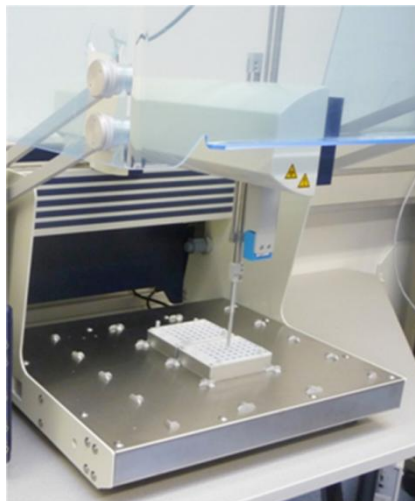


HR-MAS : Principi e tecnologia

Anna P. Minoja – Bruker Italia
Viale Lancetti 43 - MILANO



PARMA, 31 marzo 2014 - NMR DAY: HR MAS: applications in scientific and industrial fields

CONTENTS

- Introduction - What is HRMAS?
- NMR spectrometer configuration
- Compare HR vs. HRMAS vs. CPMAS :
 - Tubes & rotors
 - Lock
 - Gradient
 - Temperature control
 - Probe

NMR LINEWIDTH

The line width of an NMR resonance depends strongly on the microscopic environment of the nucleus under study.

Anisotropic interactions (i.e. chemical shift, dipole-dipole) impose dependence on the NMR frequency based on the orientation of a spin or molecule with respect to B_0 .

Averaged in liquid state by rapid isotropic motion of the molecules.

Reduced/averaged in solid state by spinning at '**Magic Angle**'.

Magnetic susceptibility of the sample and susceptibility differences within the sample lead to a broadening of the resonances.

Minimized in liquid state by the sample geometry, a cylinder parallel to B_0 .

Eliminated in solid state by spinning at '**Magic Angle**'.

What is HRMAS?

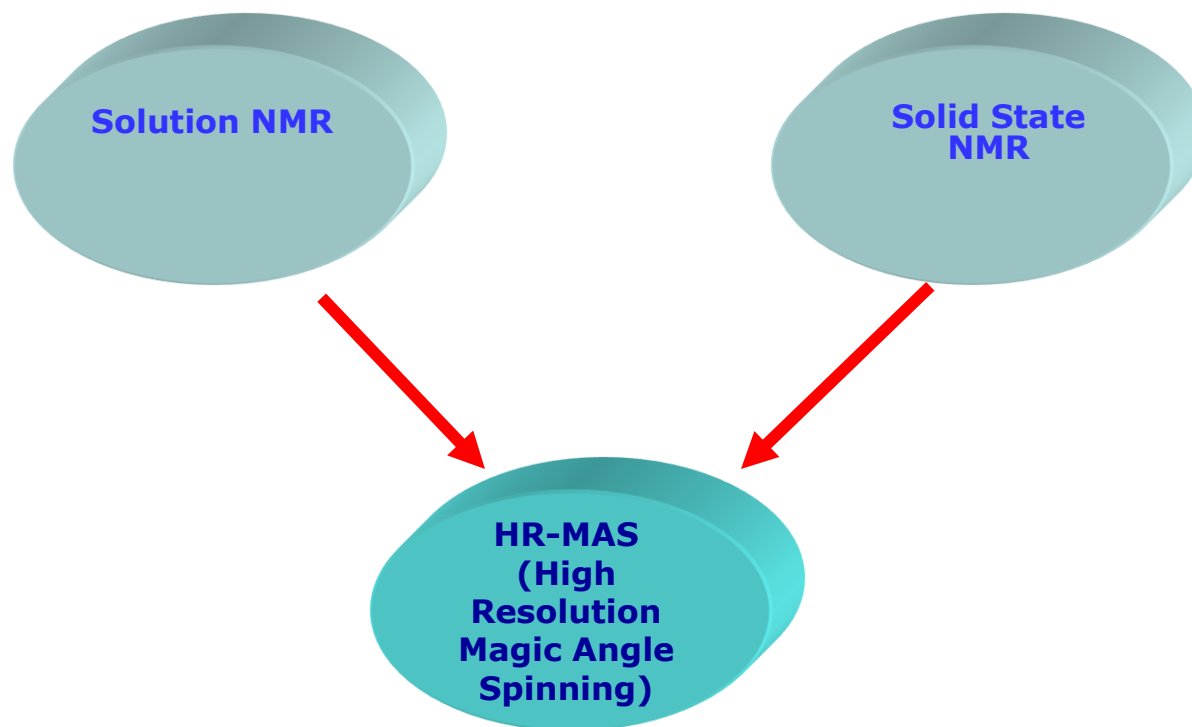
In addition to pure solids or pure liquids there is a wide range of “intermediate” materials whose mobility greatly average anisotropic interactions, but whose spectral resolution for the static samples is still much lower than that which is achieved for liquid samples due to a combination of residual dipolar interactions and variations in the bulk magnetic susceptibility.

Magic angle spinning is efficient at averaging these left-over components of the solid state line width, and leads to NMR spectra that display resolution approaching that of liquid samples. Such methods have been termed **High Resolution MAS (HR-MAS)** NMR.

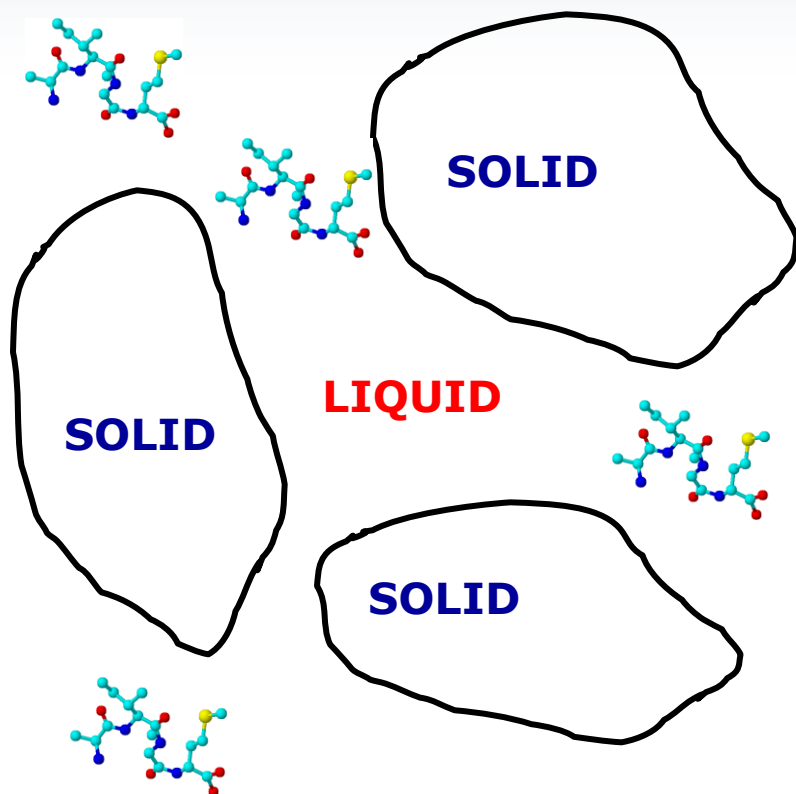
What is NMR-HRMAS?

High Resolution NMR can be used also for heterogeneous samples.

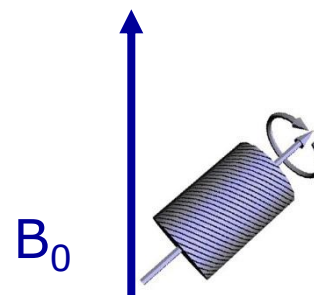
When the sample's state is intermediate between solid and liquid a peculiar configuration has to be used.



HETEROGENOUS SAMPLES

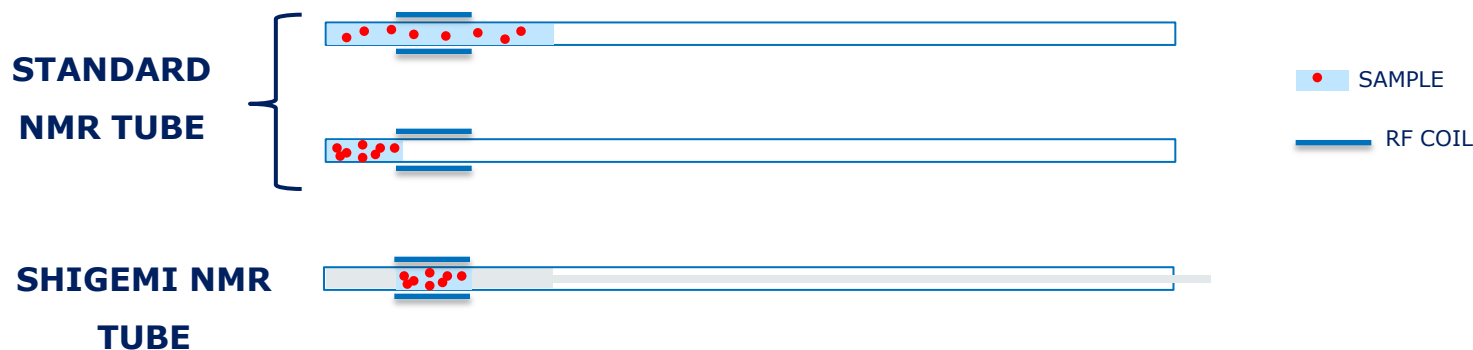


- Study of mobile compounds (metabolites) contained within heterogeneous substances (tissue)
- Main interaction: Distribution of magnetic susceptibilities
- Interaction removed by spinning the sample at the magic angle



