

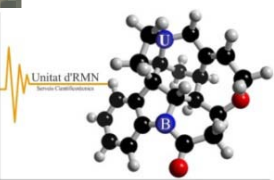


B Universitat de Barcelona



NMR Basic training Part-I 2018

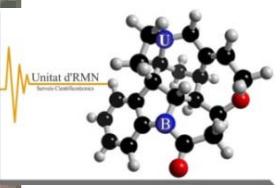




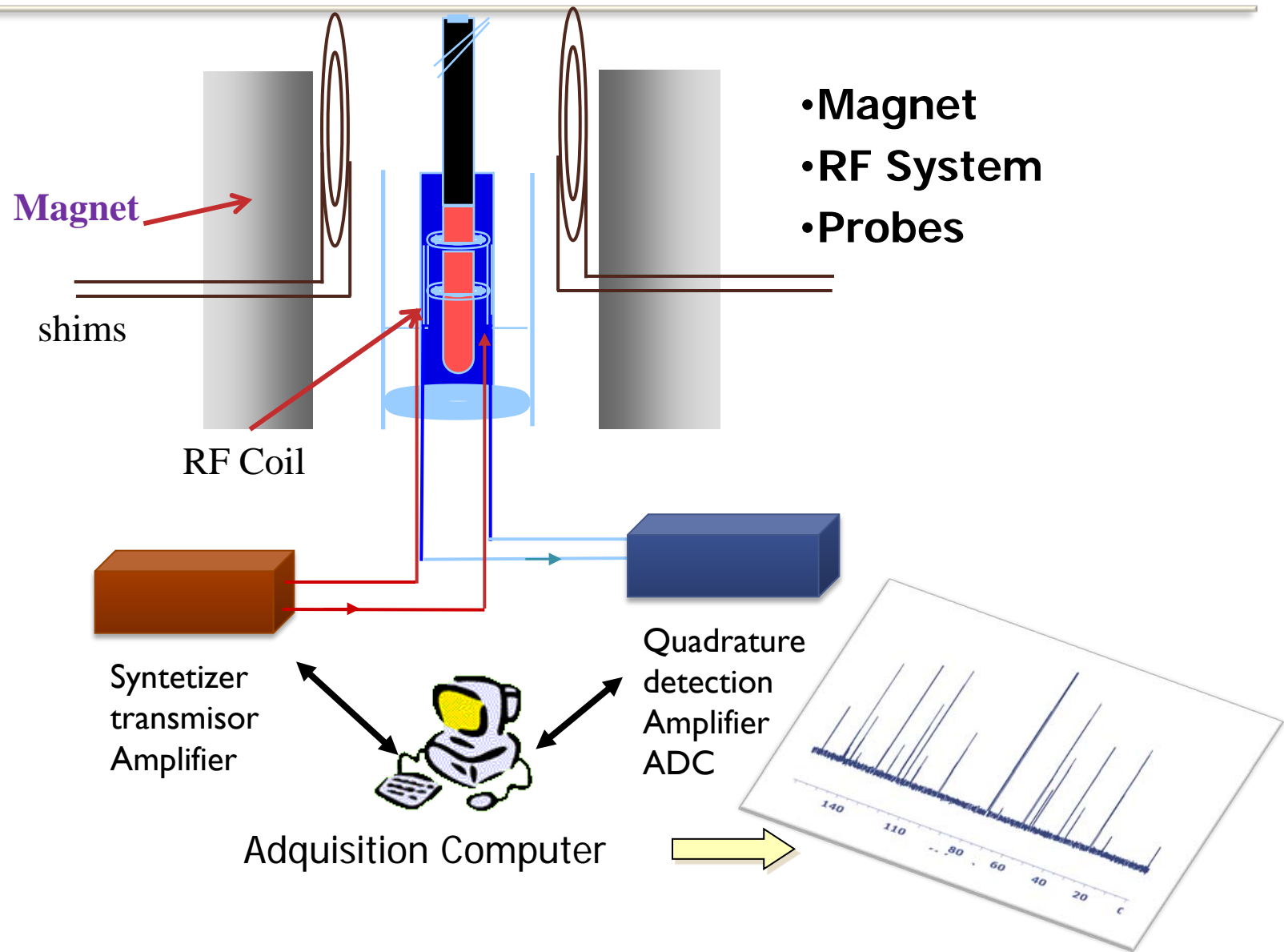
Index

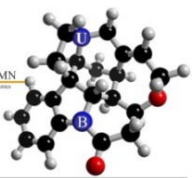
- NMR Spectrometer
 - Description
 - Lock, shimming
- NMR Sensitivity
- Security: Magnetic Field cautions
- Rules
- Samples
 - Sample preparation
 - NMR tube





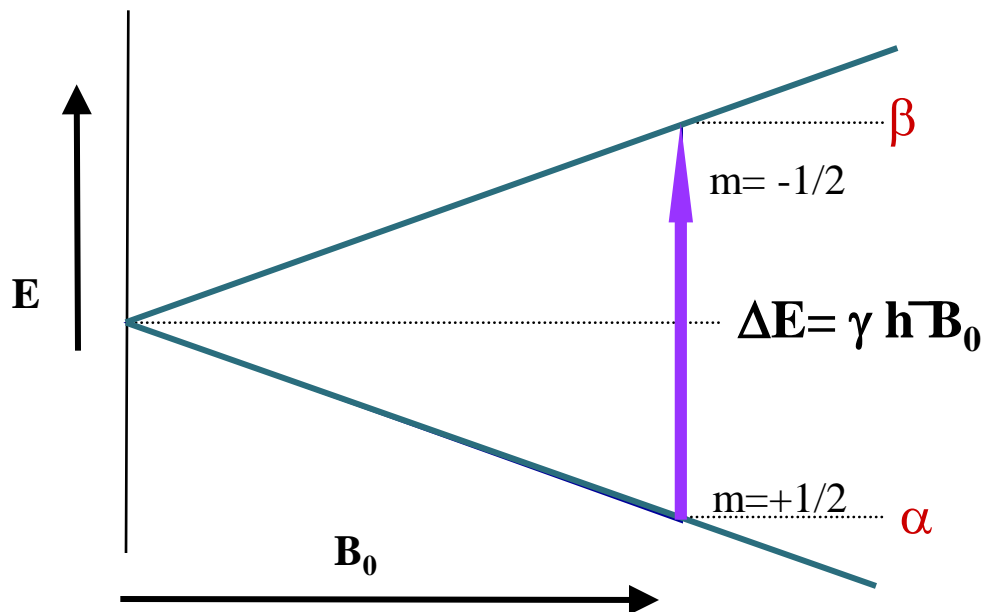
NMR Spectrometer, Block Diagram





RMN sensitivity

Higher the field strength \longrightarrow higher the sensitivity



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

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

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

$$K_B = 1.985 \text{ cal/Kmol}$$

1H

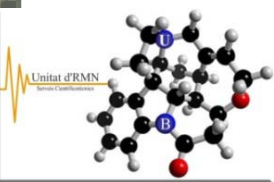
$$B_0 = 1.41 \text{ T (60 MHz)} \quad \frac{N_{\beta}}{N_{\alpha}} = 0.9999904$$

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

Low Sensitivity

Differences \approx 1-20 ppm





Magnetic field

Resolution



60MHz
(≈1965)

Dispersion

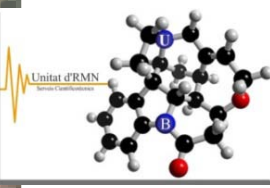


1.000 MHz
(2009)

Sensibility

Higher the field strength → higher the sensitivity and spectral dispersion





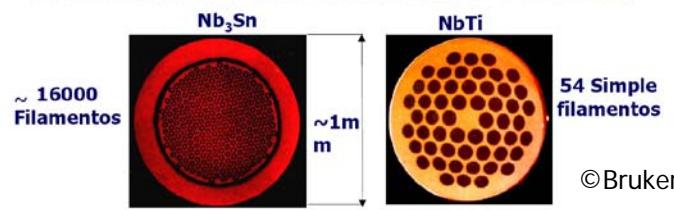
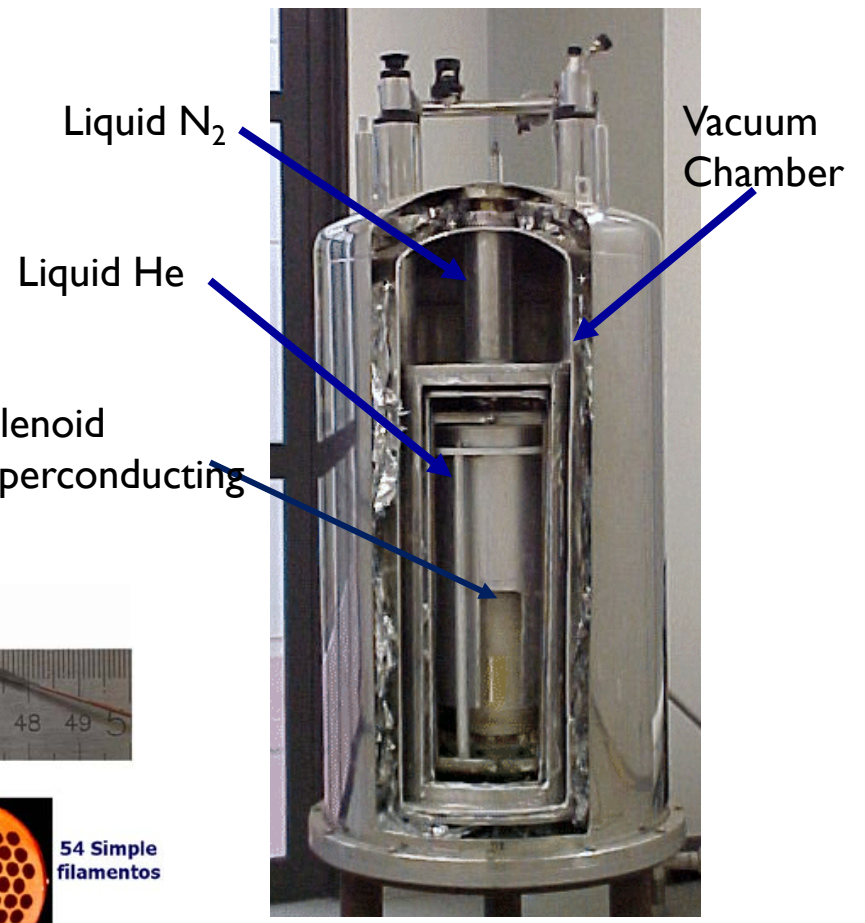
Superconducting Magnet

liquid helium temperature ($4\text{K} \approx -269\text{ }^\circ\text{C}$),
liquid N_2 ($75\text{K} \approx -198\text{ }^\circ\text{C}$)

- Solenoid coil from superconducting
- Nb-Ti Wires
- Nb_3Sn y Nb-Ti Wires
- $(\text{NbTa})_3\text{Sn}$ Wires (magnets at 2°K)

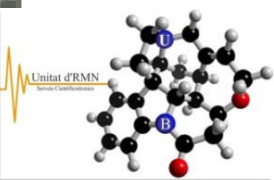
Use up to 300 Km of wire!

Magnet

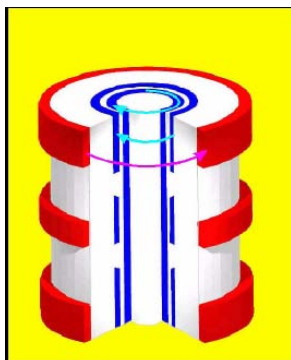


©Bruker



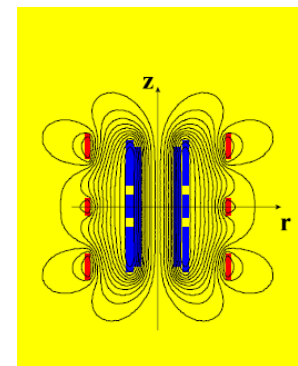


Shielded Magnets



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

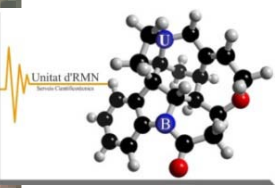
3 Mercury-400 MHz NMR

1 VNMRS-500 MHz

1 Bruker Avance III 600 MHz

1 Bruker Avance III 400 MHz





The Stray field in the NMR spectrometers

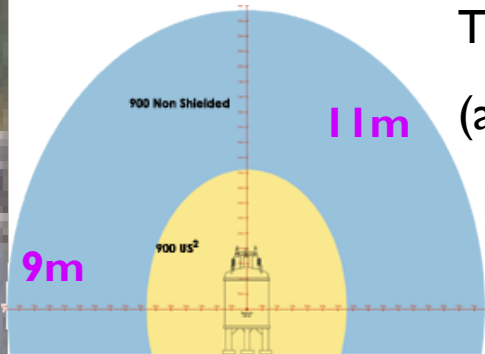
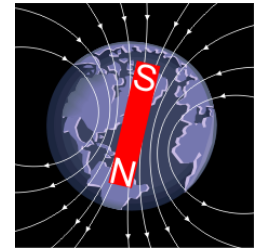
NMR magnetic field: Tesla or specifying the ^1H Larmor frequency for the magnet.

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

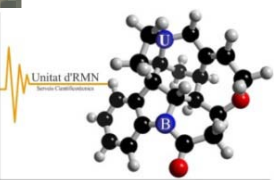


The 5 gauss Level

Magnets in NMR Facility

Field (Teslas)	^1H frequency (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

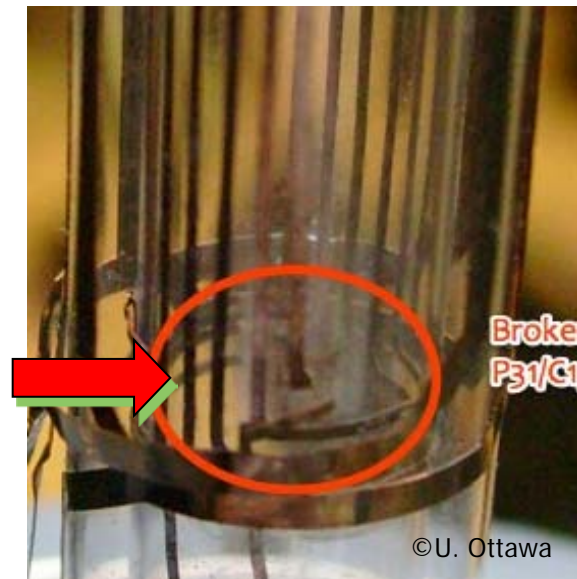


Tuning the probe to optimize:

- RF pulses
- Detecting NMR signal

The probe can be affected by:

- Quality NMR tube
- product outer the tube
- Dirty tubes

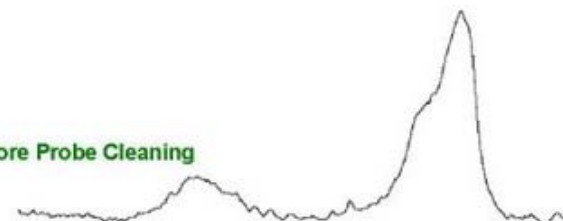


¹H NMR Spectra of an Empty 5 mm Tube in an NMR Probe Before and After the Probe Was Cleaned

