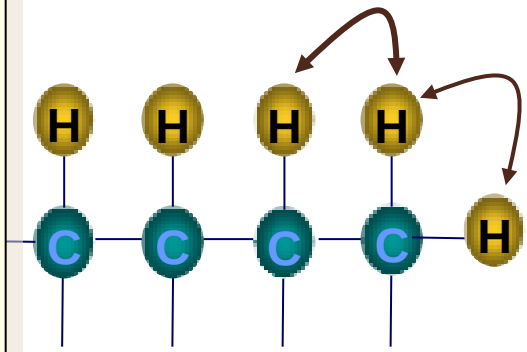






Quinine 2mg/0.8 ml

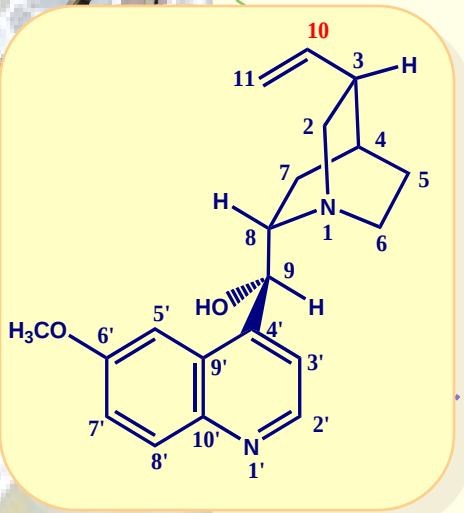
nt=2 ni=256
time 13 minutes