Magnetic susceptibility in liquid samples

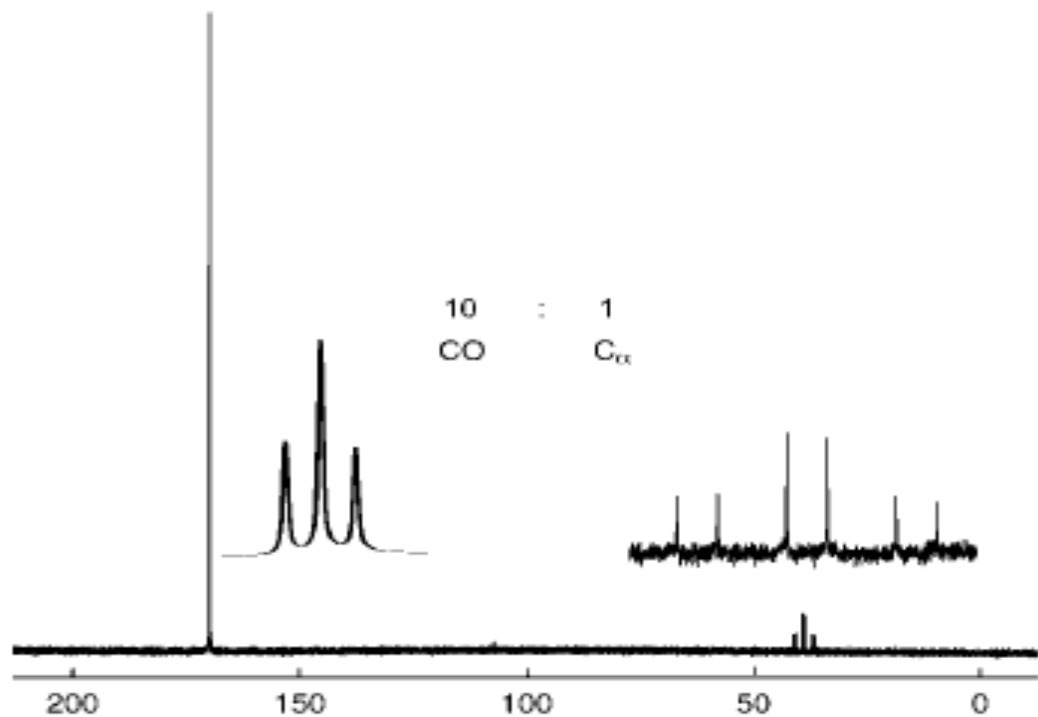
SHIGEMI NMR TUBES: designed for using small amount of sample volume



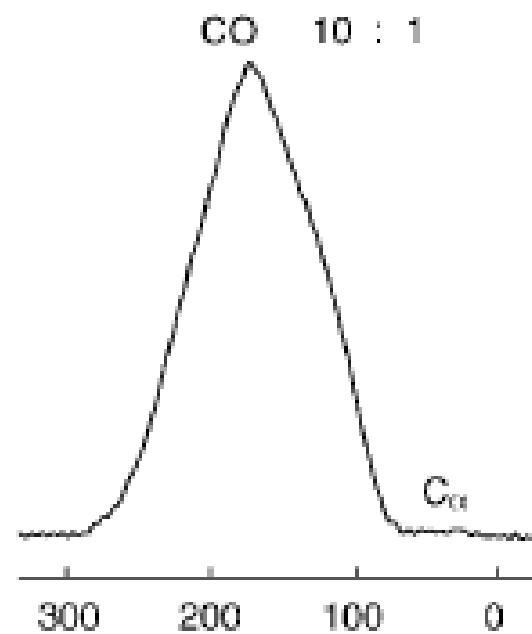
SOLVENT	χ_m	$\rho(\text{g/cc})$
CDCl_3	0.74	1.48
H_2O	0.72	1.00
D_2O	0.70	1.10
DMSO-d_6	0.68	1.10
C_6D_6	0.61	0.87
CD_3OD	0.53	0.79
Aceton- d_6	0.46	0.78

The top and the bottom have the same susceptibility of the solvent

^{13}C Spectrum of glycine ^{13}C -enriched

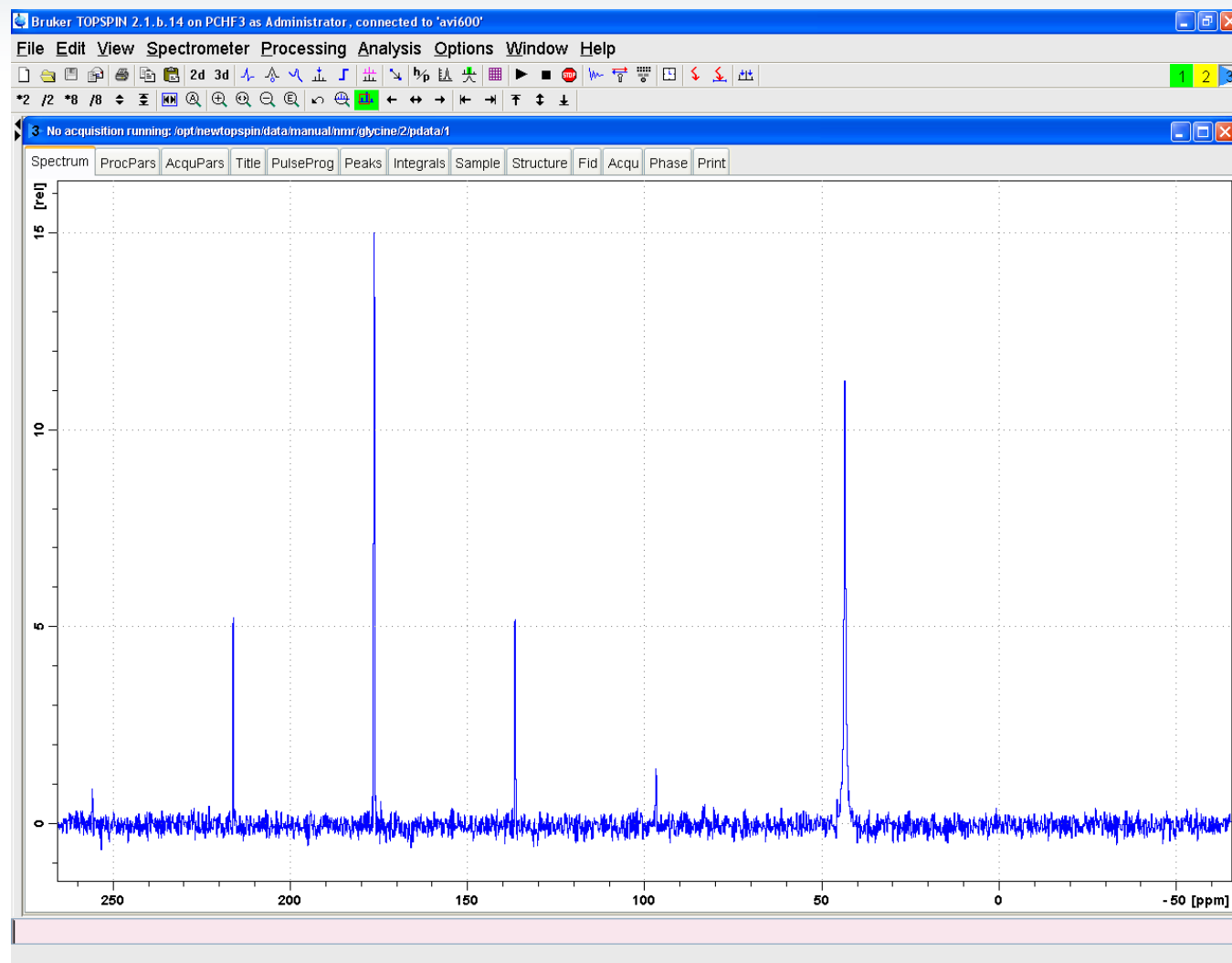


10mM solution in D_2O



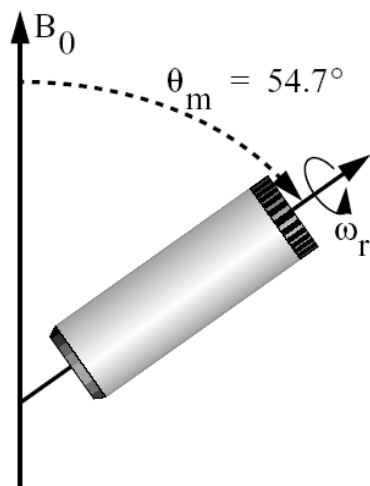
90mg solid state sample

$^{13}\text{C}\{^1\text{H}\}$ CP-MAS Spectrum of glycine



What is NMR-HRMAS?

As the mobility is intermediate the configuration of the system is intermediate.



STATOR

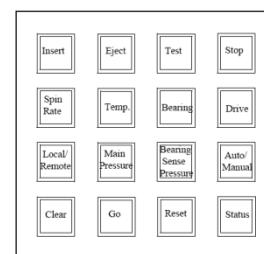
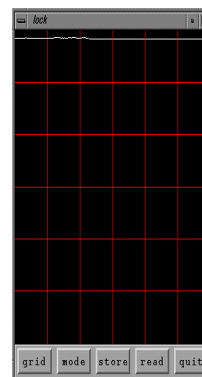