After Probe Cleaning



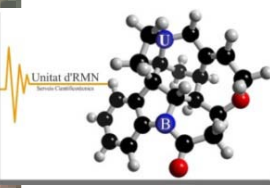
Before Probe Cleaning



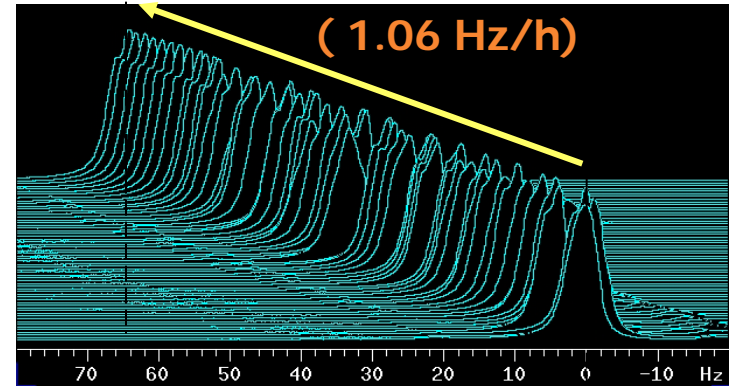
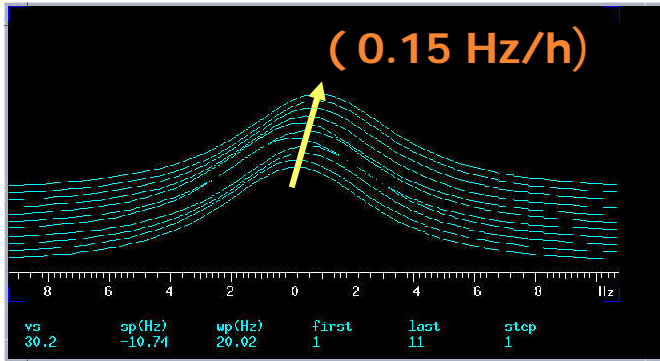
Probe repair ≈ 10.000 \$ (3 months)

New probe ≈ 60.000 \$





Lock System, Field Drift

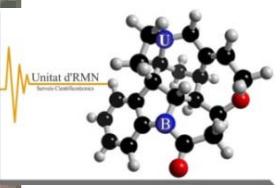


NMR magnetic field slowly drifts with time

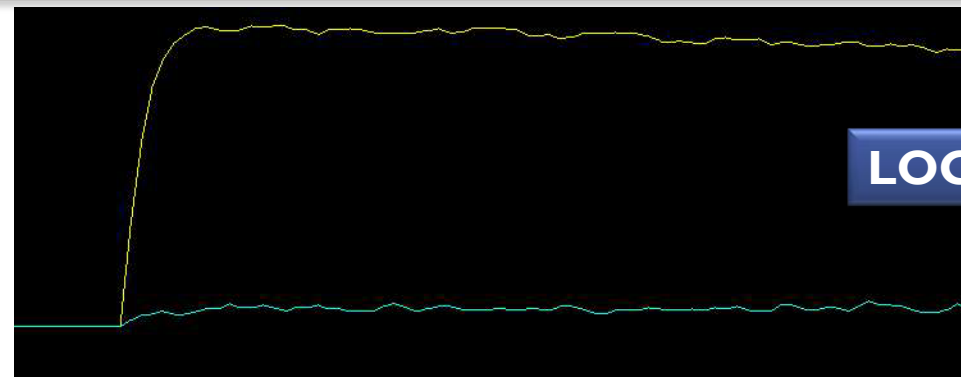
The field drift can be affect to the NMR Signals

- 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



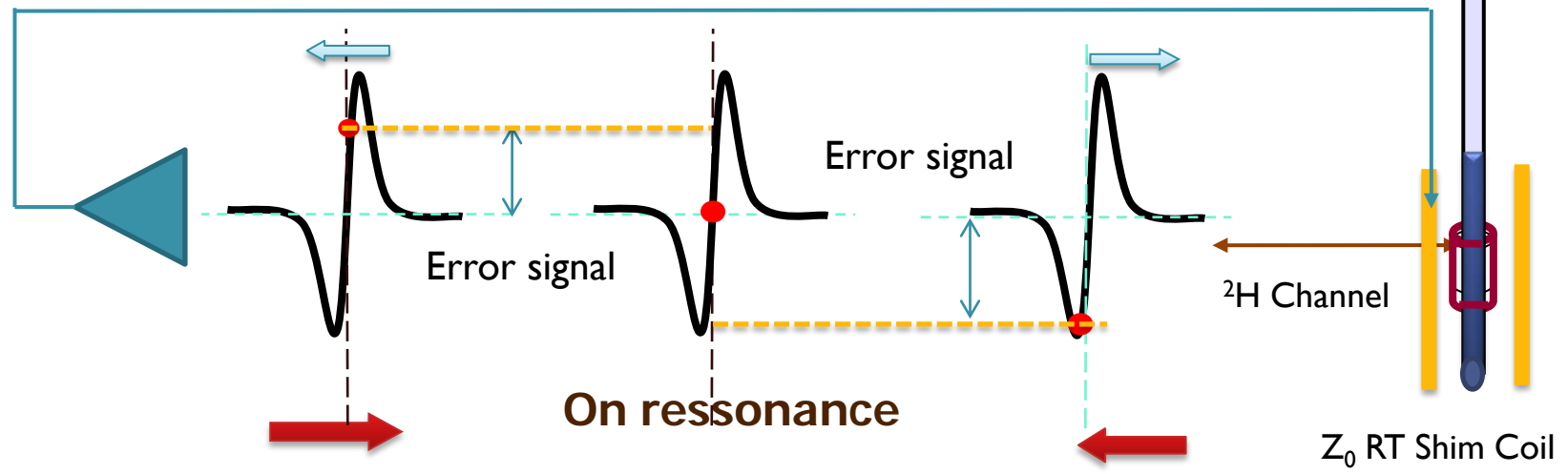


The feed back del lock System



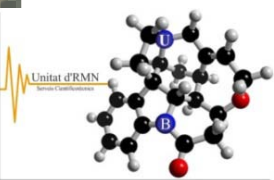
LOCK on-resonance

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

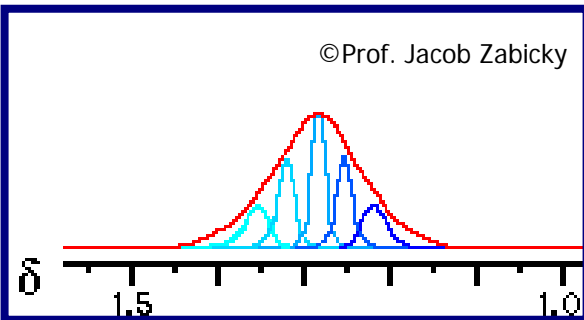
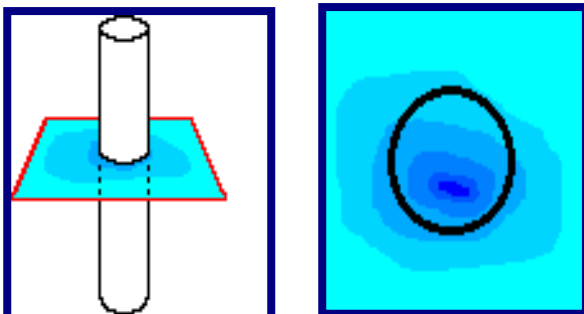


With a shim coil (Z_0) creates a small magnetic field to moved the main field to place and the lock-signal back into resonance





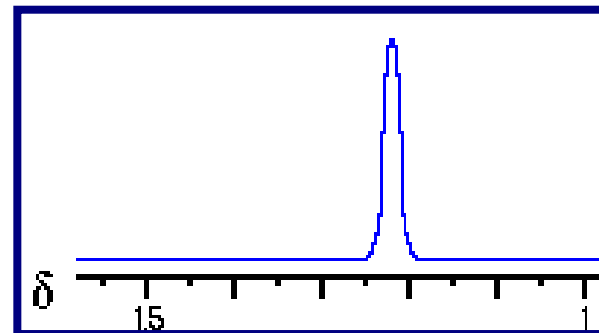
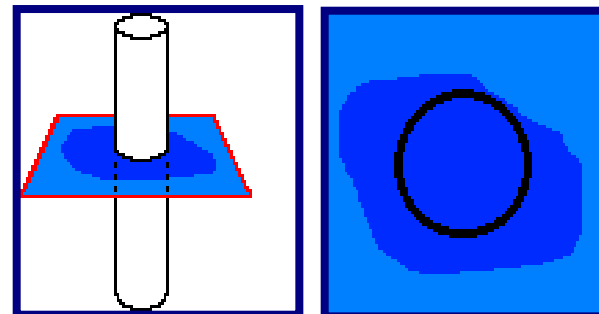
Homogeneity in the magnetic field



If the magnetic field is heterogeneous across the sample

broader NMR signals

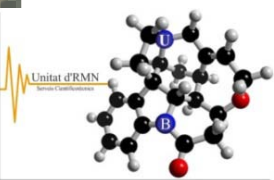
The same proton experience different B_0 magnetic field



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

narrow NMR signals

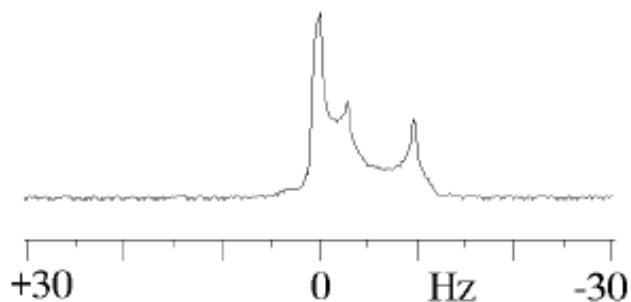




Magnetic Field Homogeneity

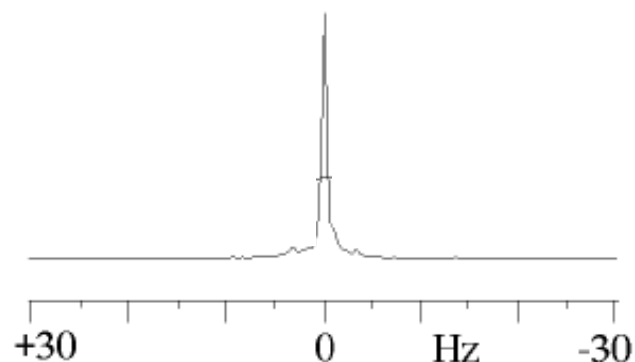
Frequency of absorption:

$$\nu = \gamma B_0 / 2\pi$$



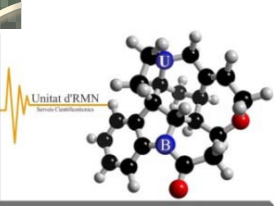
Poor Homogeneity → multiple peaks at different effective B_0

Resonance depends on position in NMR sample



Good Homogeneity → single peak with frequency dependent on B_0





Adjust the homogeneity : Shim Coils

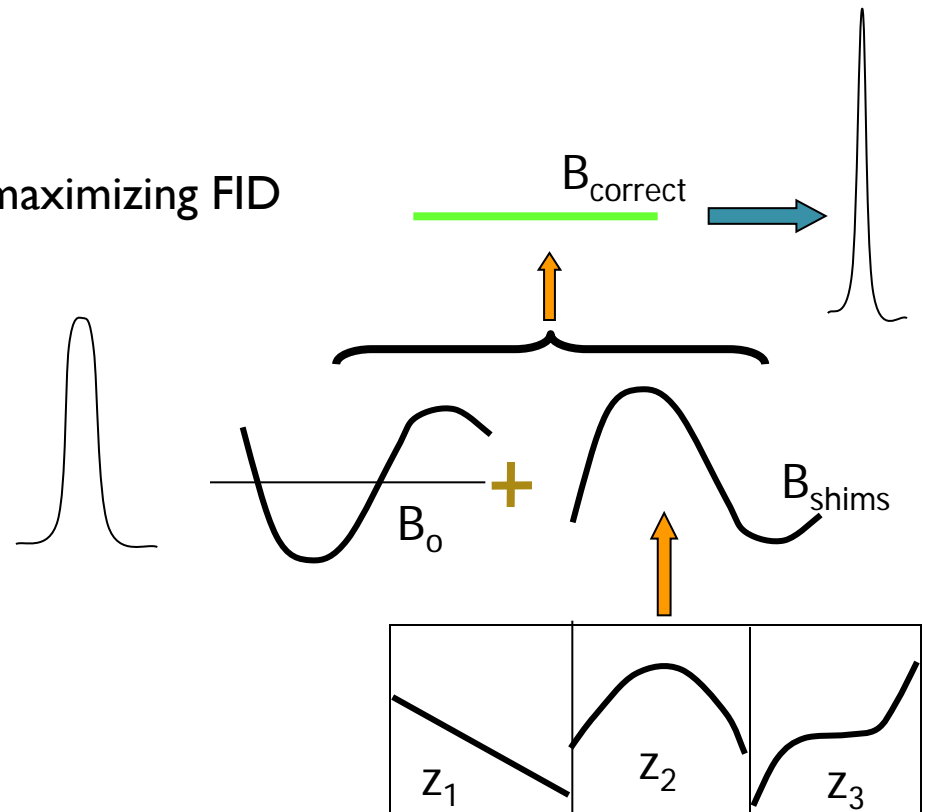
The electric currents in the shim coils create small magnetic fields which compensate the inhomogeneities in the magnet

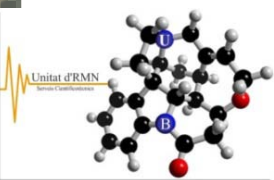
The coils have different geometric orientation and function

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

Optimize shims by:

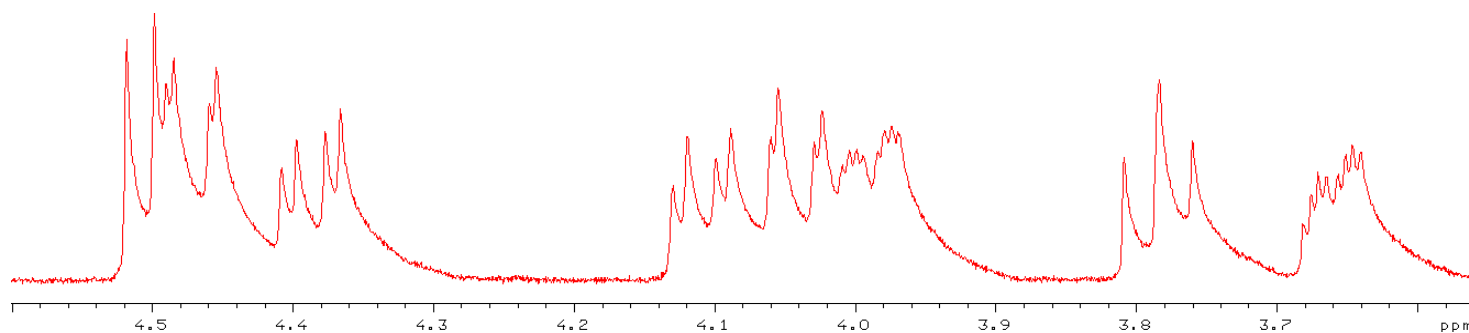
- minimizing line-width
- maximizing lock signal or maximizing FID





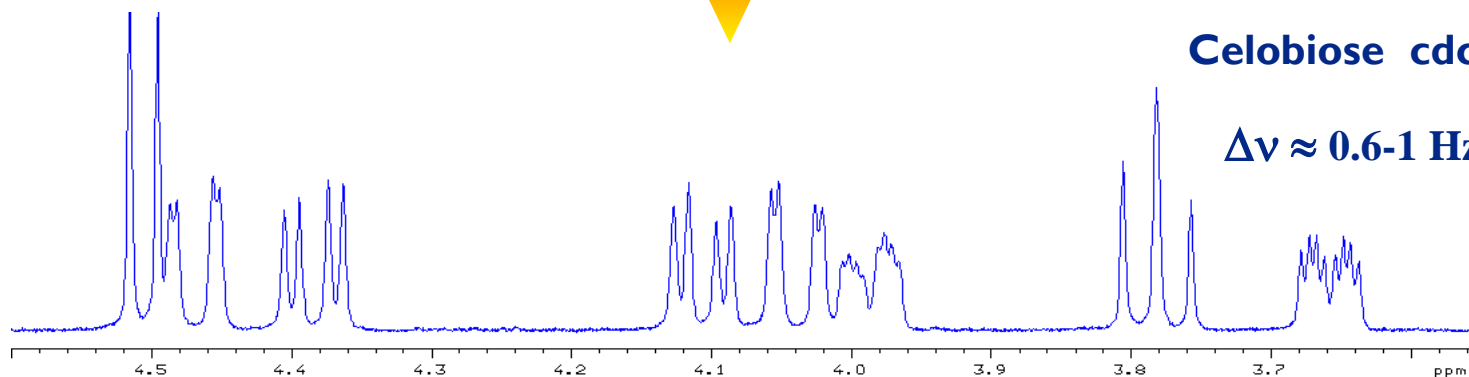
Gradient Shimming

Mercury and VNMR5 Gradient map (^2H or ^1H)