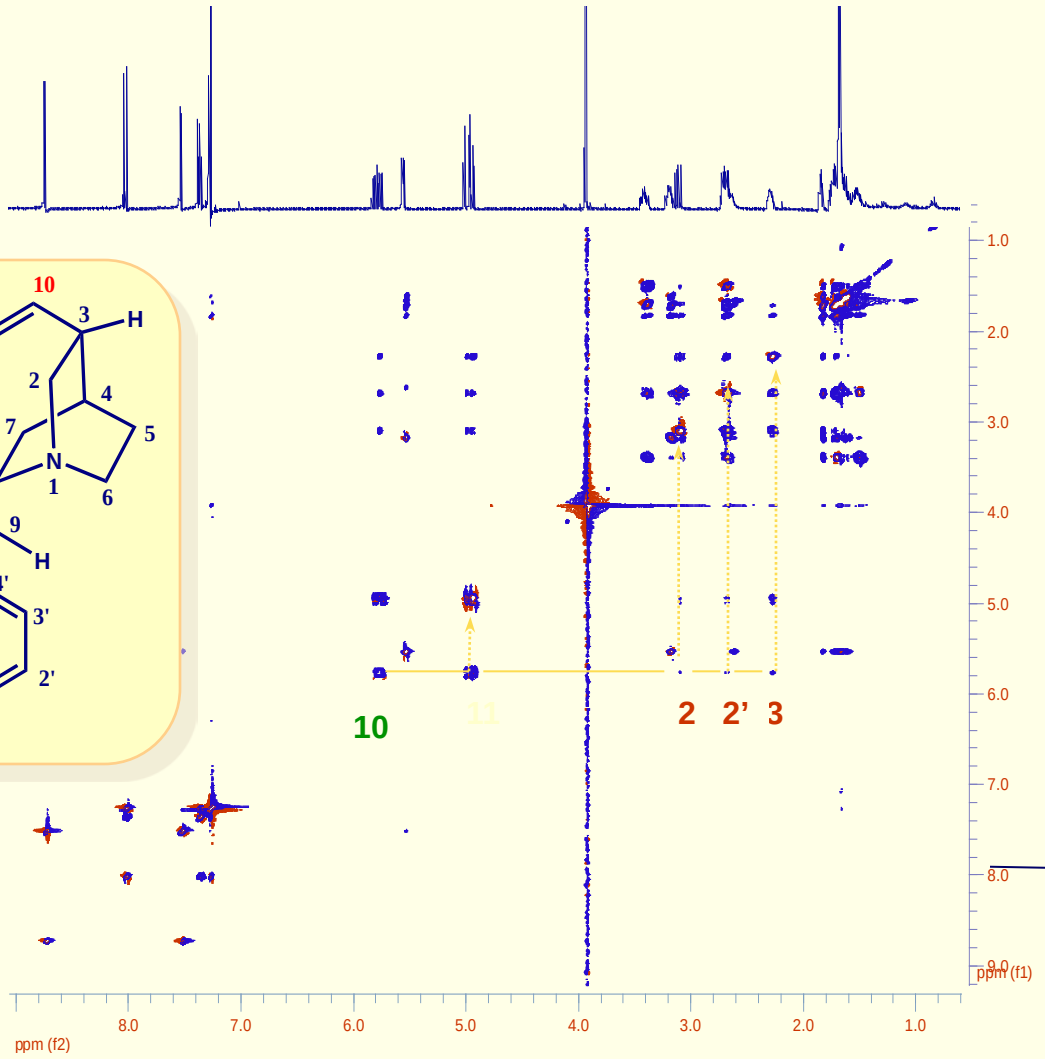


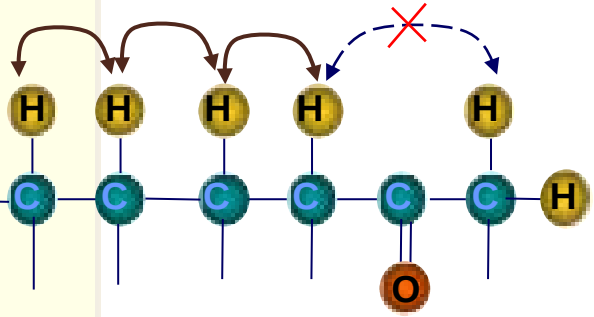
# TOCSY

Quinine 2mg/0.8 ml

TOCSY
nt=4 ni=256 mix=80 ms
≈1h