Figure 9.1. Front Panel Keypad

PNEUMATIC
UNIT

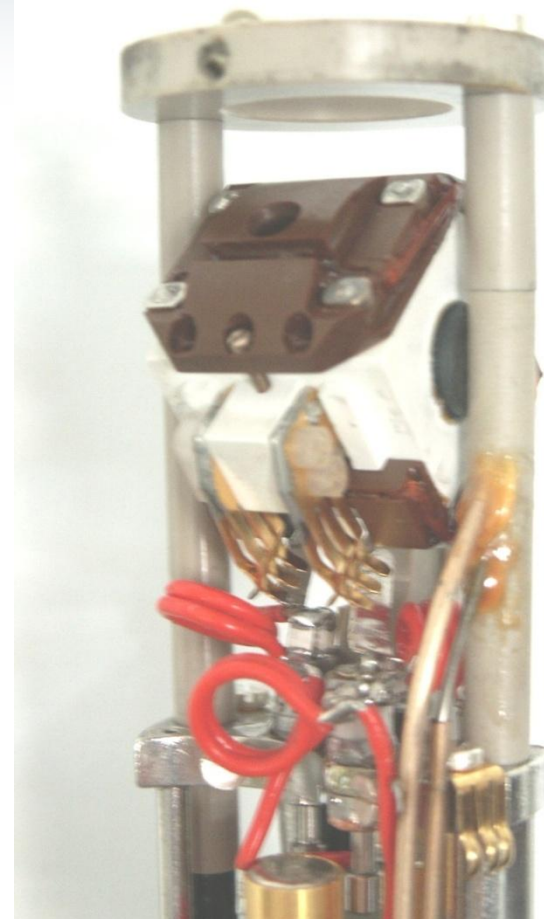


LOCK
CHANNEL

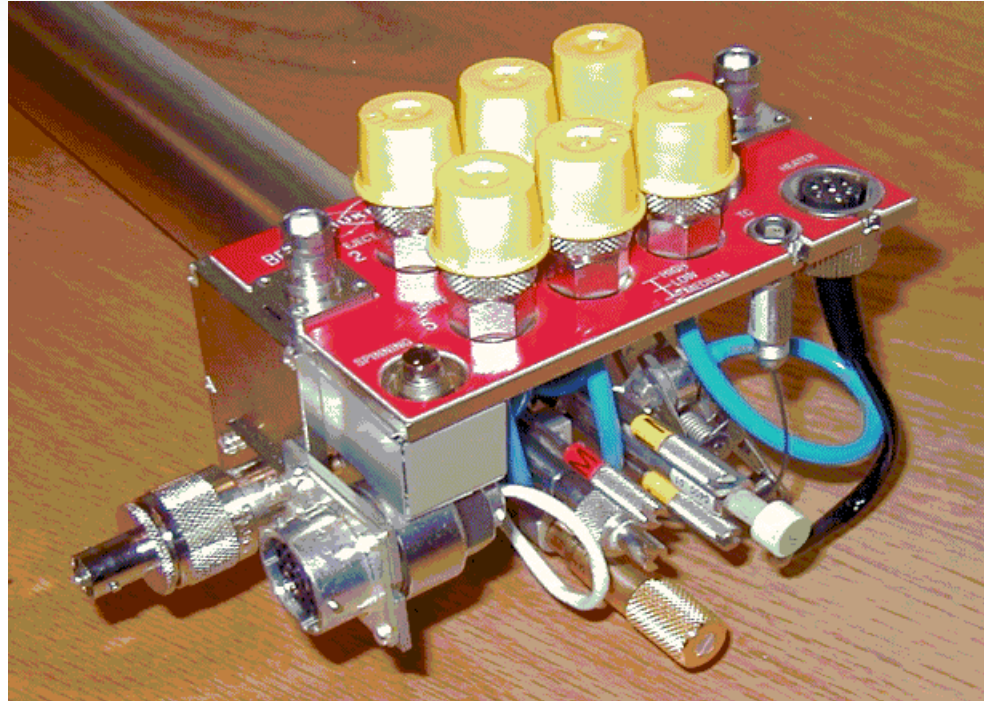
THE HR-MAS PROBE: IN ...

Properties of the HR-MAS probe:

- RF coil: Solenoid (different configuration)
- Gradient coil oriented at the magic angle
- MAS. Maximum spinning rate depends on rotor types (diameter, inserts, material,...).
- Sample insertion/ejection without removal of the probe.
- Magic angle adjustment with micrometer screw at probe bottom.
- Optical spin rate counter.



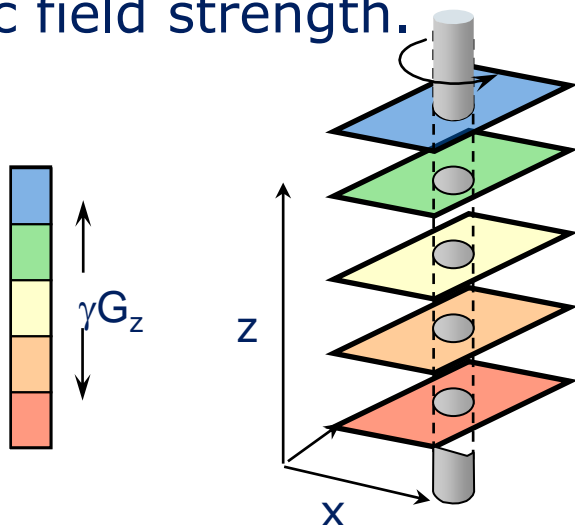
... and OUT



Magic Angle Gradients

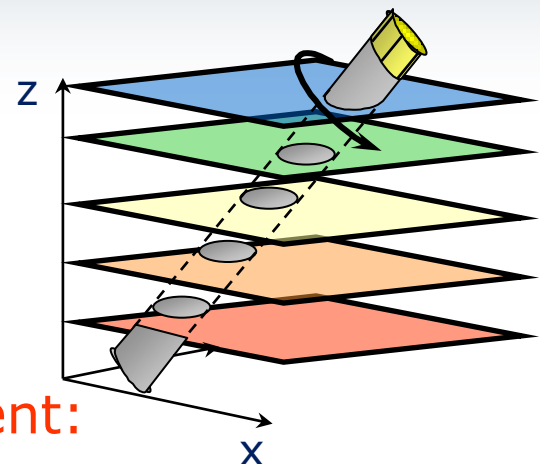
Conventional spectroscopy with a z-gradient:

By rotating sample around an axis which is parallel to B_0 then a rotating spin will always experience the same magnetic field strength.



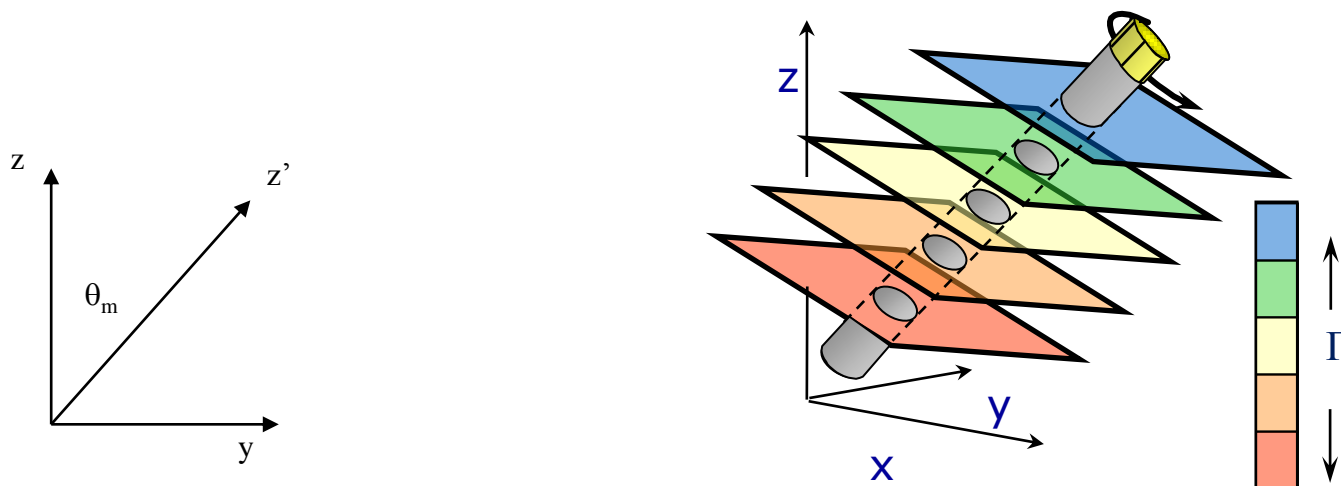
MAS spectroscopy with a z-gradient:

By applying a z-gradient in MAS spectroscopy, spinner axis and gradient direction are different: a rotating spin samples different magnetic field strengths.



Magic Angle Gradients

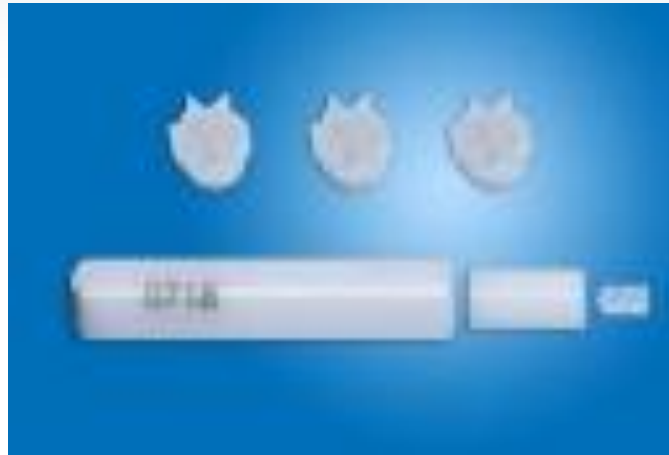
In order to properly combine Magic Angle Spinning with magnetic field gradients, the **gradient field is directed along the magic angle axis**, so that the gradient interaction is not temporally modulated by the spinning. Furthermore the gradient field has to be such that spins in a plane perpendicular to the spinner axis experience the same gradient field.



Compare HR vs. HRMAS vs. CPMAS TUBES and ROTORS



TUBES



ROTORS



Reduced Volume 4 mm Rotors

4 mm ZrO₂
rotor with
Teflon / Kel-F
insert.

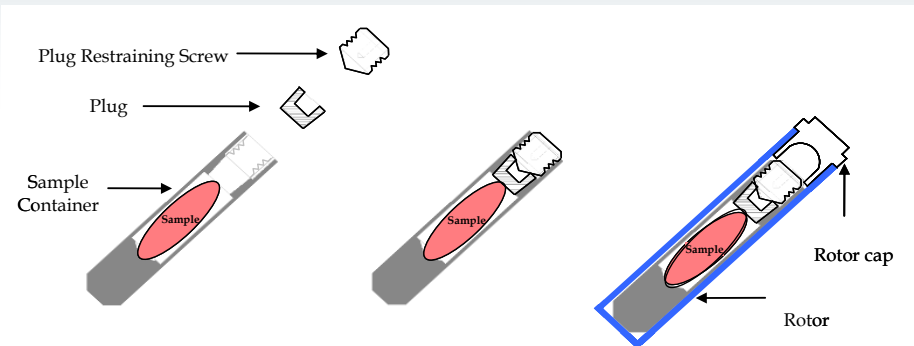


Volume: 50 μ l

Volume: 12 μ l



DISPOSABLE INSERT



- Kel-F® components
- Biologically and chemically inert
- Leak-proof
- Higher throughput
- Can be frozen to -80°C with the sample using insert storage rack

HR-MAS Sample Preparation

The setup and acquisition of NMR experiments are exactly the same as in HR, but **sample Preparation is a critical step** for any HR-MAS experiment.

- Good resolution and sensitivity strongly depends on the preparation.
- Different HR-MAS Applications different rotors.

Different Rotors - Different Applications

INSERT SHAPES:

- spherical insert well adapted for liquid-like samples (better B1 homogeneity)
- full rotors for polymers, rubbers....