N° iterations (10 max)

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



Celobiose cdCl_3

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

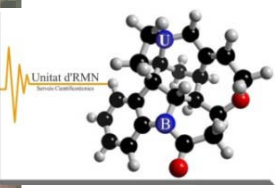
Line widths near of 1 Hz



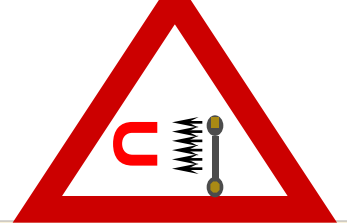


Safety

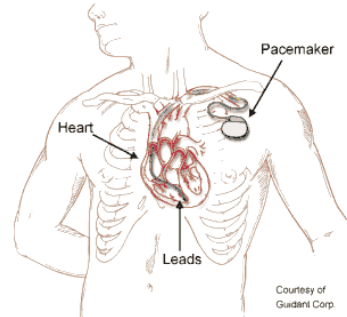




Magnetic forces



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



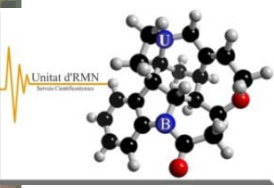
CAMPS MAGNÈTICS MOLT INTENSOS

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

CAMPOS MAGNETICOS MUY INTENSOS

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





RESTRICTED ACCESS AREA



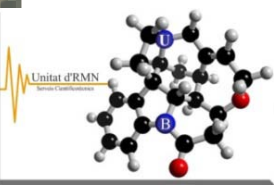
Only allowed to:

- 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 superconductivity in the magnet's main coil that produces a rapid evaporation helium liquid to gas

Quench Video-

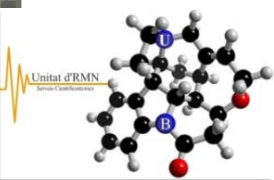


Don't

- 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

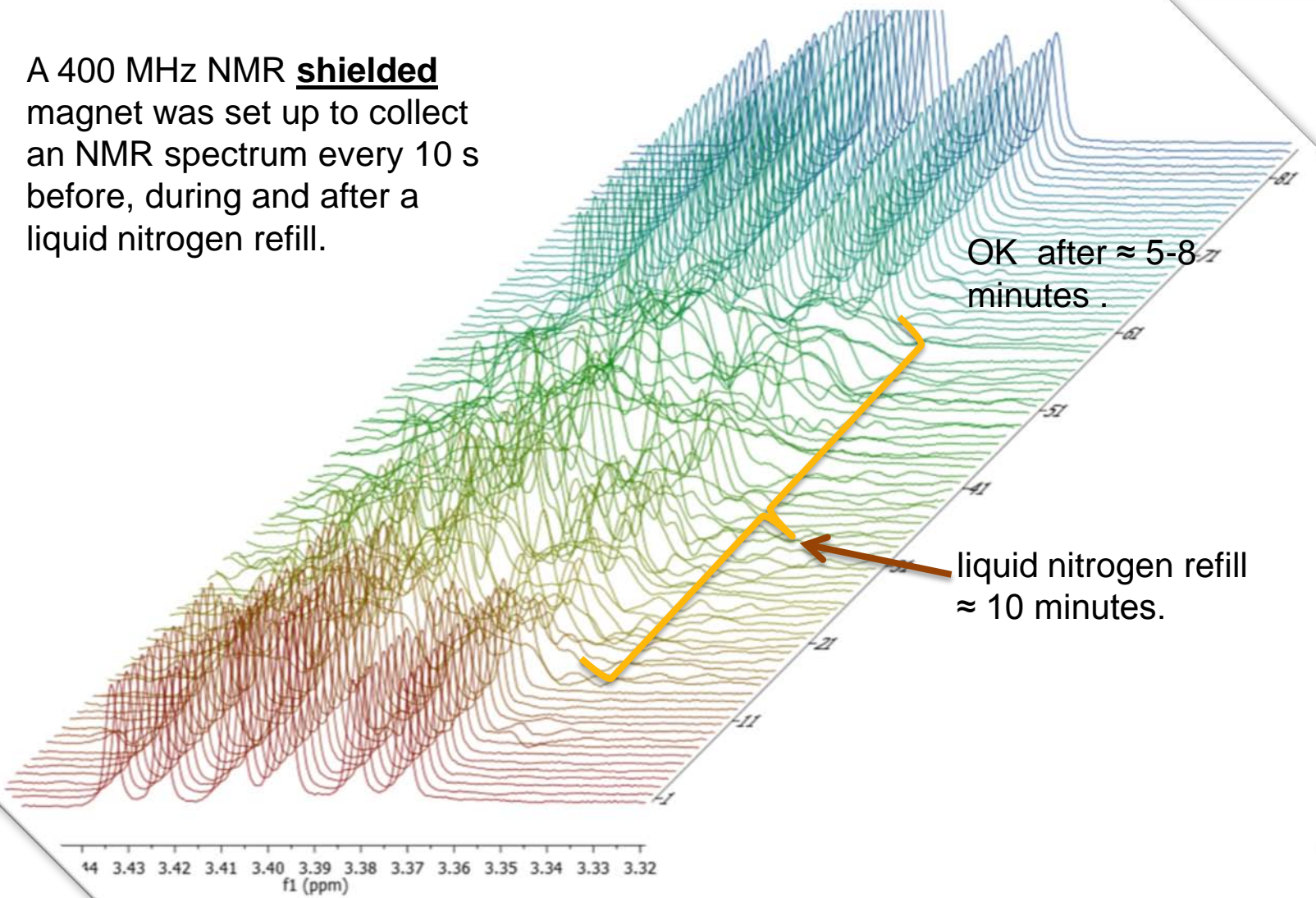
- Refill criogenic liq
 - **N₂ y He liq**
- Checking the NMR performance
- Calibrations
- Preventive Maintenance
- Updates and improvements

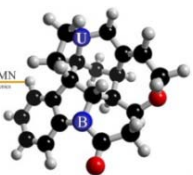




Resolution in the Cryogenic's Refill

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.

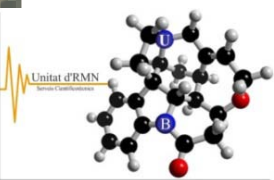




NMR Facility Rules

- 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





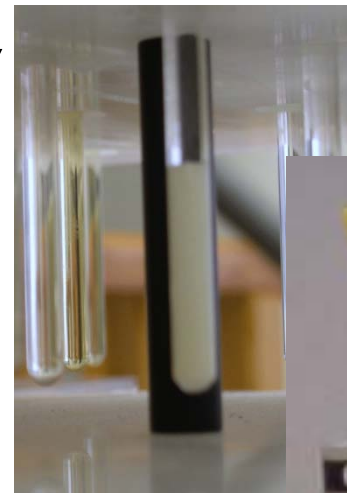
Sample Preparation





The Sample

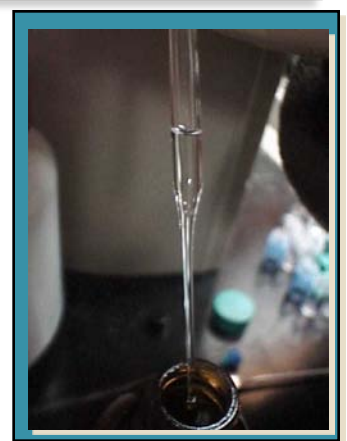
- Adjust the sample concentration to the solubility
Avoid product precipitation
- Use a single deuterated solvent
Reference for lock
- Avoid heterogeneous samples.
Avoid air bubbles, suspended particles, sample separation
- The low quality NMR tubes → distorts magnetic field homogeneity
Breaks easily → damage the NMR probe
- Adjust the Properly position NMR sample in the magnet with the NMR gauge



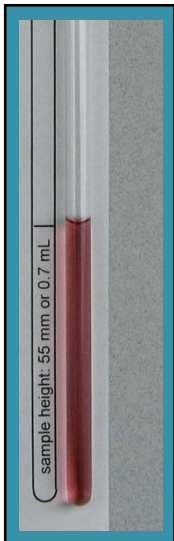


NMR Sample Preparation

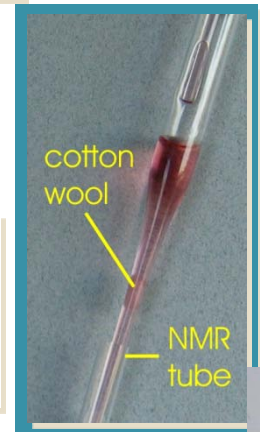
For ^1H Use about 5-25 mg of product



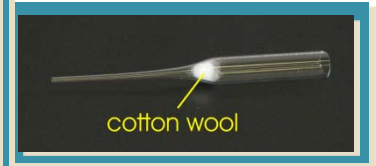
1-0.75 ml deuterated solvent



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



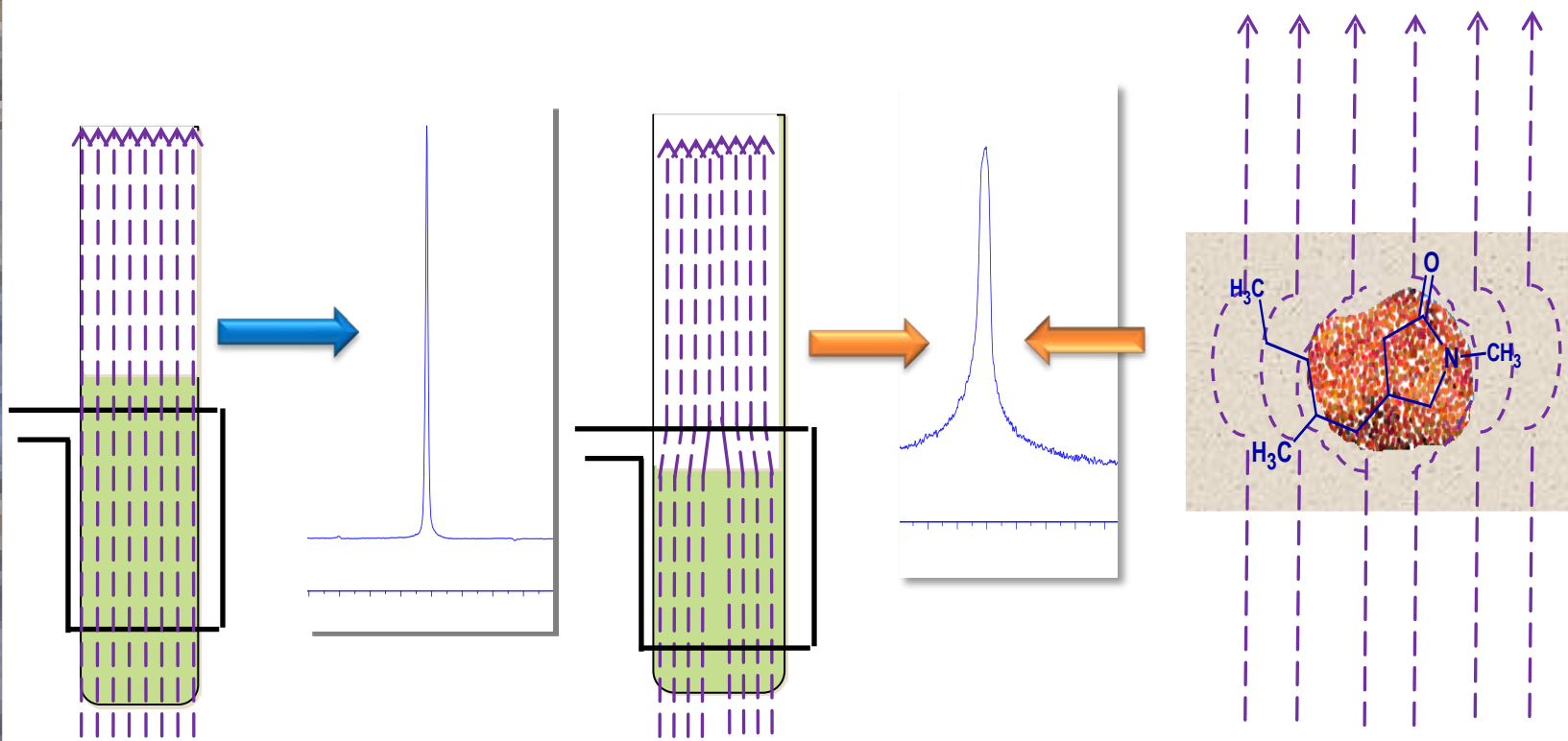
label your sample





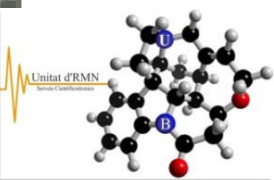
Effects due to incorrect sample preparation

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

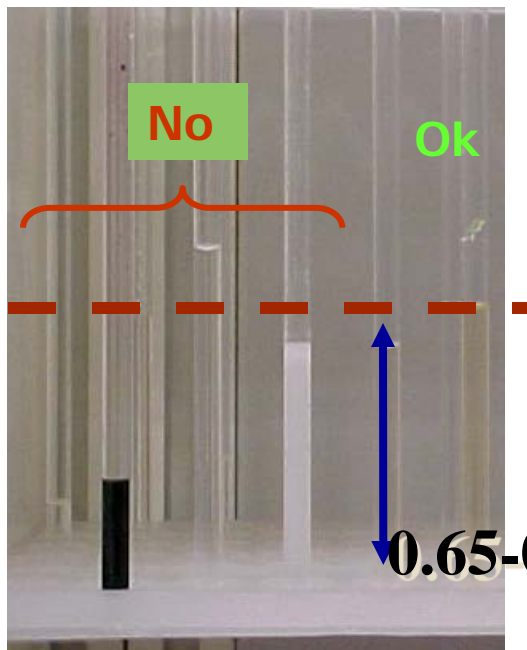




Before placing the sample in the autosampler

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?



0.65-0.75 ml

No

> 2 ml





Tubos de RMN encontrados mal preparados

Volumen correcto
0.7-0.9 ml

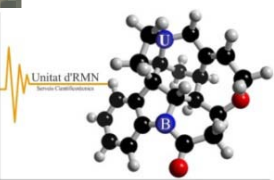
Tubo correcto

Tubos
excesivamente
cortos (ponen en
riesgo la sonda)

Volumen excesivo

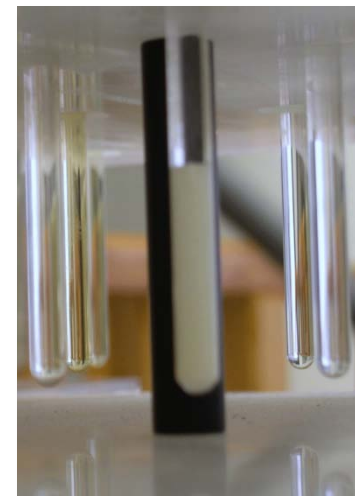
Tubos con muy poco
volumen (perdida de
homogeneidad)





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.