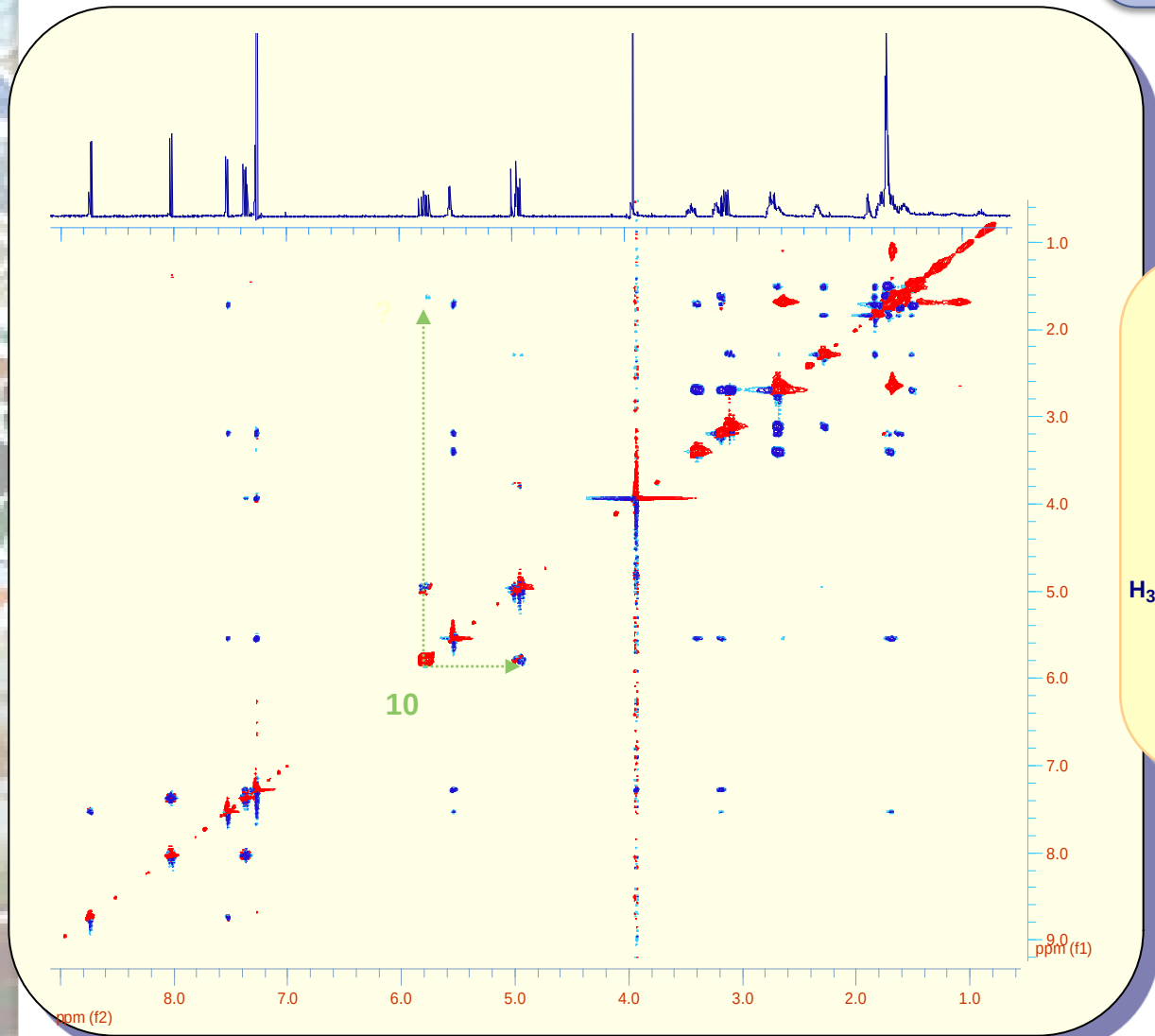
# NOESY

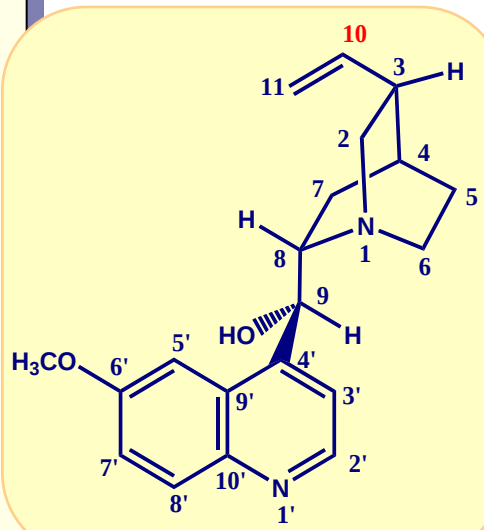
Quinine 2mg/0.8 ml

NOESY

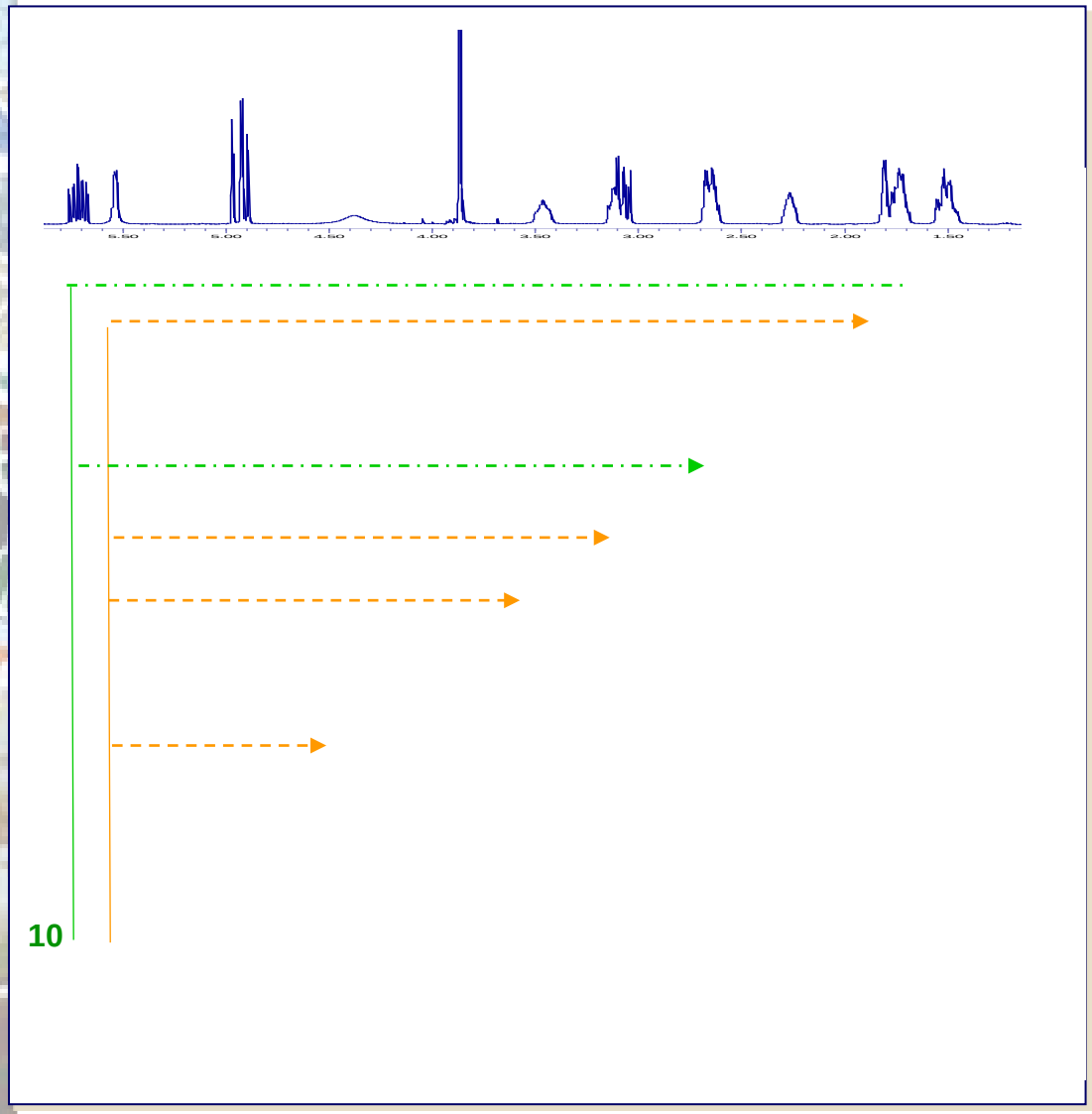