ROTOR CAPS:

- Kel-F Caps for room temperature experiments
- Boron Nitride Caps for low temperature experiments

MATERIAL INSERTS

- Kel-F inserts for organic solvents
- Teflon inserts for samples in water

NMR Experiment

Once the sample is inside the magnet and it spins, it is exactly as to be in front to a HR-NMR spectrometer for analyzing solution samples: same procedures, same experiments (PULPROG, rpar,).

The only difference that you have to take into accounts are:

- Speed rotation may interfere with the experiments (i.e. TOCSY spin lock)
- High speed may change the sample temperature

NMR experiment & temperature

Temperature is a crucial parameter for any NMR sample/experiment:

- Resonance assignment problems in Bio-NMR:
Shift differences due to heating caused by NMR pulse sequences or techniques (spinlock, decoupling, high spinning rate)
- Exact AND reproducible chemical shift values:
Metabonomics
Protein chem. shift database
General comparability of NMR data

NMR experiment & temperature

Temperature is a crucial parameter for any NMR sample/experiment:

- Exact determination of:

Thermodynamic parameters (enthalpy, entropy, equilibria)

Motional behavior (dynamics of biomolecules from NMR relaxation data)

Diffusion coefficient

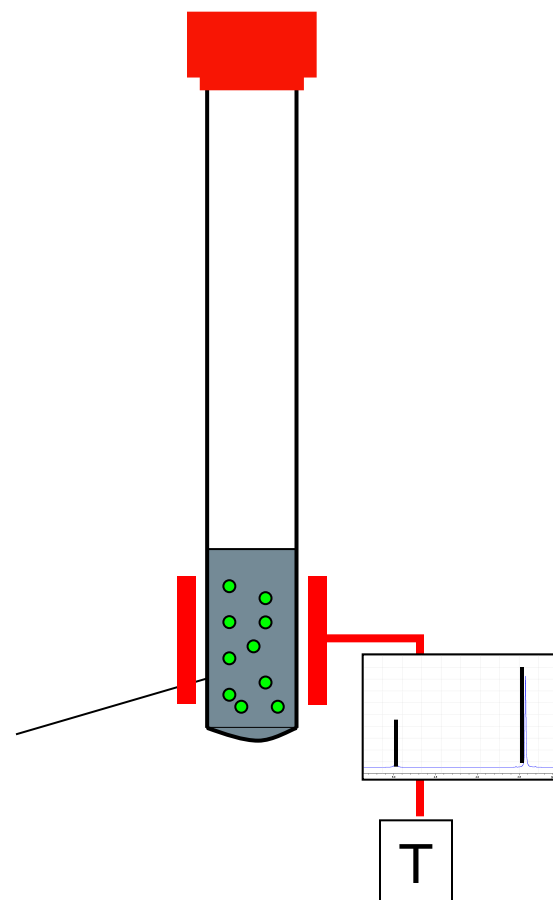
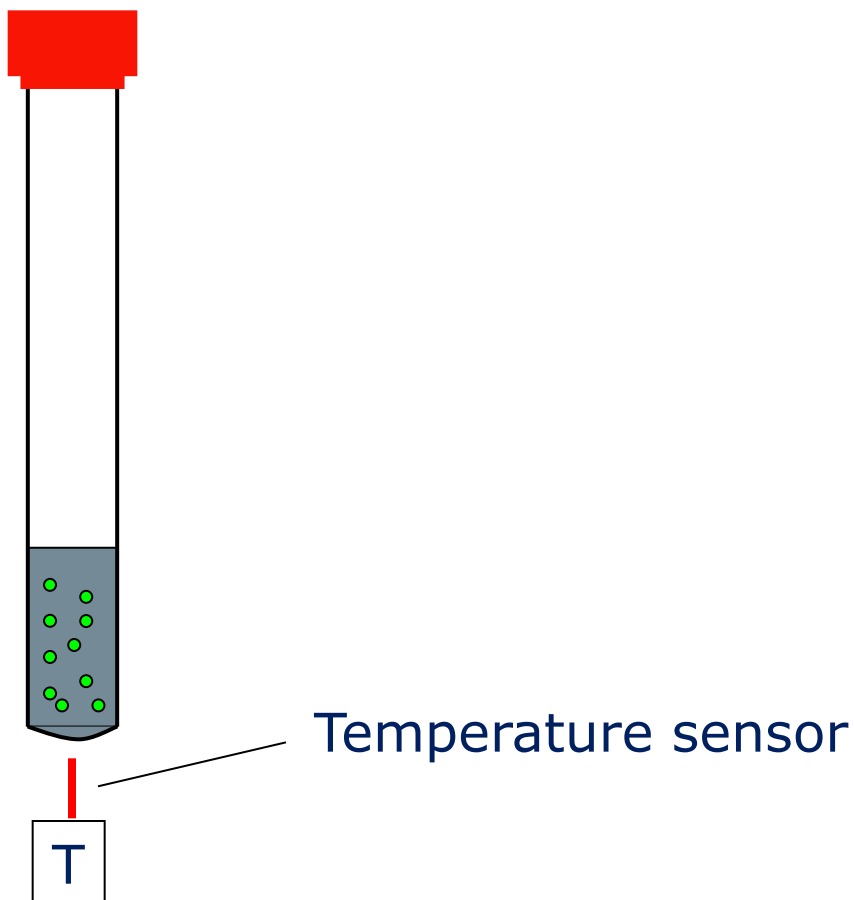
- Harm to the sample material
- ...

TEMPERATURE MEASUREMENT

TEMPERATURE SENSOR

On the probe

Inside the sample



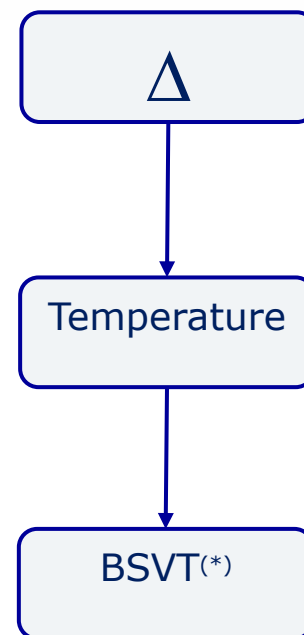
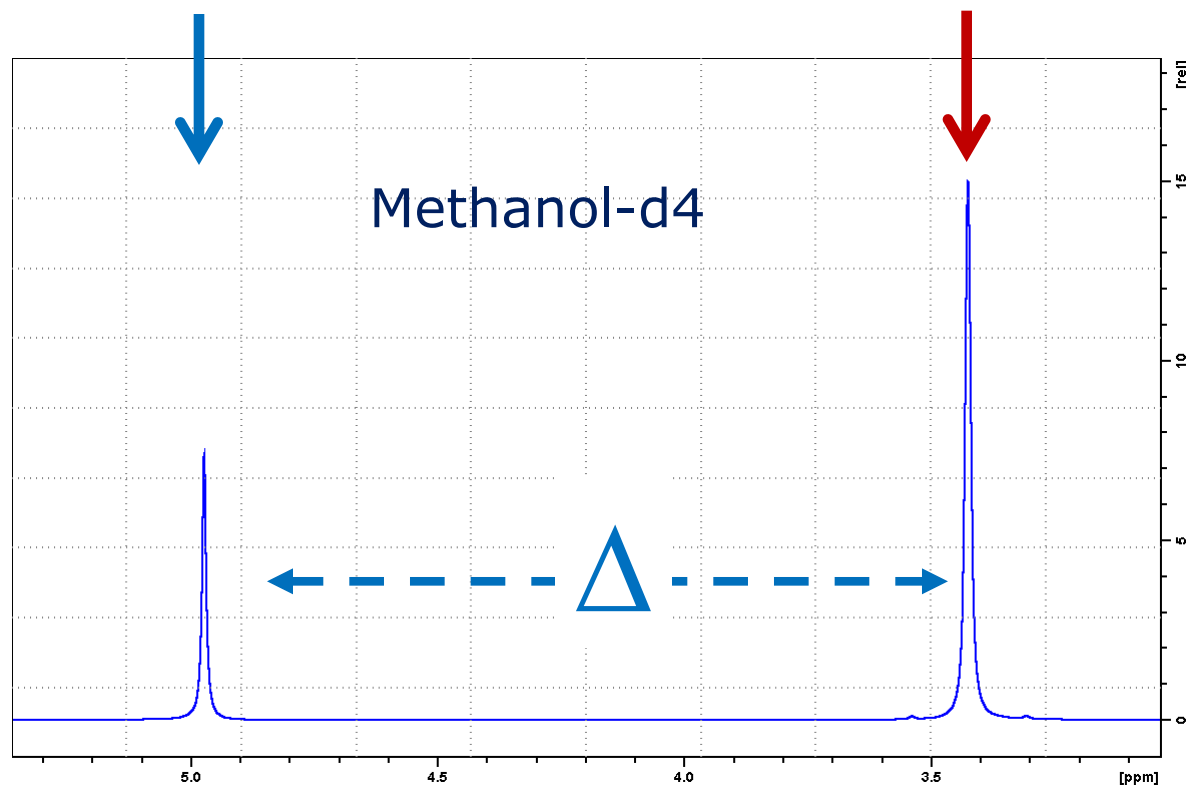
The answer: NMR Thermometer™

- Use sample temperature in addition to the probe T-sensor for temperature regulation
- Accurate and easy temperature calibration
- Monitoring of the internal sample temperature
- Exact and stable sample temperature independent from RF load or spinning speed (HR-MAS) : chemical shifts independent from experiment and system.
- Trigger the start of the experiment after reaching the set point (stability) of the sample temperature

How does the NMR Thermometer work?