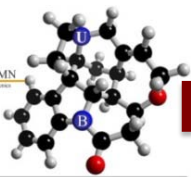


Placed at PCB,
Chemistry and
Pharmacy



Placed only at
Pharmacy





Unitat d'RMN

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 <math><2-3\text{€}</math> a piece to >math>50\text{€}</math>. 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>

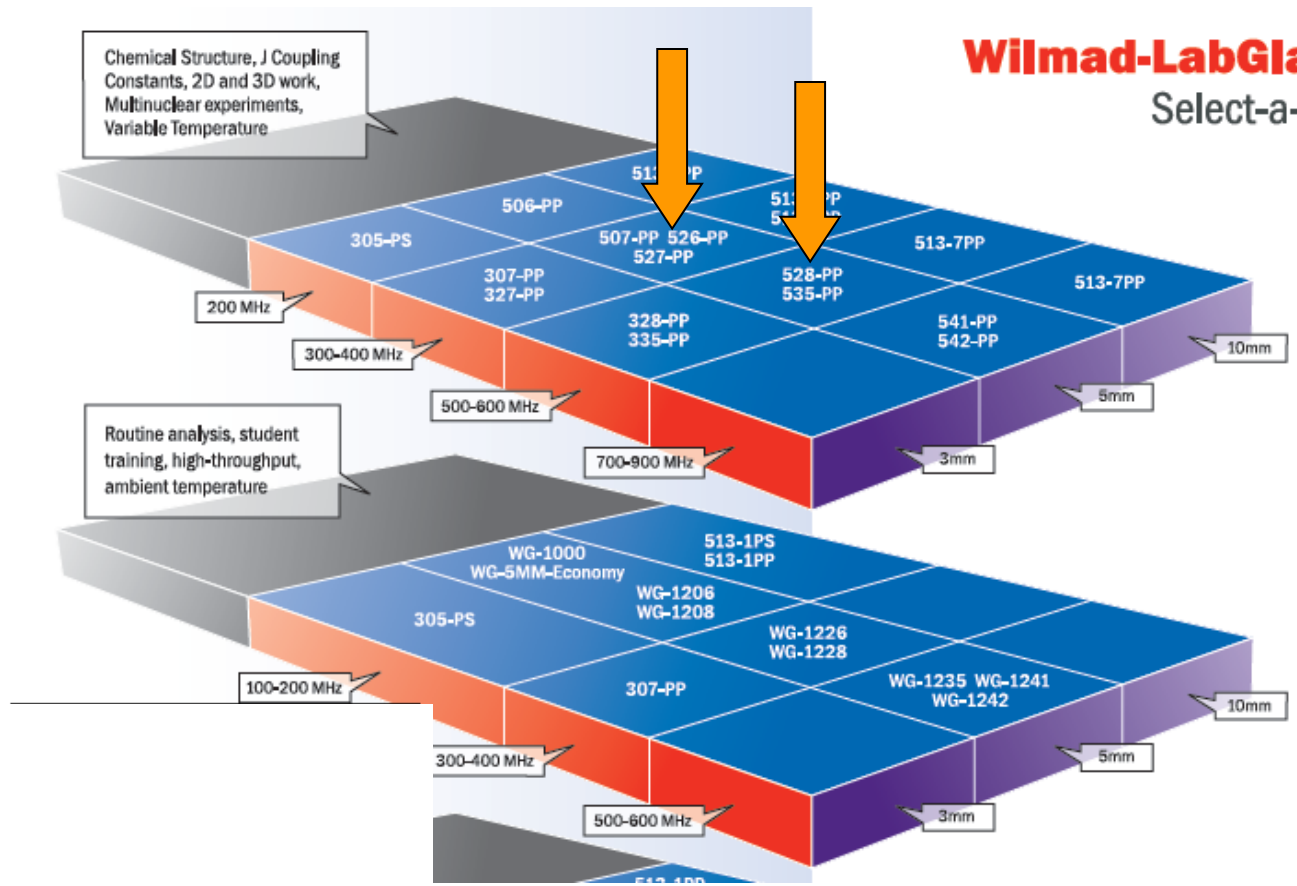
<http://www.newera-spectro.com/>

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





NMR sample Tubes



Wilmad-LabGlass NMR
Select-a-Tube Guide



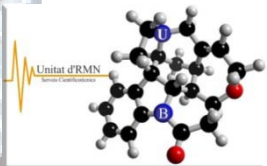


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NMR Training part II 2018





Operating Modes



9 samples

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



50 Samples (3) or 9 samples (1)

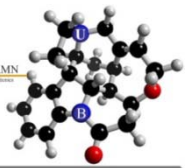
- Autosampler
- Preset conditions
- Easy and flexible use
- Operation in continuous mode

• *24h a day every day of the year*



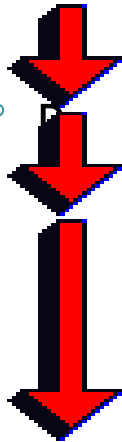
50 samples





Operative system

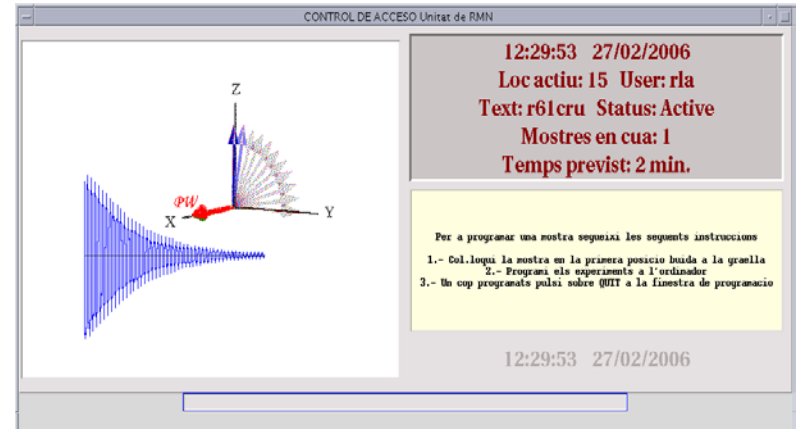
- Two systems: **the same way**
VNMR 6.IC (old System G300)

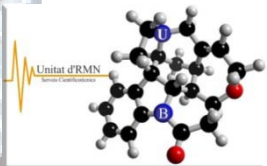


Evolution to Java

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

**B400 MHz NMR
Fac. Chemistry**



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



**500 MHz NMR
spectrometers
PCB**





Detection & Sensitivity

Signal-to-noise of an NMR measurement depends:

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

$$\gamma \text{ } ^1\text{H} = 26,753 \text{ rad/G}$$

$$\gamma \text{ } ^{13}\text{C} = 6,728 \text{ rad/G}$$

$$\left. \begin{array}{l} \gamma \text{ } ^1\text{H} = 26,753 \text{ rad/G} \\ \gamma \text{ } ^{13}\text{C} = 6,728 \text{ rad/G} \end{array} \right\} \text{Ratio } (\gamma \text{ } ^1\text{H} / \gamma \text{ } ^{13}\text{C})^3 \approx 64$$

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

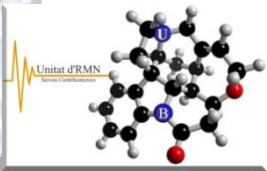
^1H is 6400 times more sensible than ^{13}C

$\gamma \text{ } ^{15}\text{N} -2,71 \text{ rad/G}$, $A \approx 0,27\%$ ^1H is $\approx 27 \cdot 10^5$ 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

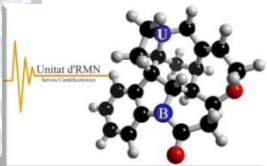
- 4 x 400 MHz spectrometers shielded magnet , Gradient in Z
- 2 x ATB probe (1H/19F/13C) Mercury 400 (PCB / Chemistry),
1 x Optimized for 1H/19F y 13C high tolerance salt and solvent changes (Pharmacy)
- 1 x 1H/19F/13C/31P probe VNMRS400 (Pharmacy)

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

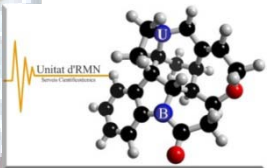




Some useful information should be required before programming the NMR experiments

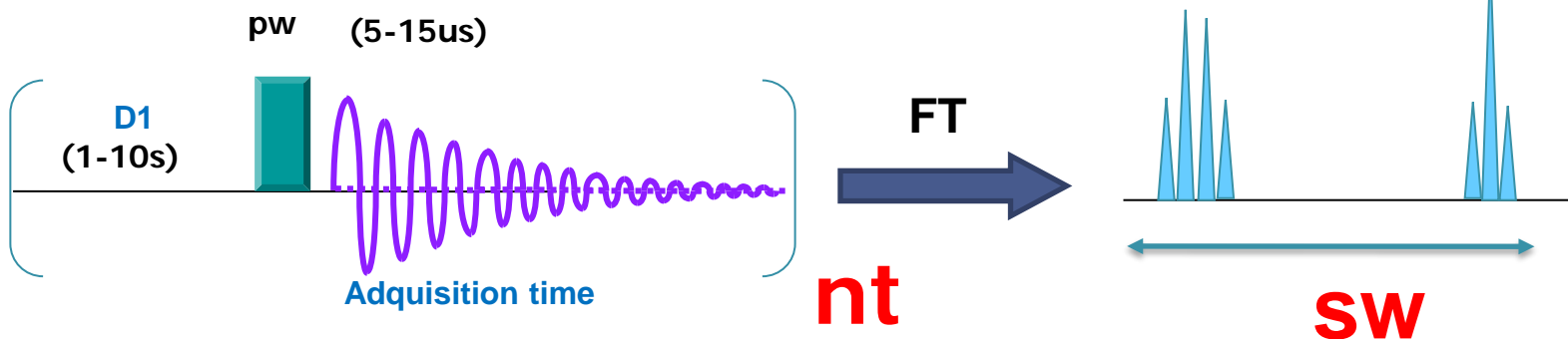
- Chemical characteristics of the compound
 - Molecular weight
 - Functional groups
 - Symmetry
- Solubility in mg/ml ?
- The product contains a transition metal ? (e.g. organometallic compound such as Pt, Pd)
- Is a mixture or a single compound ?
- The spectrum is needed for
 - Check the reaction ??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

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

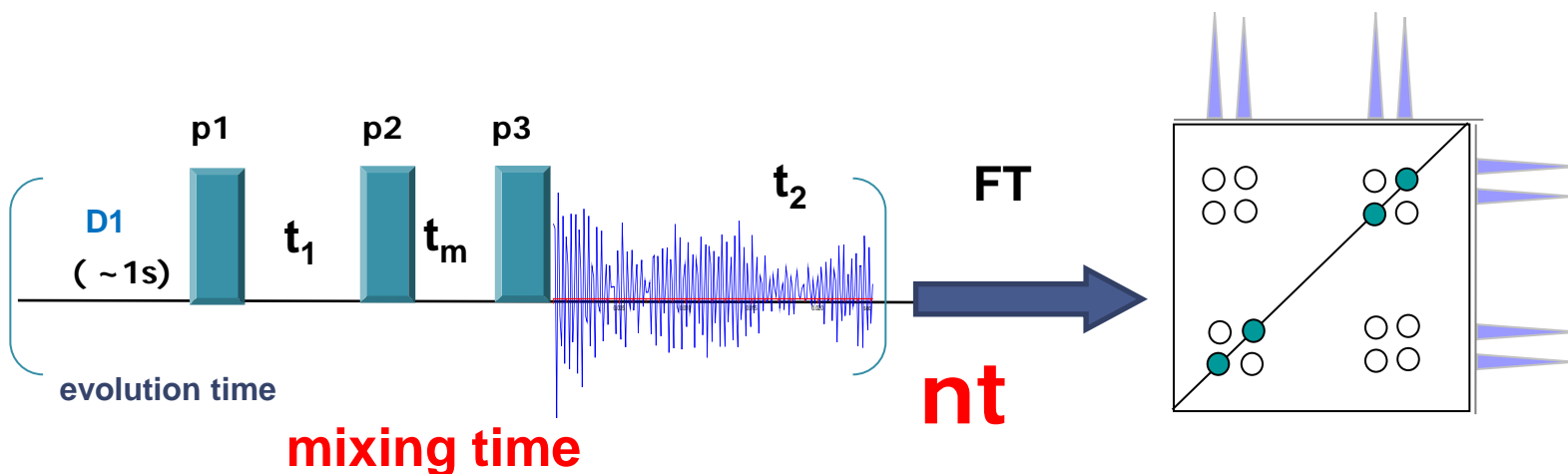


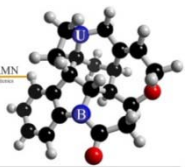
Data acquisition parameters: "the key"

- Basic 1D NMR sequence



- 2D .NMR sequence (NOESY)



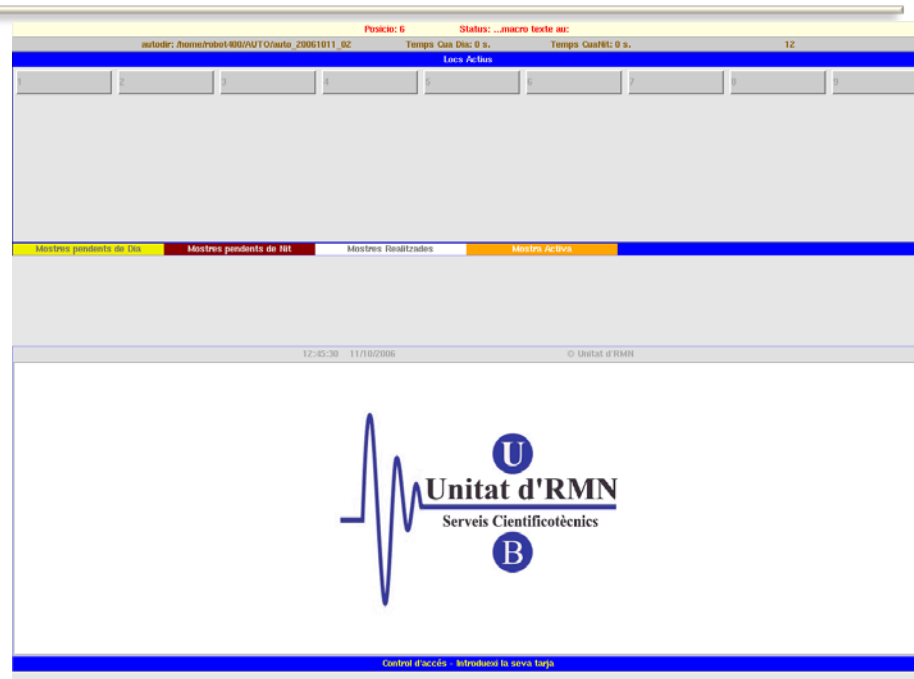


Automatic System: User Interface

Access 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

The screenshot displays the Bruker Automation Interface software. The main window is titled "Experiment Panel" and shows a grid of experiment types: PROTON, CARBON, PRESAT, wet1D, (1H) gCOSY, (1H) gHSQC, (1H) gHMBC, and (1H) NOESY1D. An arrow points from the word "Experiments" to the "PRESAT" button. Below the grid is a "Study Queue" showing "edulc-Unic (Location: 49 - to be su)".

The "Acquisition Parameters" section at the bottom right includes:

- Operator: robot400
- Sample Name: edulc-Unic
- SampleDir: (edulc-Unic_20120906_01)
- Registre: 000
- Codi Usuari: servei
- Eaddr: miguel@rmn.ub.es
- Usuari: MIGUEL FELIZ RODENAS
- Solvent: CDCl3
- Comment: Submitq / Mercury-400 cdc13 / Temp: 25C / N.Reg: 000 Usuari: servei / Mostra: edulc-Unic Nom: MIGUEL FELIZ RODENAS
- Sample Preparation: Equilibrate for: 0.5 sec, Run Sample at: 25.0 C
- AutoRun: auto_20120906_01
- Current Status: Update
- Available Location: 49
- Next Submission starts... PriorityQ: 02:47 PM, DayQ: 02:47 PM, NightQ: 11:07 PM
- Select shimmap: 2H gradient (read probe file at runtime)
- Study time: Day -

The status bar at the bottom shows "Temp 25.0 C", "Spin 19 Hz", "Lock 95.4", "Sample 48", and "Probes ATB".