nt=32 ni=256 mix=0.5 s

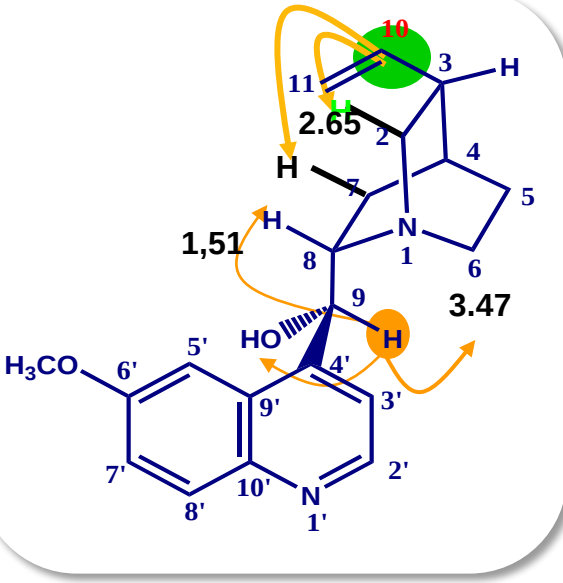
8 h 23 min



# NOESY QUININE



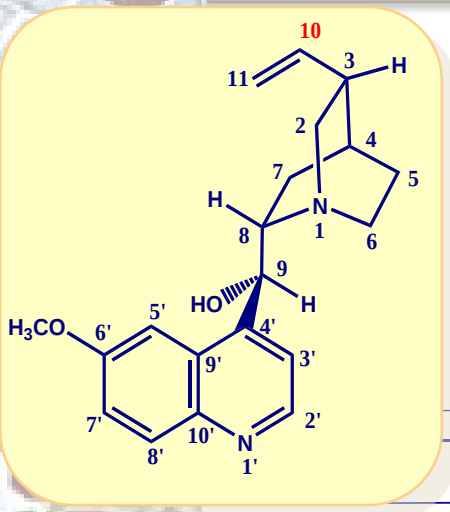