NMR thermometer

Field lock



(*) BSVT = **B**ruker **S**mart **V**ariable **T**emperature

Edit lock parameters

Solvents Edit BSMS Help

Lock Nucleus: ☒ 2H ☐ 19F Auto Phase: ☐ Auto phase during lock Auto ph: ☐ Auto ph

Solvents Lock Spectrum Reference Properties

Solvent	Probe
CDCl3	Generic
CH3CN+D2O	Generic
CH3OH+D2O	Generic
CHCl3	Generic
D2O	Generic
D2O-MeOD	5 mm CPTCI 1H-13C/15N/D Z...
DEE	Generic
Dioxane	Generic
DME	Generic
DMF	Generic
DMF2	Generic
DMF3	Generic
DMSO	Generic
EtOD	Generic
Glycol	5 mm CPTCI 1H-13C/15N/D Z...
Glycol_T	Generic
H2O+D2O	5 mm CPTCI 1H-13C/15N/D Z...
HDMSO	Generic
MeOD	Generic
MeOD_T	5 mm CPTCI 1H-13C/15N/D Z...
Naac50	5 mm CPTCI 1H-13C/15N/D Z...
Pyr	Generic
Pyr2	Generic
Pyr3	Generic
TFE	Generic
THF	Generic
Tol	Generic

Edit lock parameters

Edit lock parameters for solvent "Naac50".

Lock parameters

Probe name: 5 mm CPTCI 1H-13C/15N/D Z-GRD Z75812/0055

Probe description:

Lock power: -18

Loop gain: -3.7

Loop time: 0.31

Loop filter: 100

Lock Phase: 210

Lock power instep: 10

Temperature lock power: -22

Signals

Signal	Shift [ppm]	Relative intensity	Type	Description	Delete
1	4.7	1	Lock		<input type="checkbox"/>
2	1.8	1	Temperature		<input type="checkbox"/>

Add Signal Delete Signal

Temperature shift values

Value	Shift [ppm]	Temperature [K]	Delete
1	2.48	346.16	<input type="checkbox"/>
2	2.52	341.37	<input type="checkbox"/>
3	2.56	336.62	<input type="checkbox"/>
4	2.61	331.8	<input type="checkbox"/>

Add Value Delete Value

OK Cancel

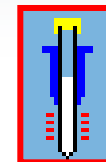
se	Shift [ppm]
220	7.24
220	4.7
220	4.7
220	7.24
220	4.7
210	4.7
220	1.07
220	3.53
220	3.3
220	2.91
278	2.72
278	8.01
220	2.49
220	1.11
220	4.7
-1	3.76
-1	3.8
220	4.7
220	2.49
220	3.3
225	3.3
210	4.7
220	8.71
278	7.2
278	7.55
220	3.88
220	1.73
220	2.09

Close

HR-MAS on biological samples



SPINNING SPEED from 2 kHz up to 6kHz



SAMPLE
TEMPERATURE

HEATER
POWER

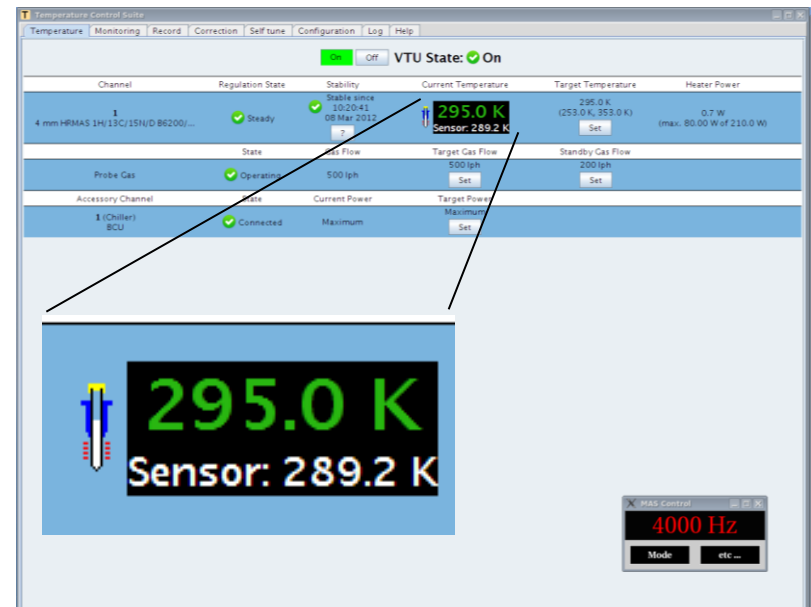
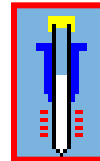
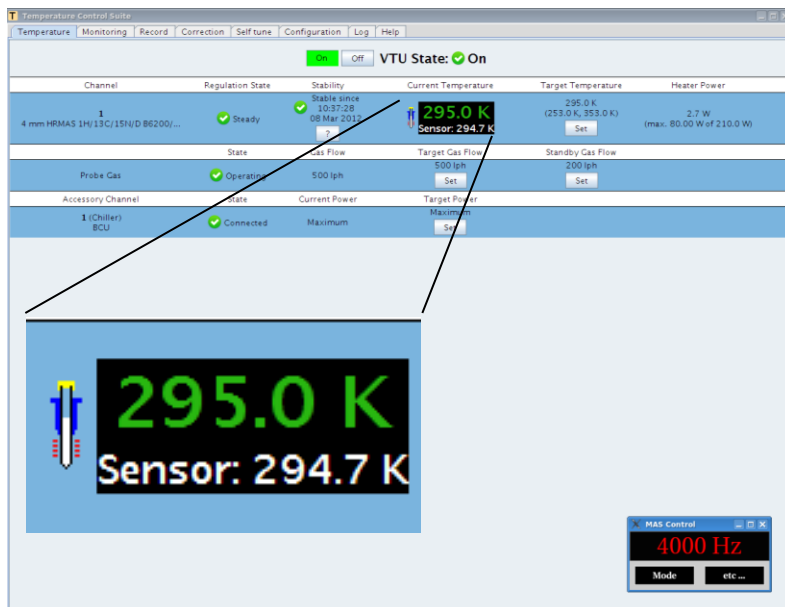
HR-MAS on biological samples

2D-TOCSY, speed 4kHz, 295K

EFFECT OF SPINNING

compensated

EFFECT OF SPINNING
+
SPIN LOCK

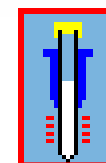


HR-MAS on biological samples

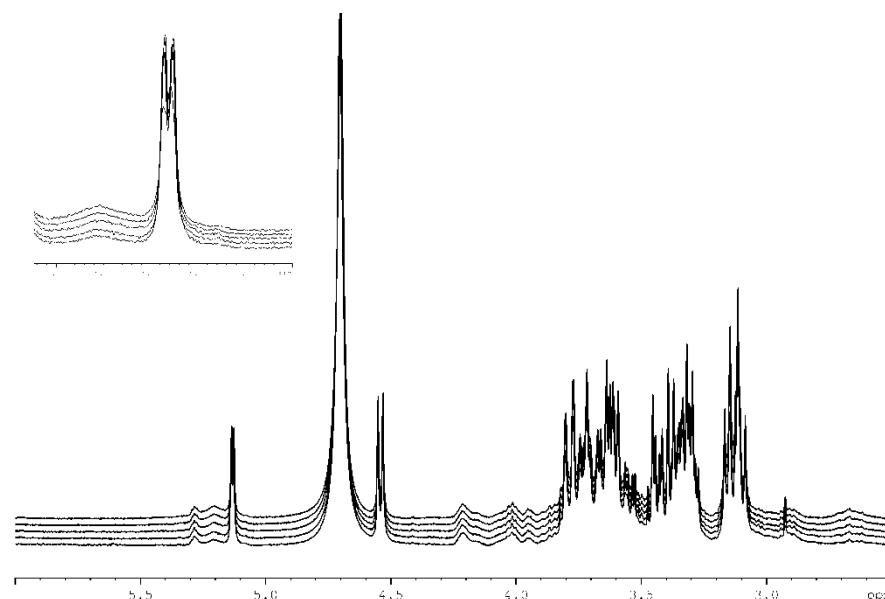
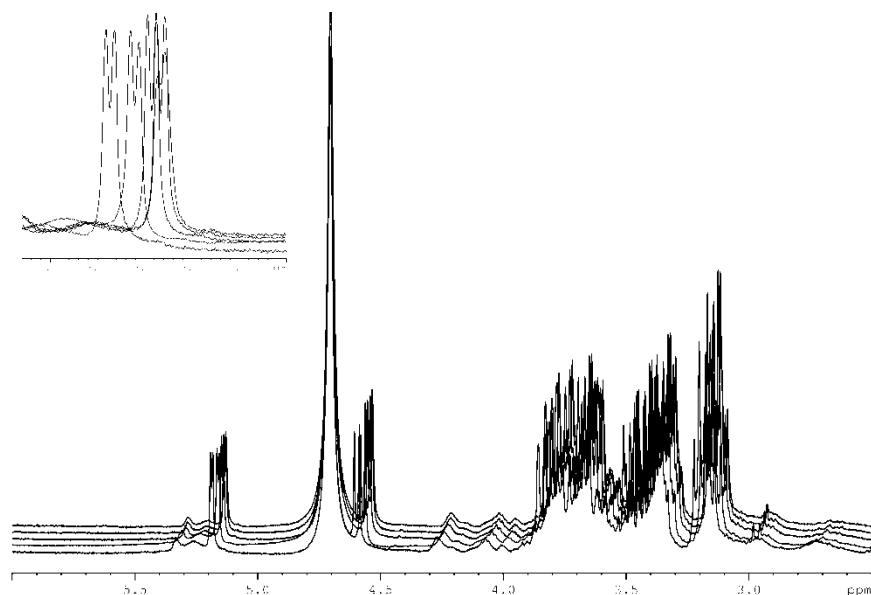
Constant temperature with HR-MAS applications