Experiments

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

Start Acquire Process **ClearSampleInfo** **NewSample**

standard

Operator : robot400 **QUIT SESSION**

Sample Name: mentol

SampleDir: mentol

Registre: miguel

Codi Usuari: servei Eaddr miguel@rmn.ub.e

Usuari FELIZ_RODENAS_MIGUEL FELIZ

Solvent **CDCl3**

Comment

H1 / Mercury-400
cdcl3 / Temp: 25C / N.Reg: miguel
Usuari: servei / Mostra: mentol
Nom: MIGUEL FELIZ RODENAS
Data: 26/09/11 / Ope.: M.FELIZ

Sample Preparation

Equilibrate for: 0.5 sec

Run Sample at: 25.0 C

After EXP Autoplot

plotter lj1_pcb [b+w]

After Queue e-message

Before 1st EXP (day/night)

Lock? Yes (alock<>n)

Shim Tune

Select shimmap: 2H gradient (read probe file at runtime)

Study time: Day - 2 min, 24 sec

AutoRun:
auto_20110926_02

Current Status: Update

Available Location: 30
Next Submission starts...
PriorityQ : 03:38 PM
DayQ : 03:43 PM
NightQ : 06:00 PM

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 NightQ**
- Click the buttons for the desired experiments (PROTON, CARBON)
- **Customizing each Experiments** you selected

Vnmrj

QUIT SESSION...

Sample#: 26 (1 study)

Studyid: 1oc26
Operator: robot400
Samplename: PMG-237ac
Solvent: d2o
Notebook:
Page:
Date: Sep 26, 2011 14:27:54
Status: Complete

1 2 3 4 5 6
7 8 9 10 11 12 13
14 15 16 17 18 19
20 21 22 23 24 25 26
27 28 29 30 31 32
33 34 35 36 37 38 39
40 41 42 43 44 45
46 47 48 49 50

Start Acquire Process

cpEXP1

Samplename: mentol Solvent: cdcl3

Std1D PROTON PRESAT wet1D
CARBON DEPT FLUORINE

Homo2D gCOSY gDQCOSY TOCSY
NOESY ROESY

Hetero2D gHSQCAD gHMBCAD

Submit next selection to:
 Short/Day Q Long/Night Q

PrepTime (3 min, 0 sec)

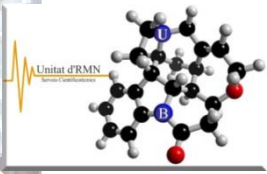
EXPLIST:
PROTON_001 (24 sec)
CARBON_001 (8 min, 40 sec)
DEPT_001 (3 min, 4 sec)
FLUORINE_001 (26 sec)
gCOSY_001 (3 min, 12 sec)
Proc/Plot (10 min, 0 sec)
TOTAL (25 min, 46 sec)

Temp 25.0 C Spin 19 Hz Lock 87.7 Sample 26

Probe ATB Idle

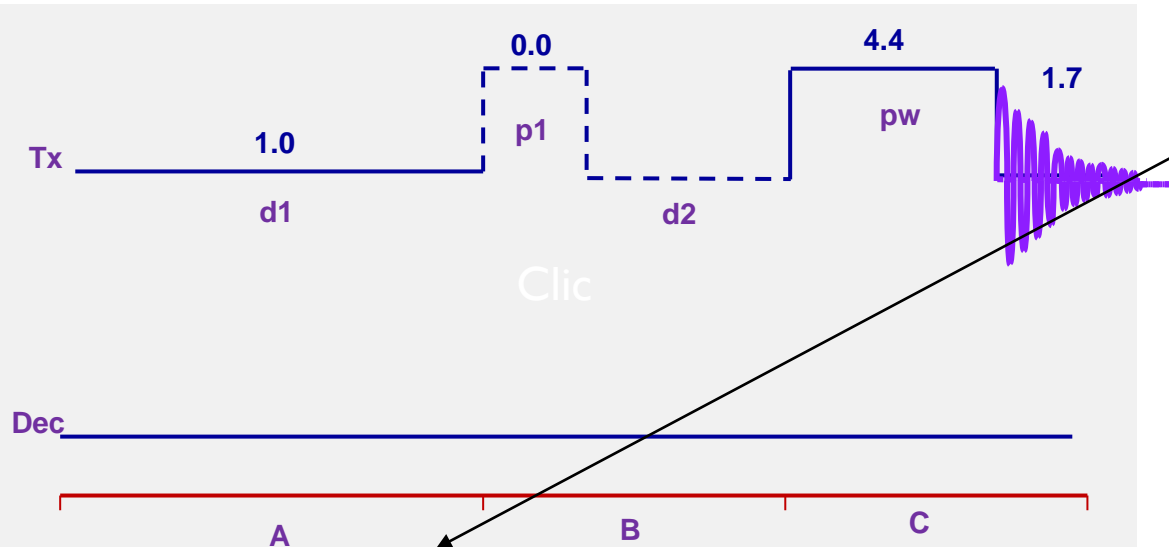
Adding FLUORINE to queue





Third step customize each experiment

for example, modifying a PROTON acquisition parameters



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

Start Acquire Process Show Time Save Quit-nosave Default Acquire Sequence Arrays Seq. Help

Default H1
ProcPlotAdv
Acquisition
Pulse Sequence
Channels
Flags
Future Actions
Overview

Experiment: PROTON Solvent: cdc13 Observe: H1 Decoupler: C13

Acquisition Options

Spectral Width (select): [] ppm
(...or enter): -2.0 to 14.0 ppm

Number of scans: 8
Relaxation Delay: 1 s
Pulse Angle: 45 degrees
Minimize SW: Auto
Calibrate pw90:

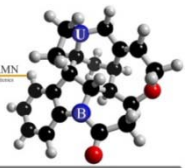
Receiver Gain (dB): 30
 Autogain
Before PROTON acq: Re-shim
After PROTON acq: Autoplot
Starting with:
Samplename: mentol
start of Q lock/shim? yes / yes
More Options: PlotProcAdv page

Select spectral window

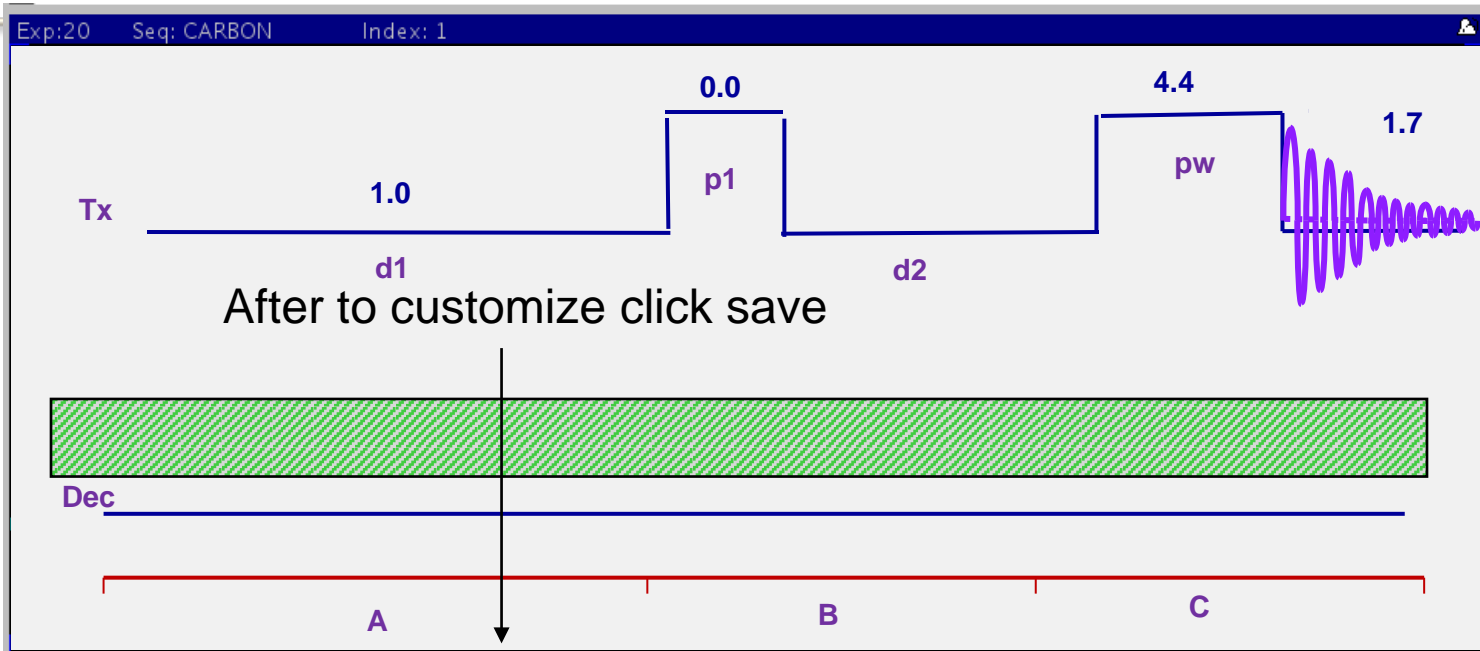
Minimize SW is very important in 2D exp
Auto

Select Scans 4 to 1024 (time limit)





¹³C Customizing parameters



¹³C

Start Acquire Process **Show Time** **Save** Quit-nosave Default Acquire Sequence Arrays Seq. Help

Default C13

Experiment: CARBON Solvent: cdcl3 Observe: C13 Decoupler: H1

Acquisition Options

Spectral Width (select): [] ppm
 (...or enter): -17.5 to: 237.5 ppm

Number of scans: 5000

Relaxation Delay: 1 sec

Pulse Angle: 45 degrees

H1 dec. mode: Decoupled + NOE

Check S/N? yes no

between: 160.0 and 100.0 ppm
 at every 256 scans & Stop at: 100

Receiver Gain (dB): 30
 Autogain:

Before CARBON acq: Re-shim
 After CARBON acq: Autoplot

Starting with:

Samplename:

start of Q lock/shim? yes / yes

More Options: PlotProcAdv page

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

Start Acquire Process Show Time Save Quit-nosave bk2default Acquire Sequence RefreshPanel

Default F19 Experiment: FLUORINE Solvent: cdcl3 Observe: F19 Decoupler: H1

Acquisition Options

Spectral width - From: -200.0 to: 30.0
Number of scans: 16
Relaxation Delay: 1 sec
Pulse Angle: 45 degrees

Receiver Gain (dB): 30
Autogain:
Before FLUORINE acq Re-shim
After FLUORINE acq Autoplot
Starting with:
Sampname: pppp
start of Q lock/shim? yes / yes
More Options: PlotProcAdv page

Available in all 400 MHz Spectrometers

After customizing click save

Start Acquire Process Show Time Save Quit-nosave bk2default Acquire Sequence RefreshPanel

Default P31 Experiment: PHOSPHORUS Solvent: cdcl3 Observe: P31 Decoupler: H1

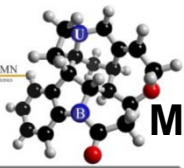
Acquisition Options

Spectral width - From: -53.7 to: 203.7
Number of scans: 64
Relaxation Delay: 1 sec
Pulse Angle: 45 degrees
H1 dec. mode: Decoupled - NOE

Receiver Gain (dB): 50
Autogain:
PHOSPHORUS acq Re-shim
PHOSPHORUS acq : Autoplot
Starting with:
Sampname:
start of Q lock/shim? yes / yes
More Options: PlotProcAdv page

Whitout Noe
Other Options

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

Study Queue

- new Sample
 - SampleInfo [Day:25:46]
 - PROTON_001_day [0:24]
 - CARBON_001_day [8:40]
 - DEPT_001_day [3:04]
 - FLUORINE_001_day [0:26]
 - gCOSY_001_day [3:12]

View: Submit Queue ***Done**

Add Next Selection to: DayQ NightQ

New Study Priority sample

Submit to Automation

Hide Tray Edit Study from Location

Clear Pending Exn from Queue

Operator : robot400 **QUIT SESSION**

Sample Name: muestra-1 Equilibrate for: 0.5 sec

SampleDir: (muestra-1_20111014_01) Run Sample at: 25.0 C

Registre: 0 After EXP Autoplot

Codi Usuari: servei Eaddr: miguel@rmn.ub.e plotter: j1_pcb [b+w]

Usuari: FELIZ RODENAS, MIGUEL FELIZ RODENAS After Queue e-message

Solvent: CDCI3 Before 1st EXP (day/night)

Lock? Yes (alock<>n)

Shim Tune

Comment

H1 / Mercury-400

cdcI3 / Temp: 25C / N.Reg: o

Usuari: servei / Mostra: muestra-1

Nom: MIGUEL FELIZ RODENAS

AutoRun: auto_20111014_01

Current Status: **Update**

Available Location: 43

Next Submission starts...

PriorityQ: 02:46 PM

DayQ: 02:52 PM

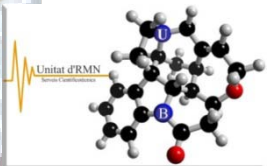
NightQ: 01:11 AM (Sat)

Select shimmap: 2H gradient (read probe file at runtime)

Study time: Day - 2 min, 24 sec

Temp 25.0 C Spin 0 Hz Lock 0.5 Sample 41 Probe ATB

FID: 2 CT: 5 00:00:06 Adding PROTON to queue

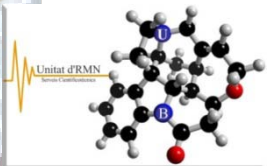


DEPT

The DEPT requires a previous ^{13}C \longrightarrow Adds ^{13}C if not scheduled

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



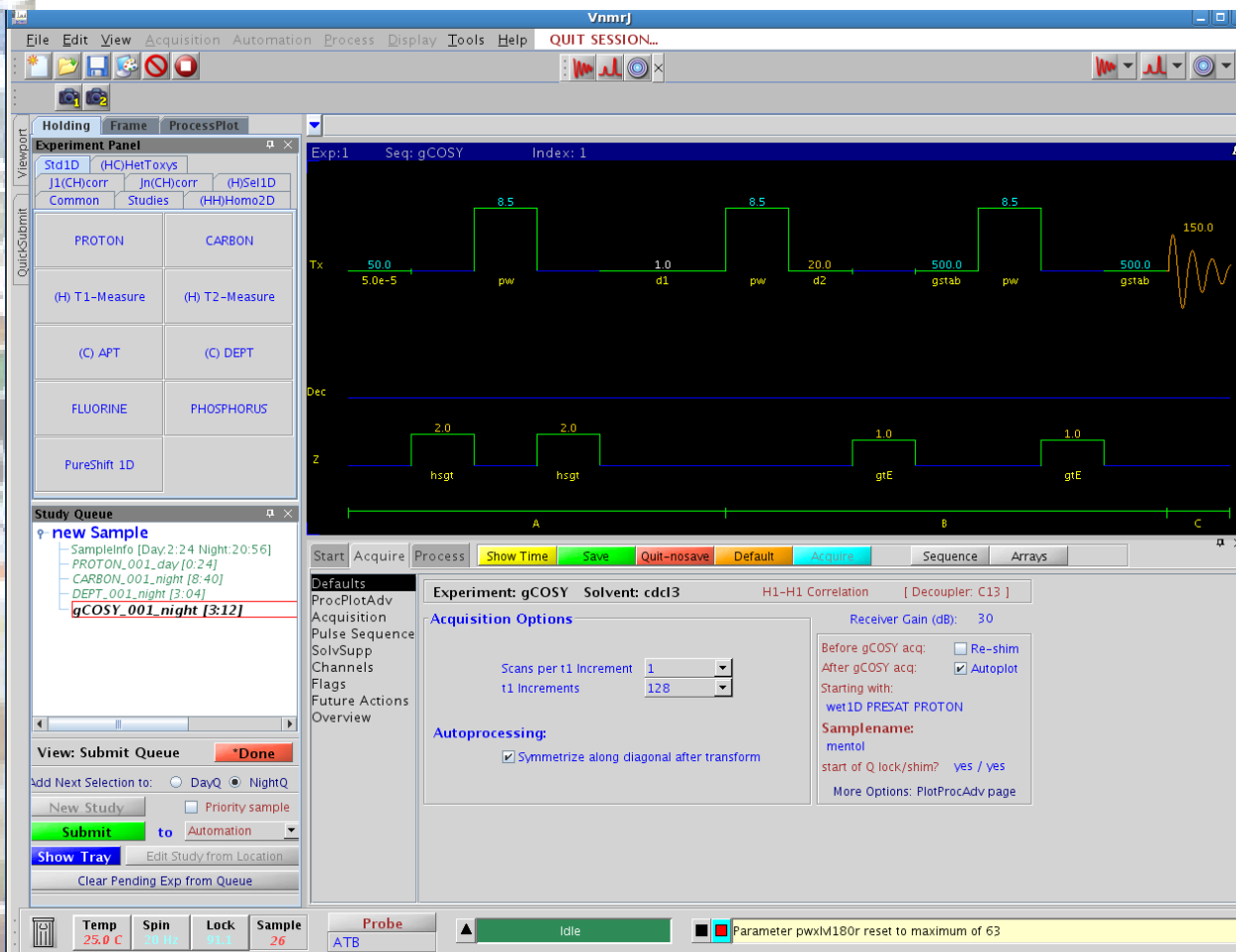
gCOSY 1H-1H correlation experiment

The 2D experiments need 1H spectrum before

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

Very easy to run :