# gHSQC

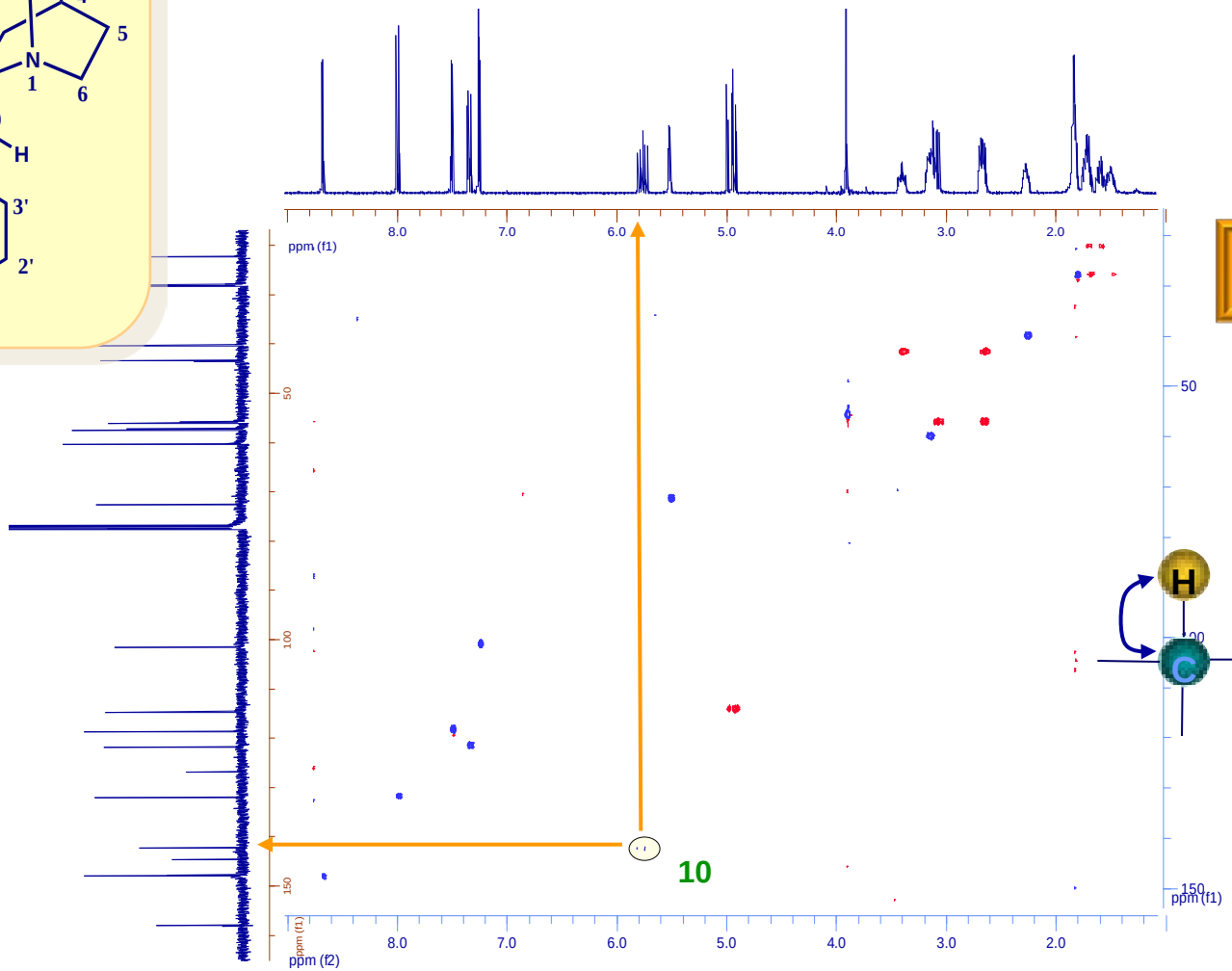
Quinine 5 mg/0.8 ml

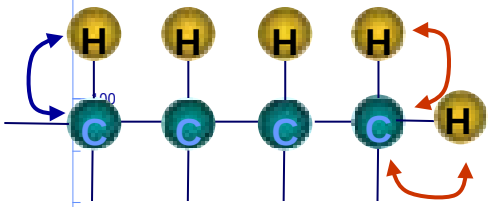
gHSQC

nt=4 ni=128 LP

26 minutes