without NMR Thermometer

with NMR Thermometer



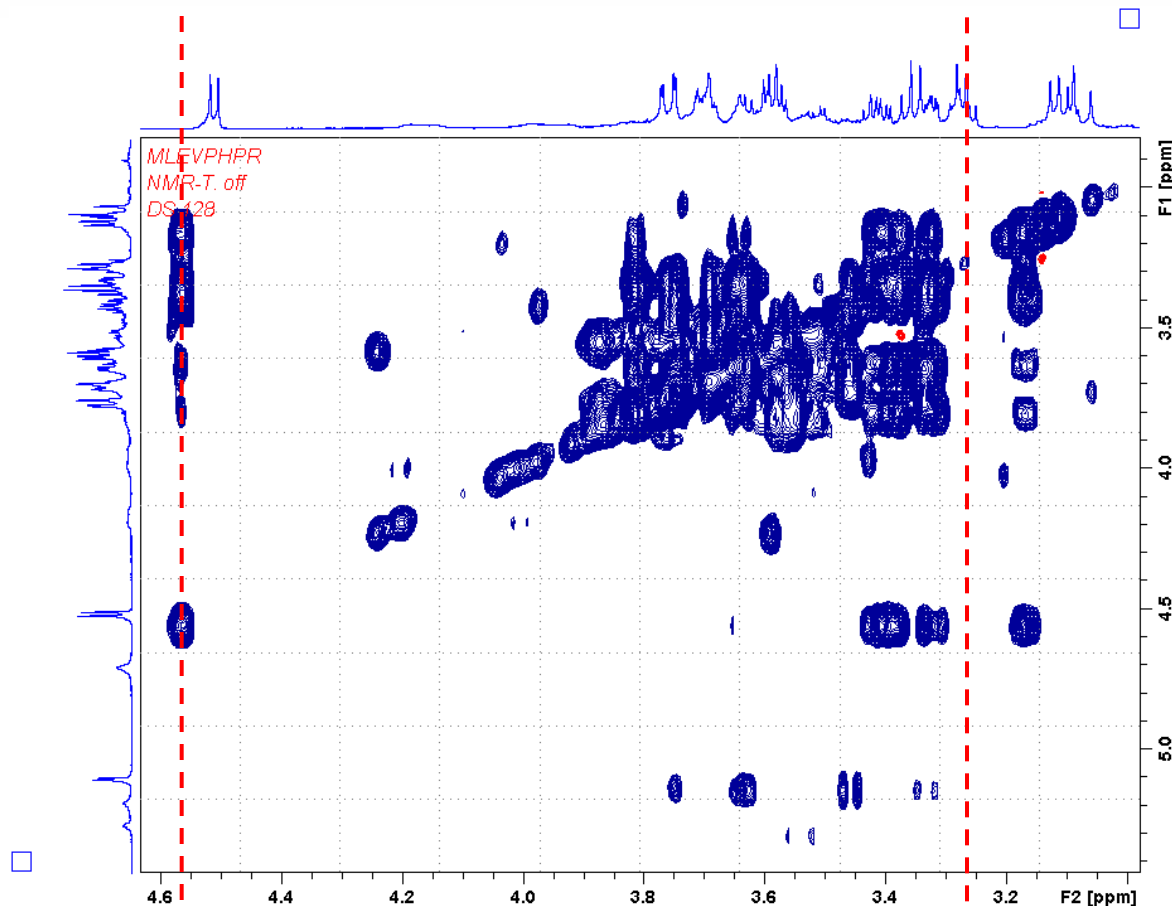
8kHz ← 1kHz



Liver sample, sodium acetate-d3 in D₂O added at 400MHz and 298K

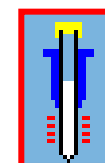
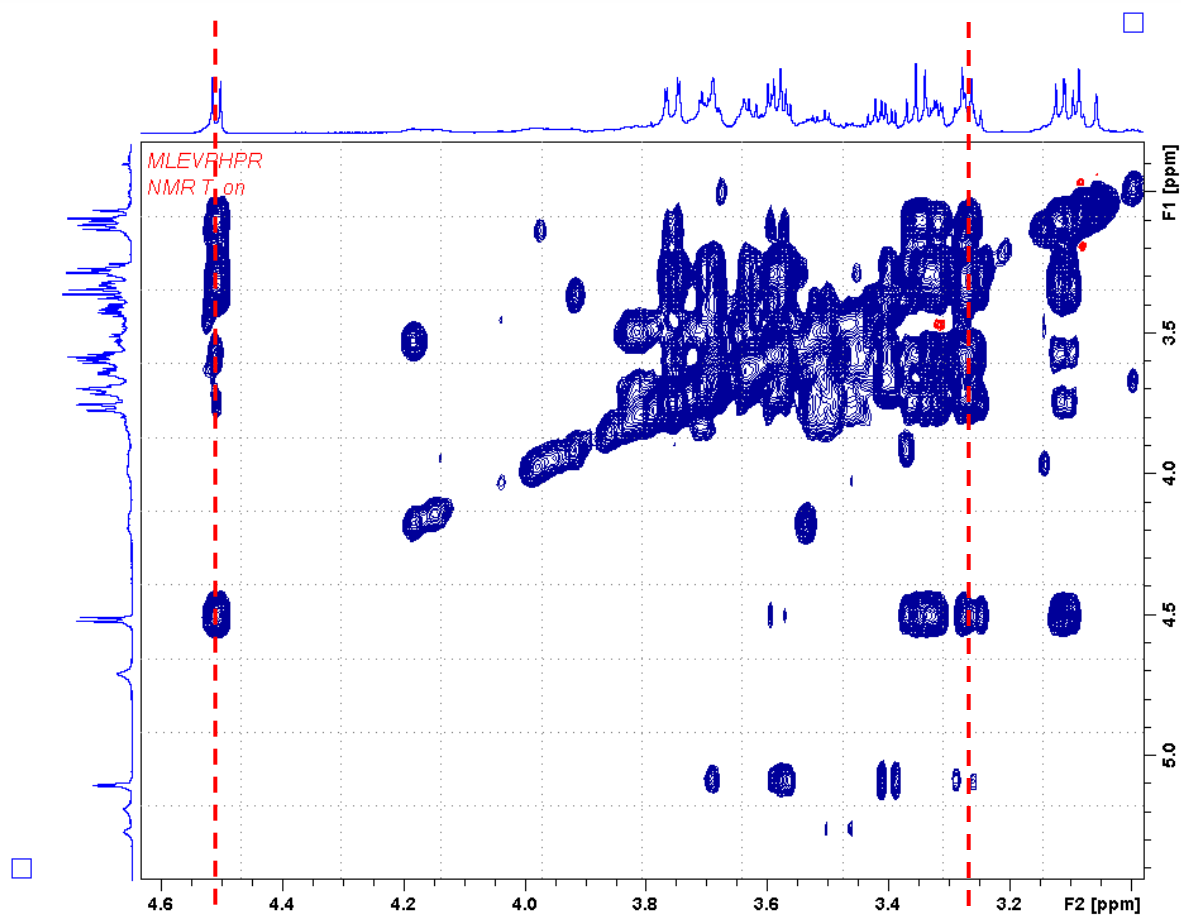
HR-MAS on biological samples