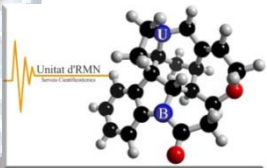
Only *nt* and *ni* need to be adjusted



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

gCOSY

gCOSY (nt=2 ni=256) time ≈ 11 min



gHSQC

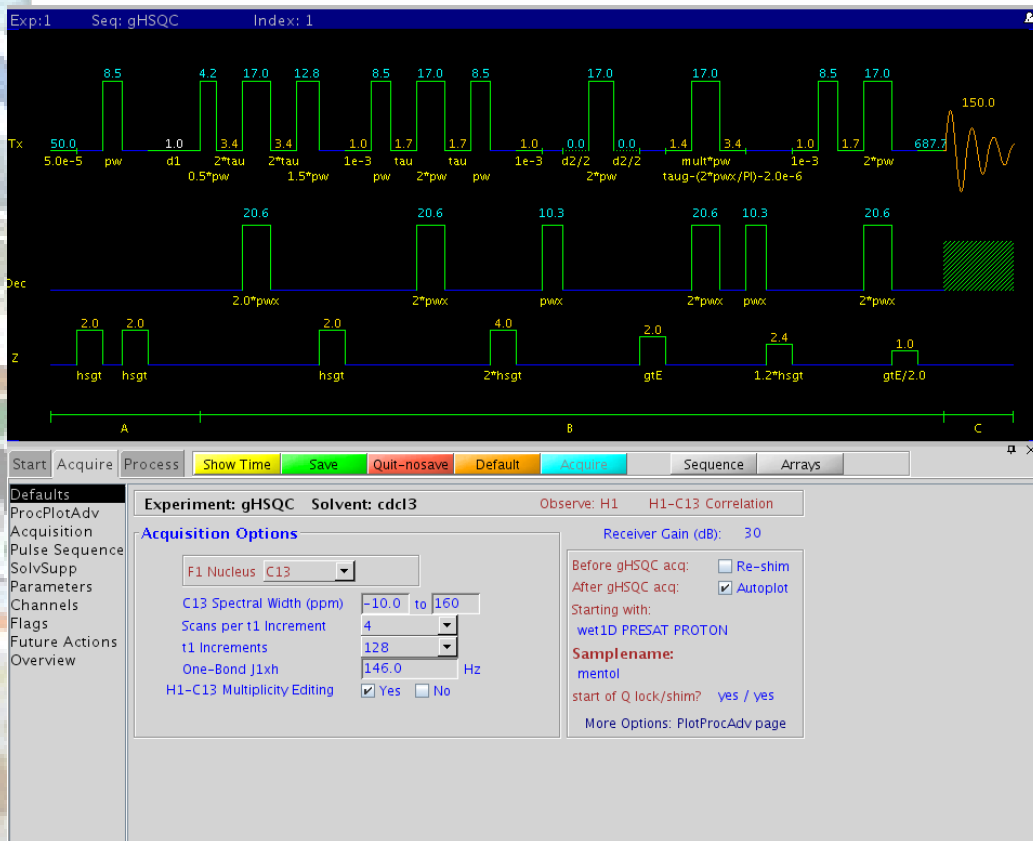
Most important parameter

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 $ni^2 - 4$

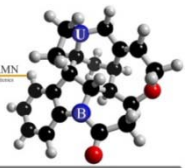
Cross peaks in gHSQC are multiplicity edited by default:
CH and CH3 are **+** and CH2 are **-**
so you get the equivalent information of a DEPT



gHSQC (nt=4 ni=128-256)

Time \approx 20- 60 minutes





TOCSY (Total Correlation Spectroscopy).

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 function:

gaussian

Linear prediction in F1

$n_i^2 - 4$



Tocsy

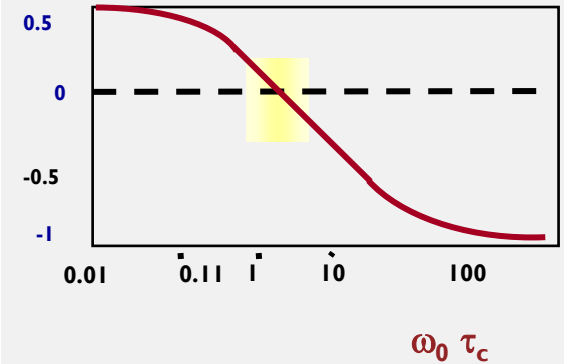
TOCSY (nt=2 ni=256) time \approx 46 min



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.

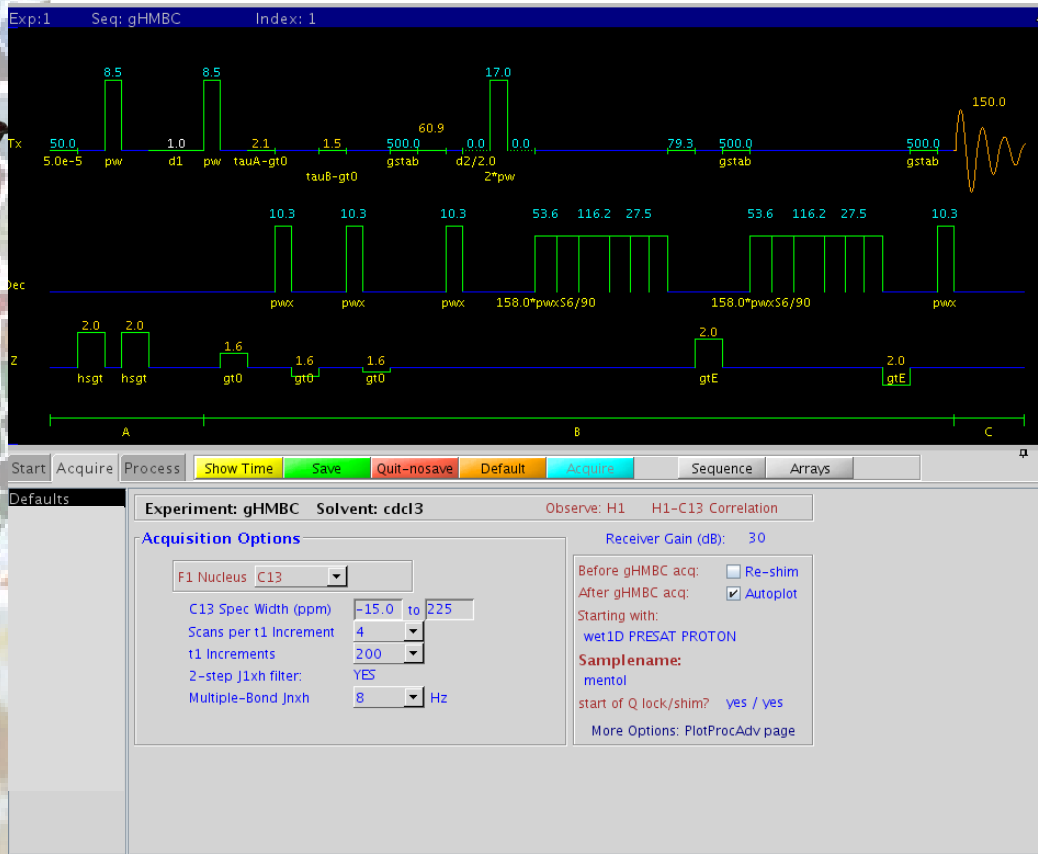
Phase sensitive;
Apodization function:
Gaussian
Linear prediction in F1
 $ni^*2- 4$

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

Noesy



gHMBC



Most important parameter

2-3 Bond $J_{n_xH} \approx 3-8$ 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 (nt=16 ni=256) time \approx 1-1.5h

gHMBC



The last but not least

The screenshot shows the Bruker software interface. The 'Experiment Panel' on the left lists parameters for 'PROTON' and 'CARBON' experiments, including 'T1-Measure', 'T2-Measure', 'APT', 'DEPT', 'FLUORINE', 'PHOSPHORUS', and 'PureShift 1D'. The 'Study Queue' shows a 'new Sample' with 'SampleInfo [Day:2:24]' and 'PROTON_001_day [0:24]'. The 'Sample Preparation' section shows 'Operator: robot400', 'Sample Name: mentol', 'SampleDir: mentol', 'Registre: miguel', 'Codi Usuari: servei', 'Eaddr: miguel@rmn.ub.e', 'Usuari: FELIZ RODENAS_MIGUEL FELIZ', 'Solvent: CDCl3', and 'Comment: H1 / Mercury-400, cdc13 / Temp: 25C / N.Reg: miguel, Usuari: servei / Mostra: mentol, Nom: MIGUEL FELIZ RODENAS, Data: 26/09/11 / Ope.: M.FELIZ'. The 'Study time' is 'Day - 2 min, 24 sec'.

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

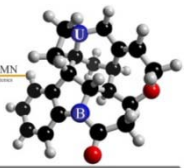
- Status
- Mail
- Info web



Time experiment guidelines

Quinine sample (5mg in 0.8 ml CDCL₃)

	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**

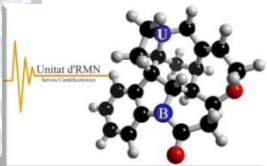


In Night-Queue **DON'T TAKE IT NEVER**



Error it can be
reprogrammed again

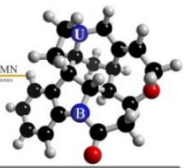




More frequent user failures

- **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**

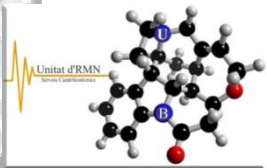
Receiver Overflow

very high concentration of sample

**Splitted or broad
peaks**

**Concentration/ product precipitation /
Poor Homogeneity**





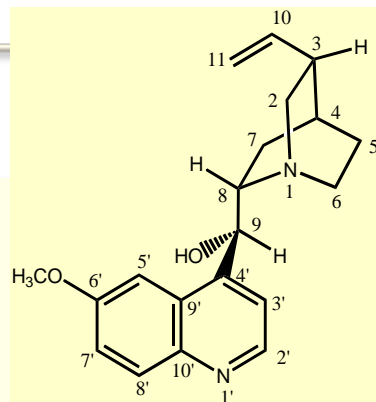
**WHAT KIND OF INFORMATION GIVE US
THE EXPERIMENTS ?**



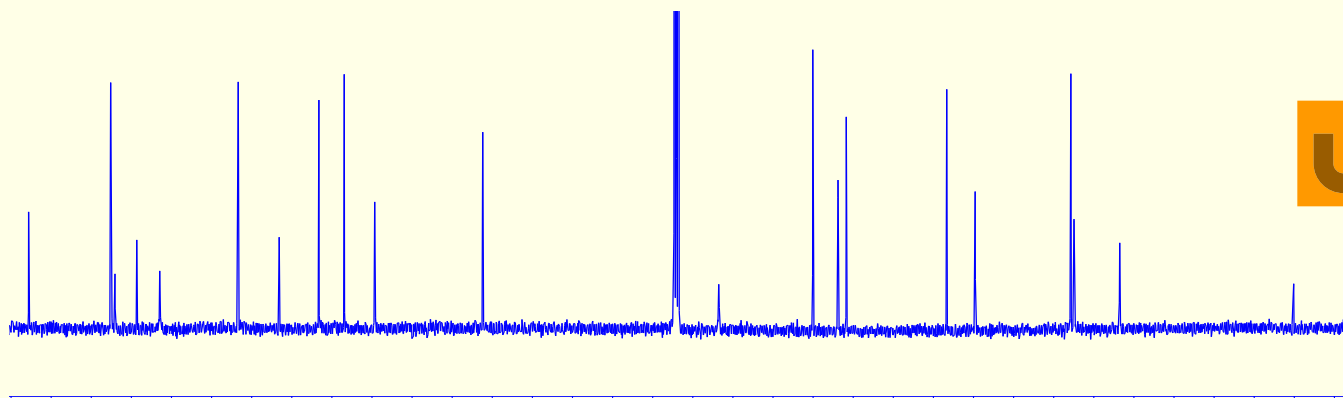
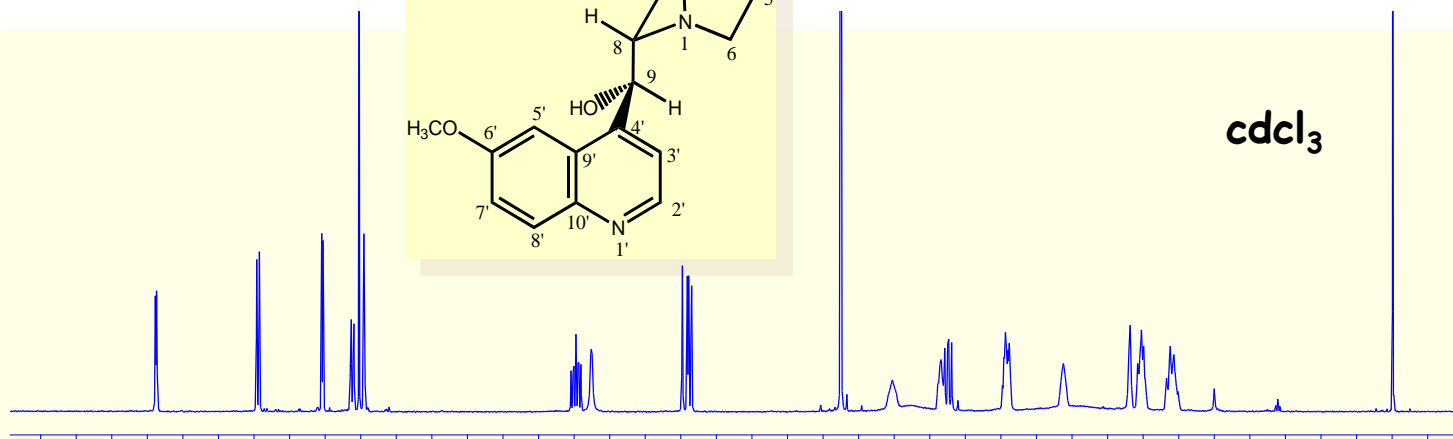