# gHMBC

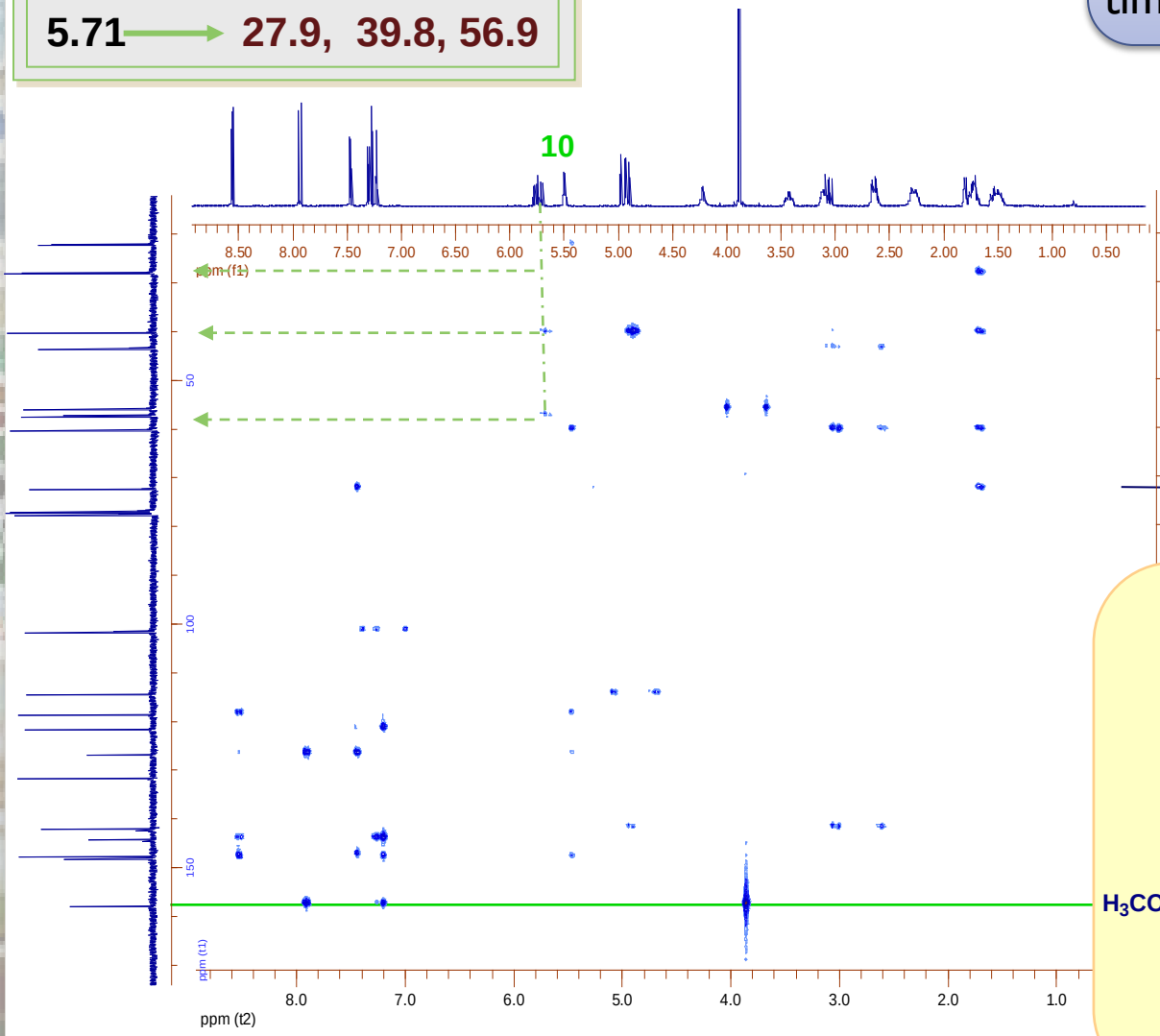
**Quinine 5 mg/0.8 ml**

GHMBC

nt=16 ni= 256 jnxh=8 Hz

time= 2h 20 min

**5.71 → 27.9, 39.8, 56.9**




HMBC

HSQC