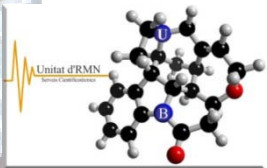
^1H and ^{13}C

Quinine 5mg/0.7 ml



cdcl_3



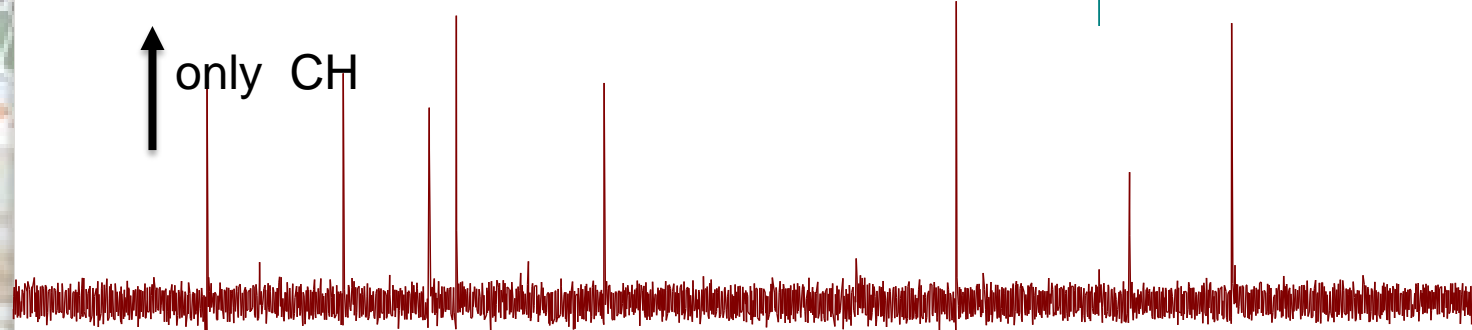
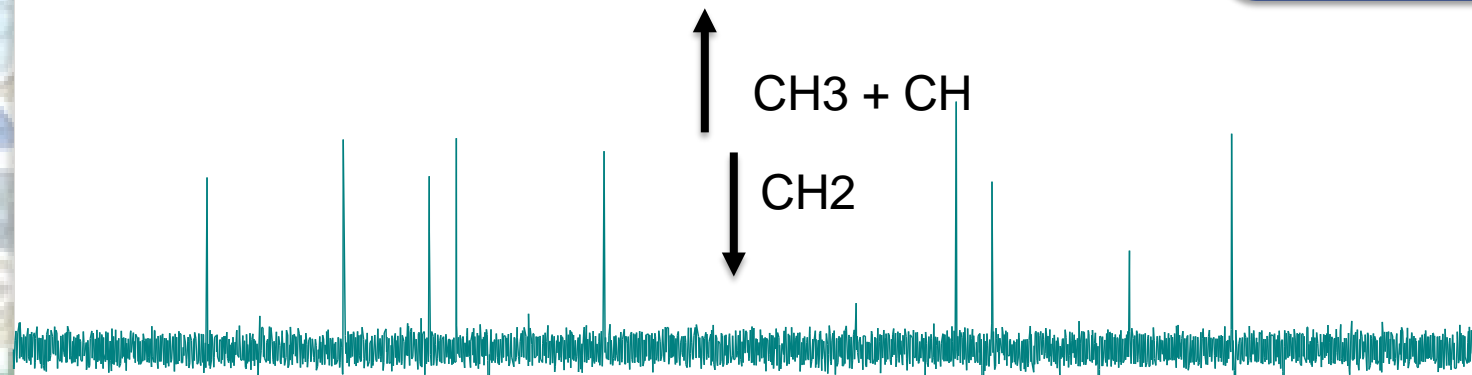


Dept

Quinine 5mg/0.7 ml

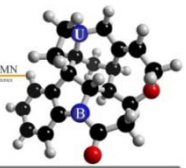
DEPT nt=256

20 minutes



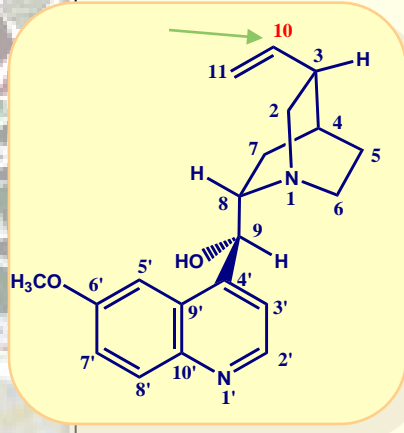
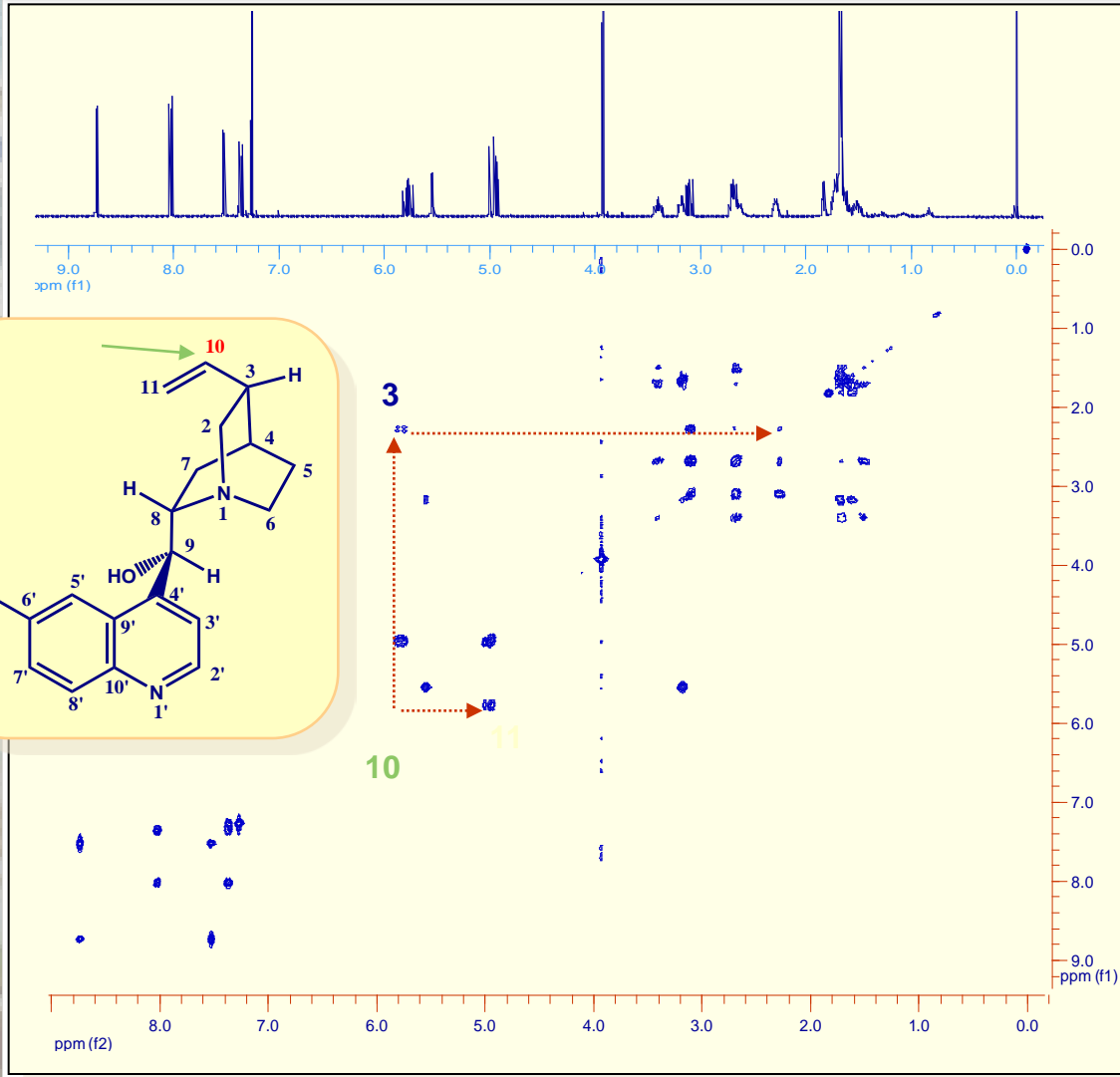
CH₂ = CH₃ 0



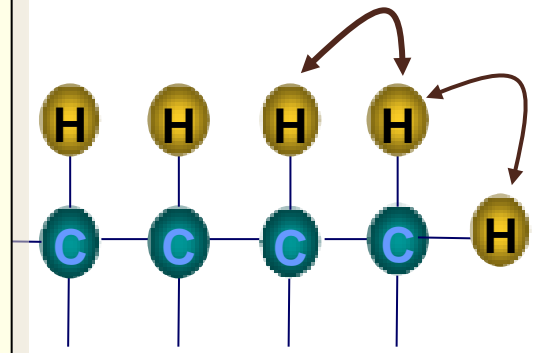


COSY

**¹H-¹H Homonuclear correlation
direct couplings**



Quinine 2mg/0.8 ml
nt=2 ni=256
time 13 minutes





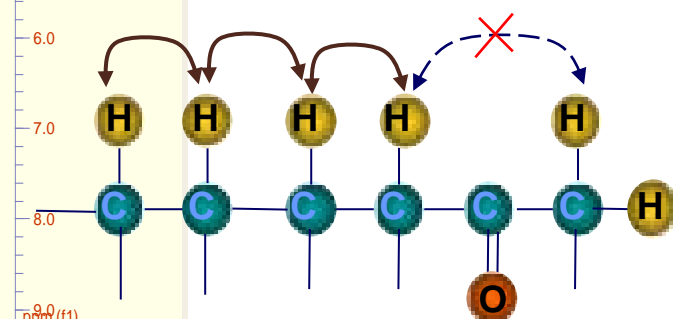
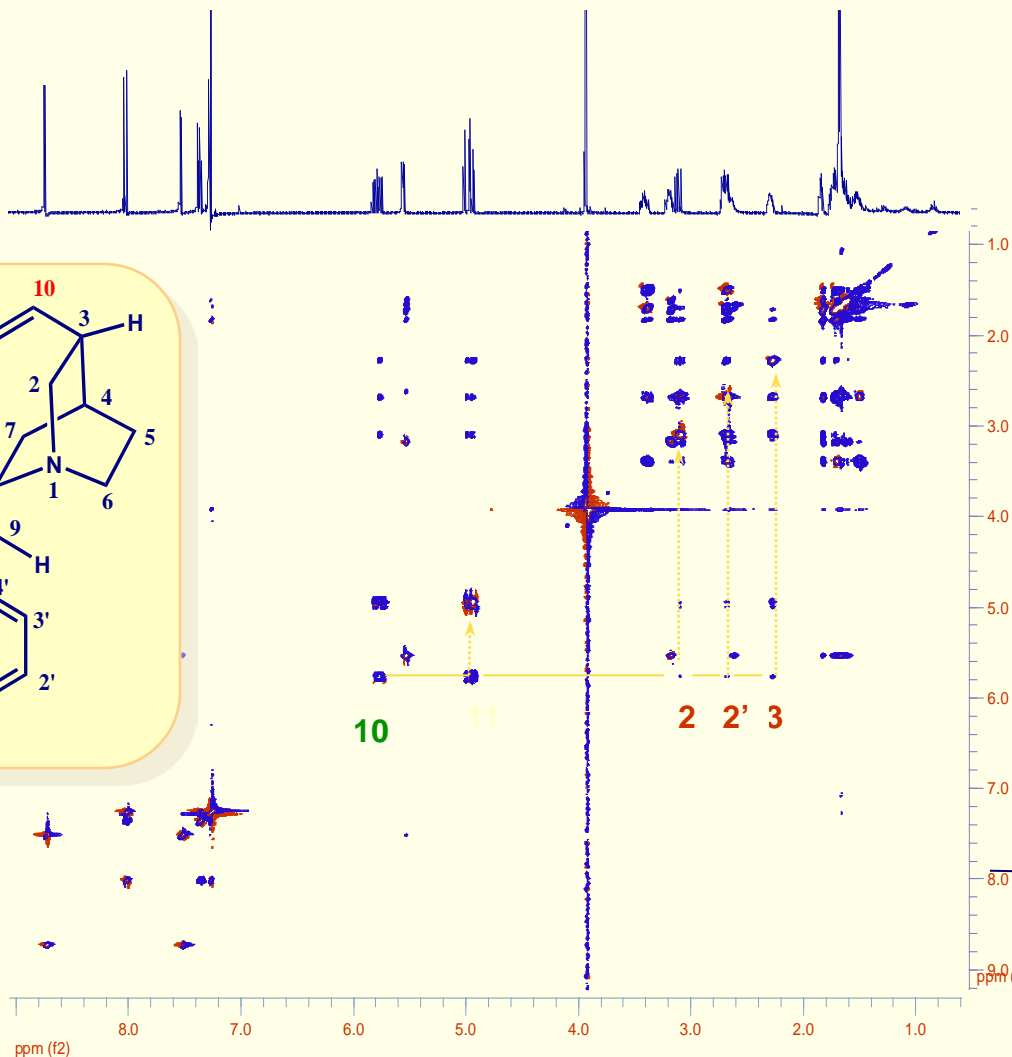
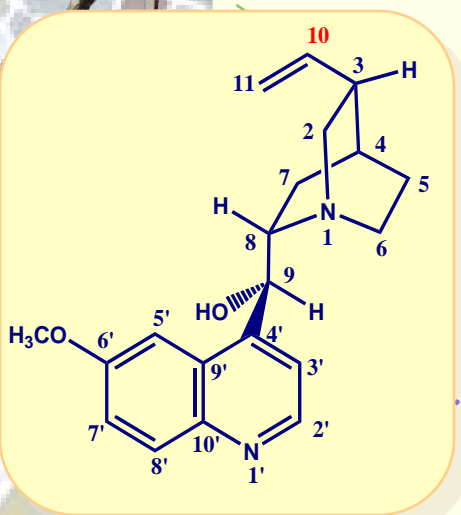
TOCSY

Quinine 2mg/0.8 ml

TOCSY

nt=4 ni=256 mix=80 ms

≈ 1h





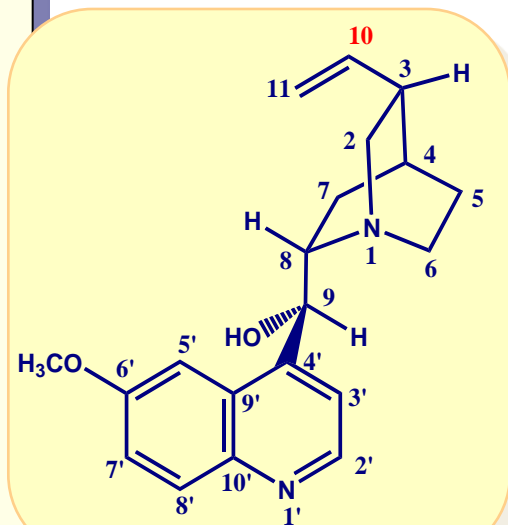
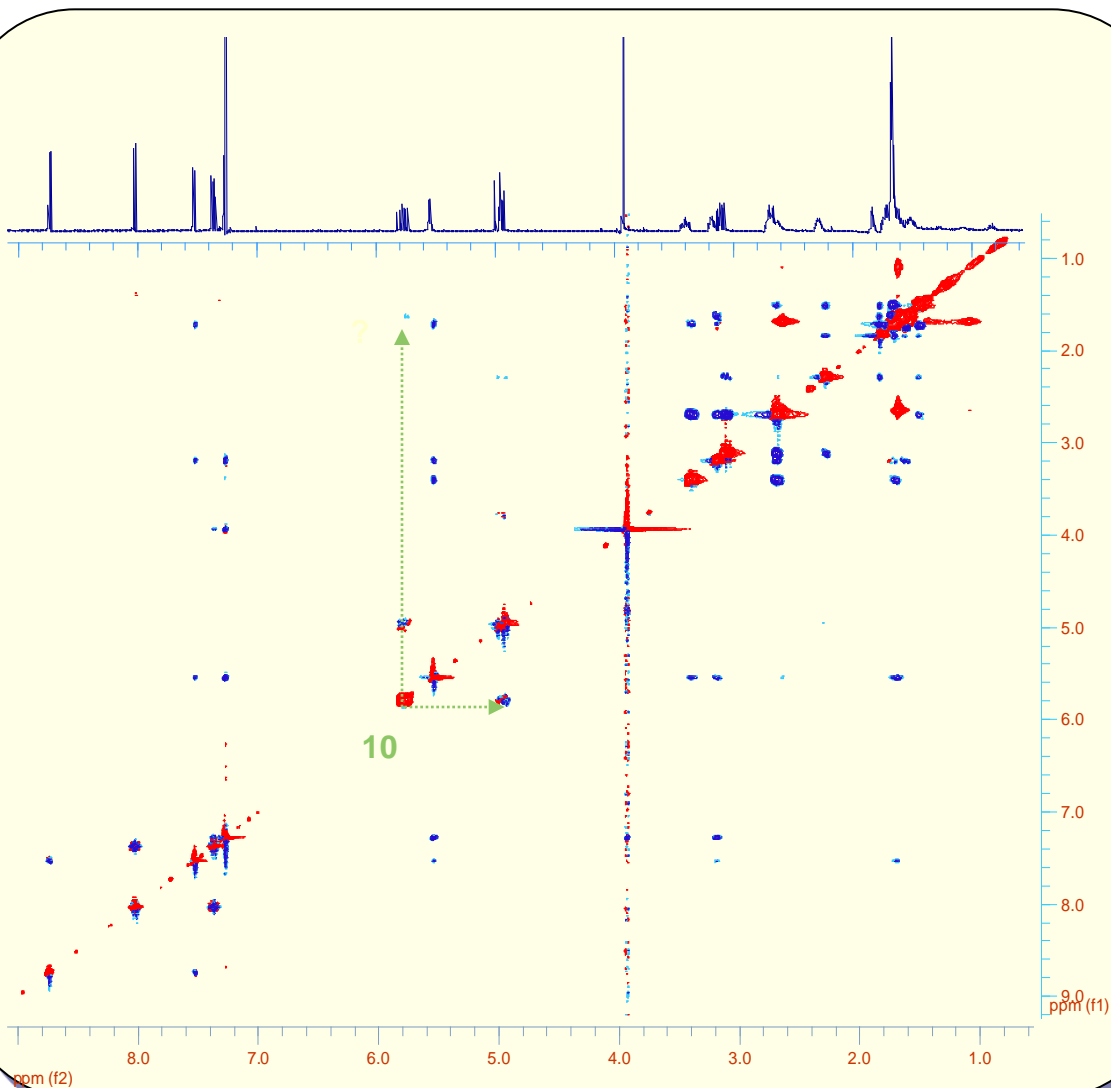
NOESY

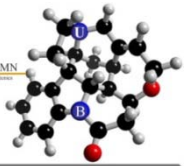
Quinine 2mg/0.8 ml

NOESY

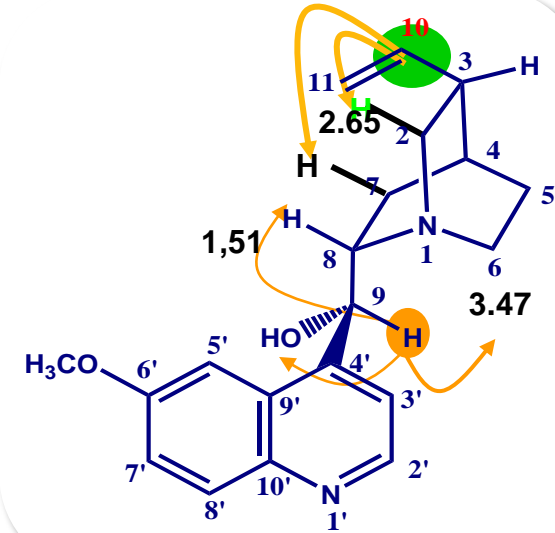
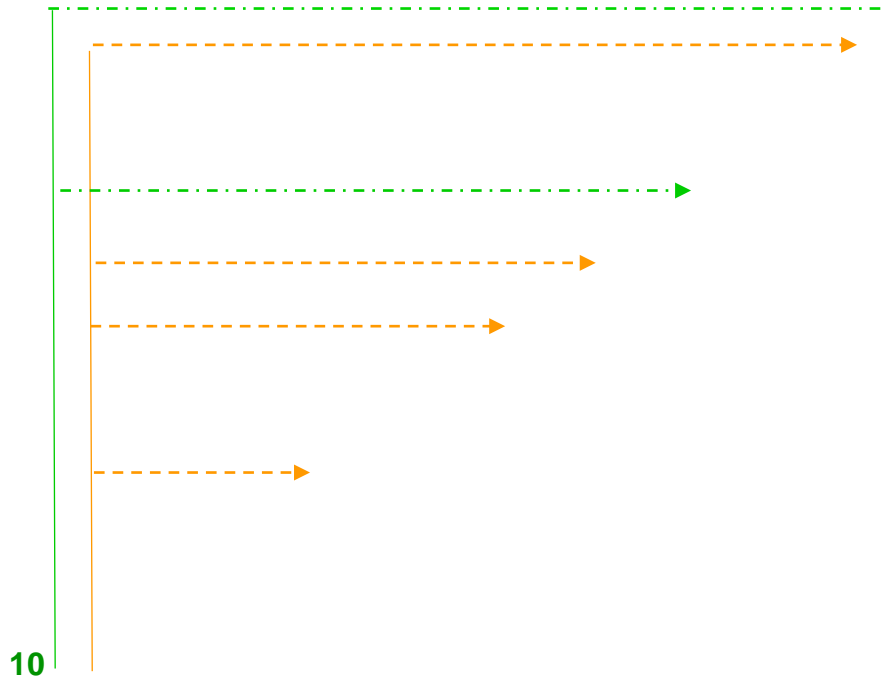
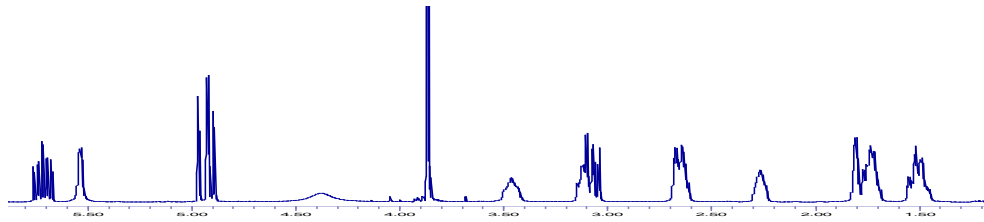
nt=32 ni=256 mix=0.5 s

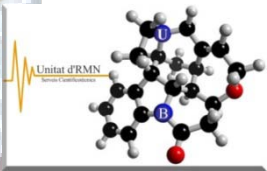
8 h 23 min





NOESY QUININE





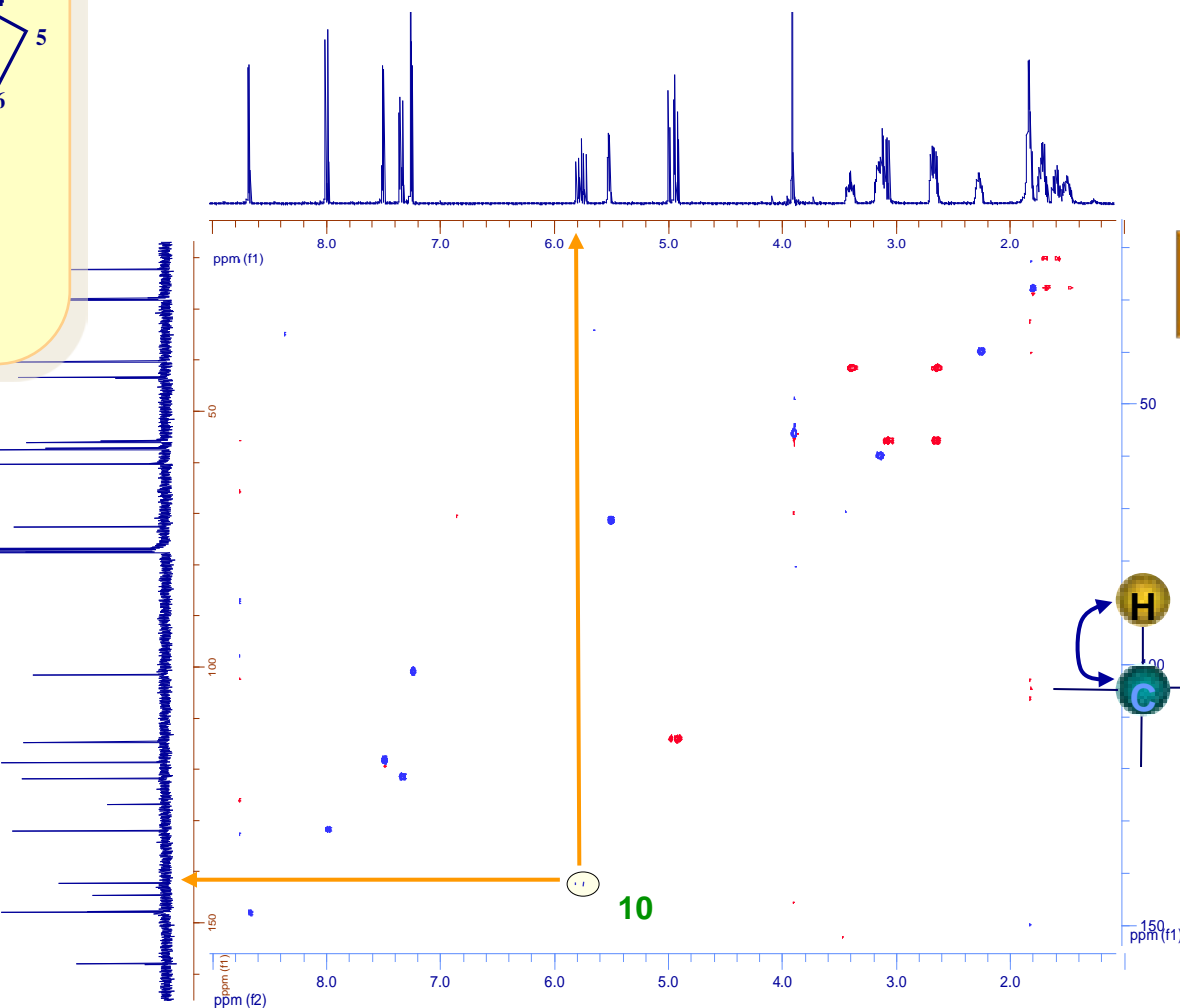
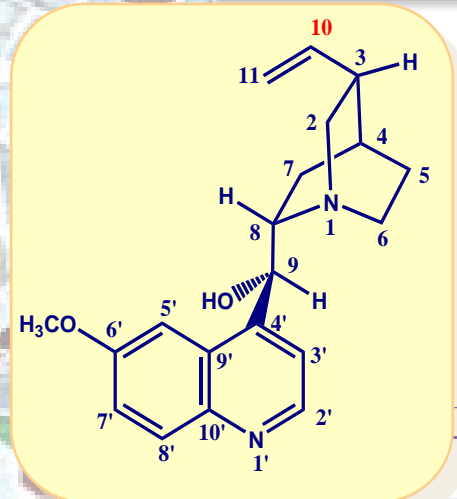
gHSQC

Quinine 5 mg/0.8 ml

gHSQC

nt=4 ni=128 LP

26 minutes

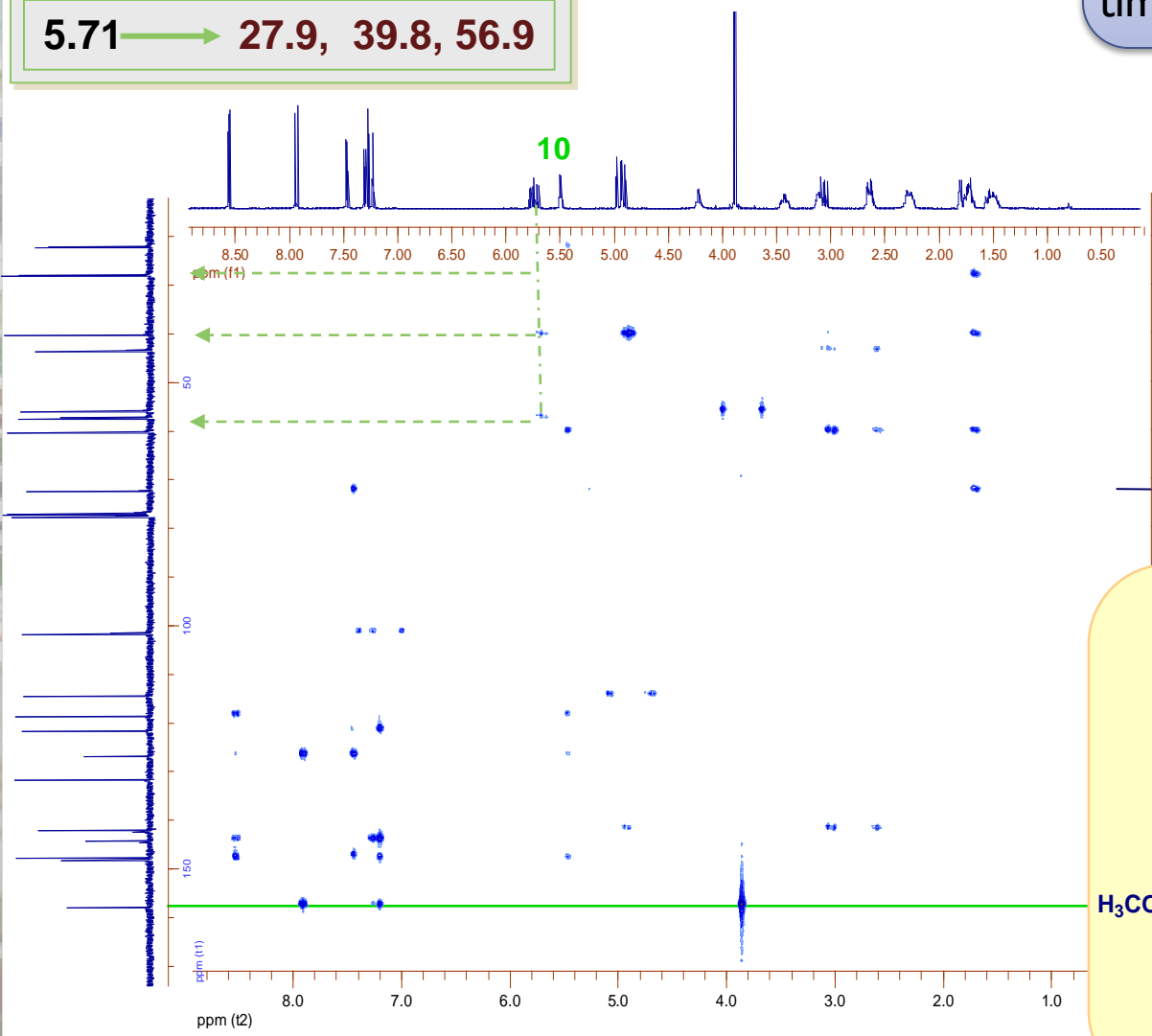




gHMBC

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