1D - 2D TOCSY comparison - NMR Thermometer **off**



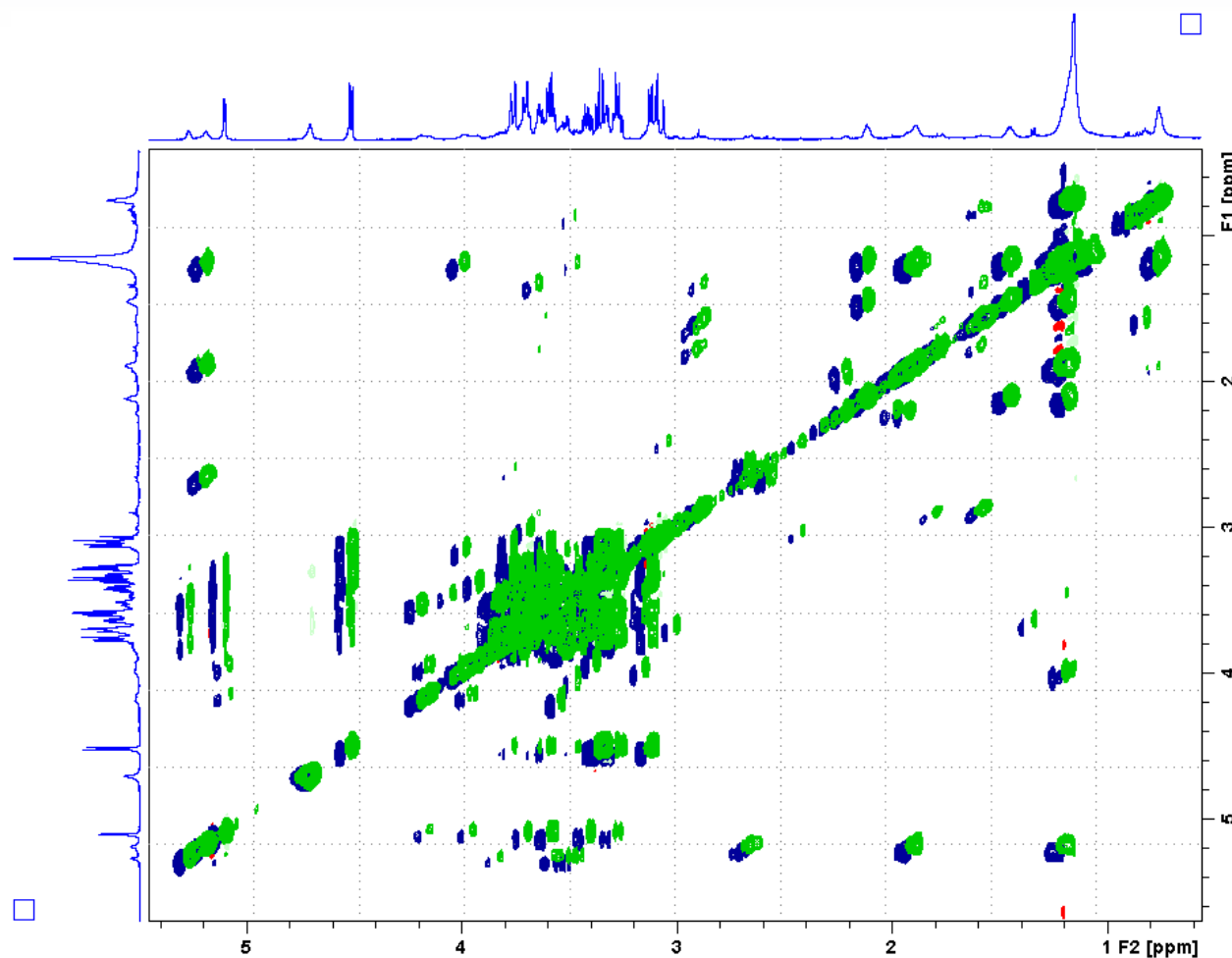
HR-MAS on biological samples

1D - 2D TOCSY comparison - NMR Thermometer **on**



HR-MAS on biological samples

2D-TOCSY, spinning speed 4kHz, 295K



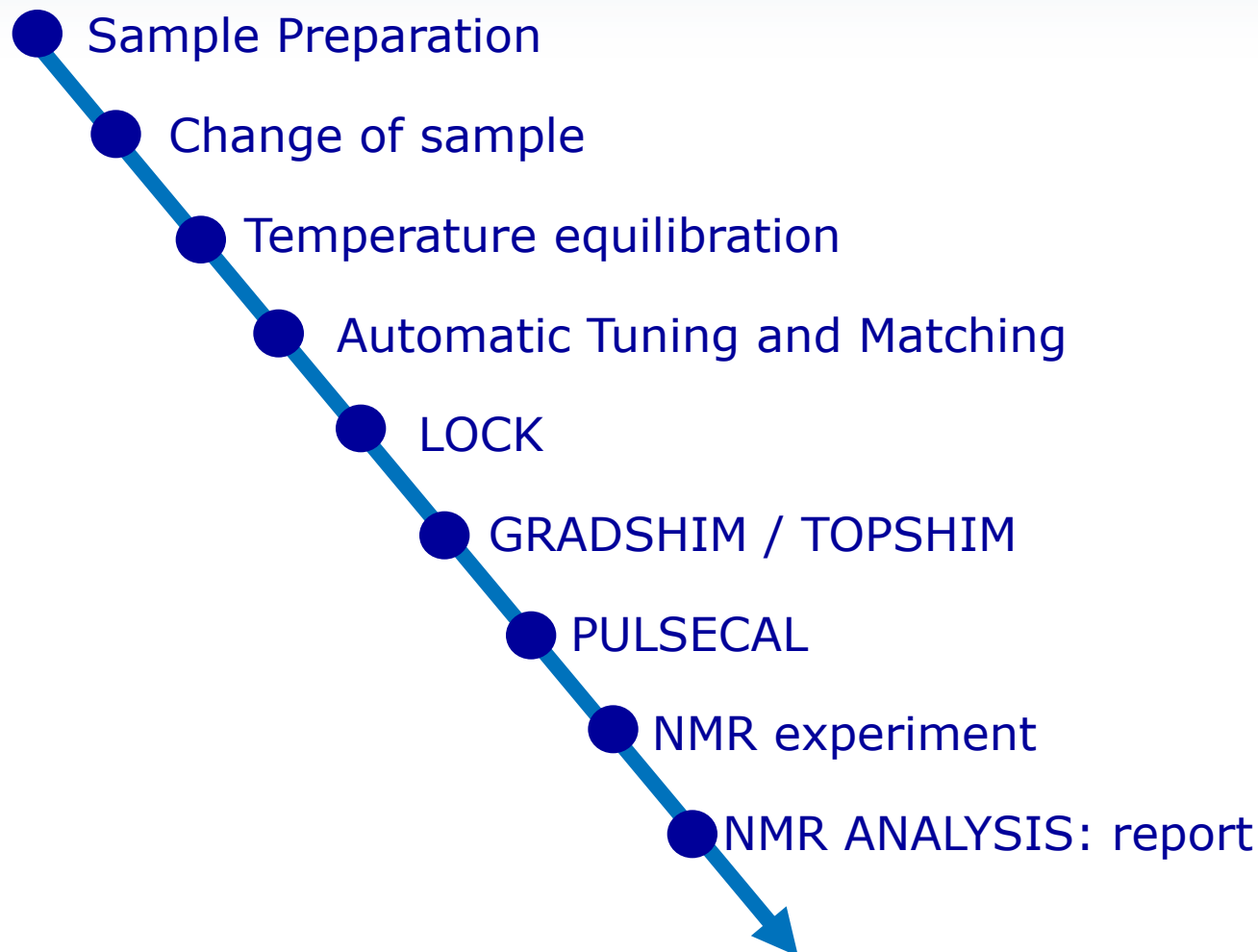
NMR Thermometer
on
off

Compare HR vs. HRMAS vs. CPMAS AUTOMATION

In the past decade automation NMR on liquid sample has dramatically improved.

Nowdays, NMR screening application are fully automated: from sample preparation up to spectra analysis.

Fruit Juices and Wine: Screening by Proton NMR



Compare HR vs. HRMAS vs. CPMAS AUTOMATION

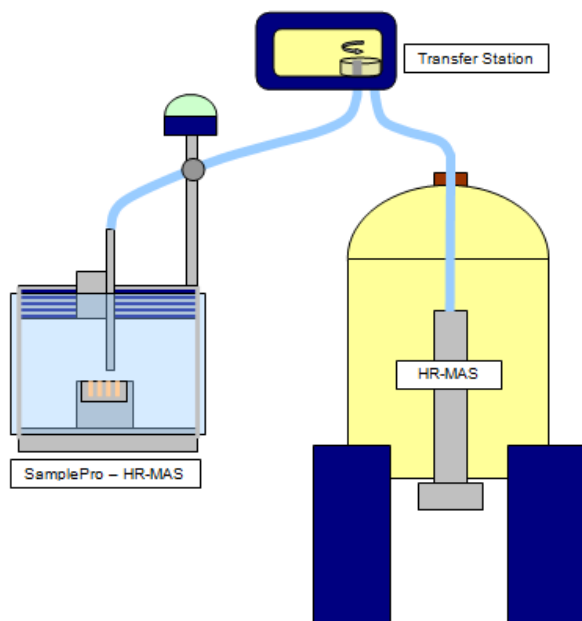
Which is the degree of automation on HR-MAS ?

Again screening applications are the driving force for such developments.

HR-MAS: sample changer Function principle

Changer based on storage device with cooling and transfer-unit

PNEUMATIC TRANSFER OF HR-MAS ROTORS



- Freely positionable storage device and transfer unit
- Transfer-tube tested up to 10m Length

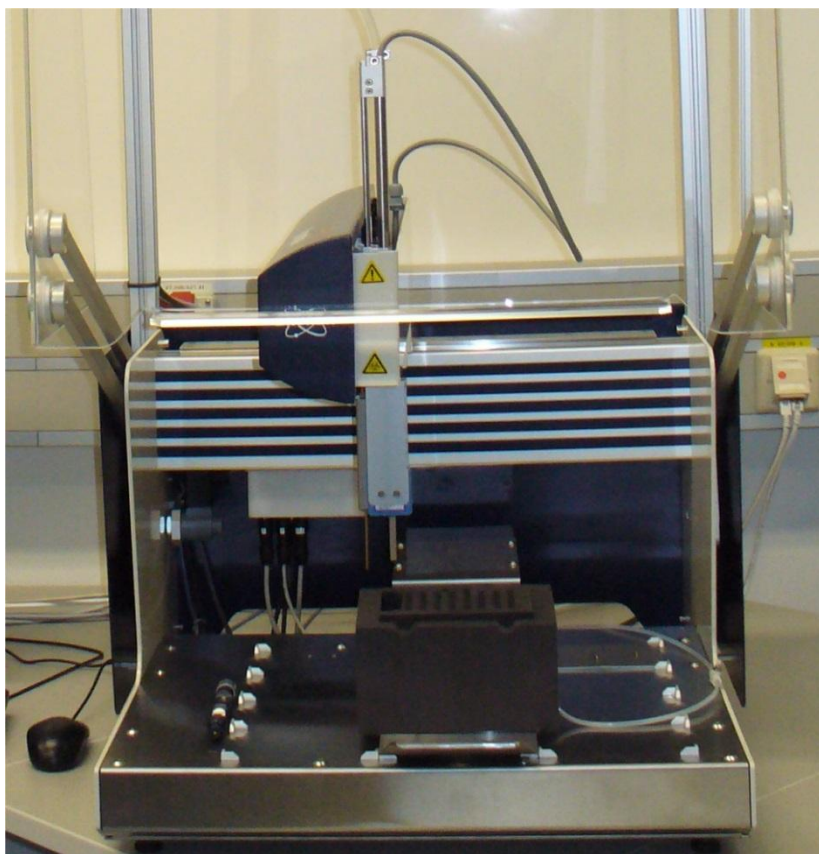
Requirement:

- Transfer in a few seconds
- Works with underpressure

SamplePro-HR-MAS™: Technical Realization I



Storage device for rotors



One arm robot with:

- Barcode reader rotors
- Barcode reader wellplates (Optional)
- 4 Tray Positions
- Safety Cover
- Sucking tube holder arm mounted
- Status light

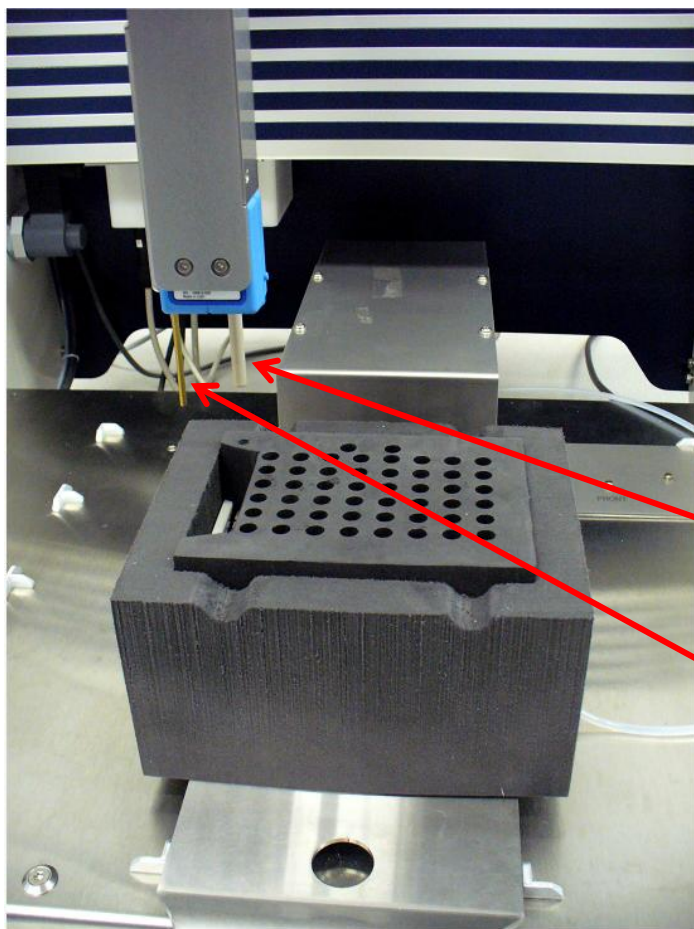


SamplePro-HR-MAS™:

Technical Realisation II



Storage Wellplates -16 ° C



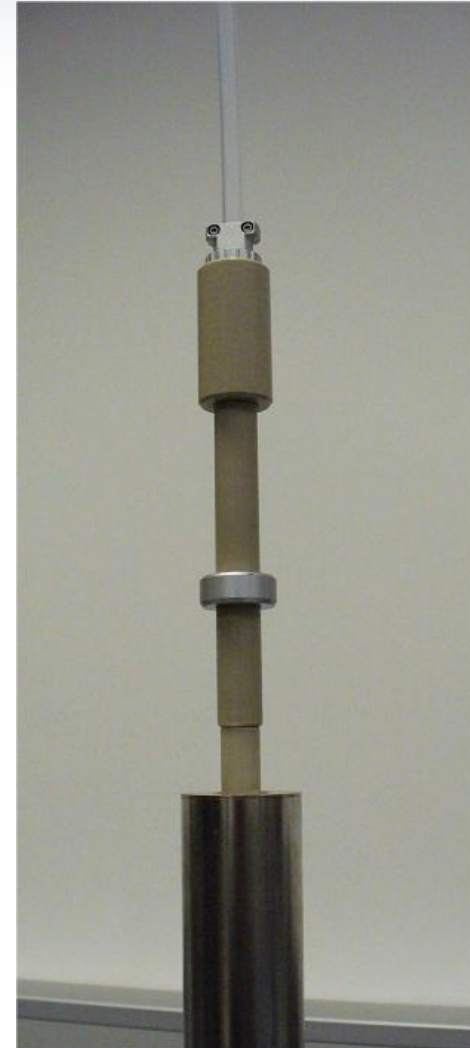
- 48 Positions per wellplate
- Full coverage and Isolation
- Top coverage shifting mechanism to suck up rotors and for back transfer into the wellplate
- Sucking Tip
- pin to check if rotor contained, open and close top wellplate cover lid
- Nitrogen degassing (prevents condensation)

SamplePro-HR-MAS™: Technical Realisation V



Sucking tube end at probehead

Length transfer-tube
maximum 10m



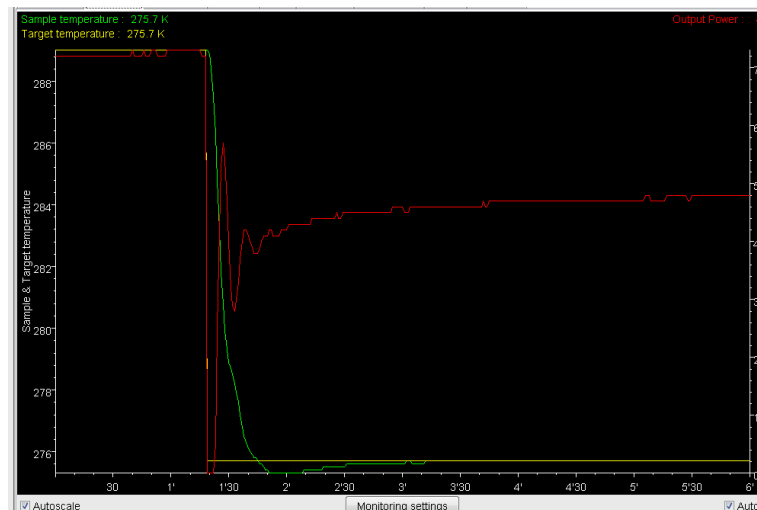
Temperature adjustment of frozen rotor in the probe (after transfer)



5 Minutes after transfer of the Rotor at -16deg. C the system is stable for measurement

Time includes:

- Transfer from cooled wellplate into the probe
- Start and adjustment of rotation
- Temperature stabilization to 0,1° accuracy

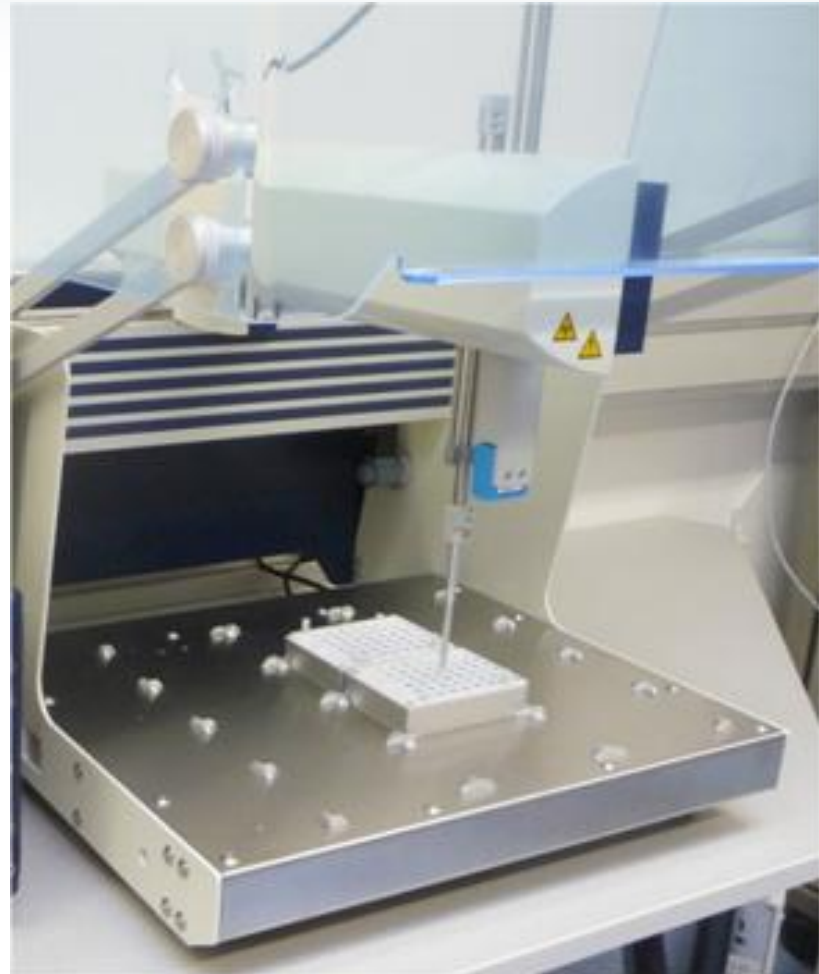


Rotation @4,5 kHz

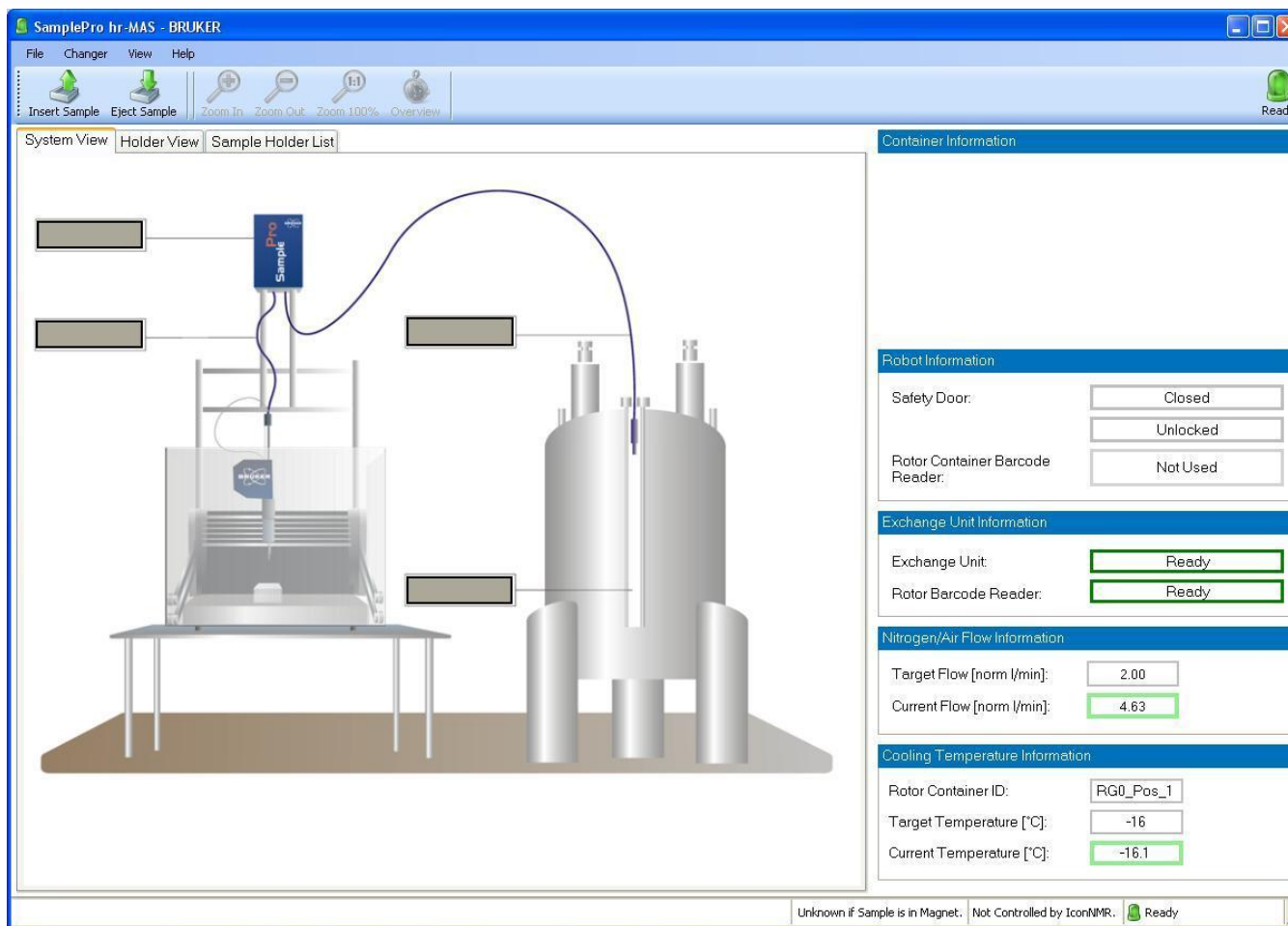
Version for Solids-NMR and non-temperature controlled HR-MAS samples



- Identical basic system
- Wellplates without temperature control and geometry as HR-MAS

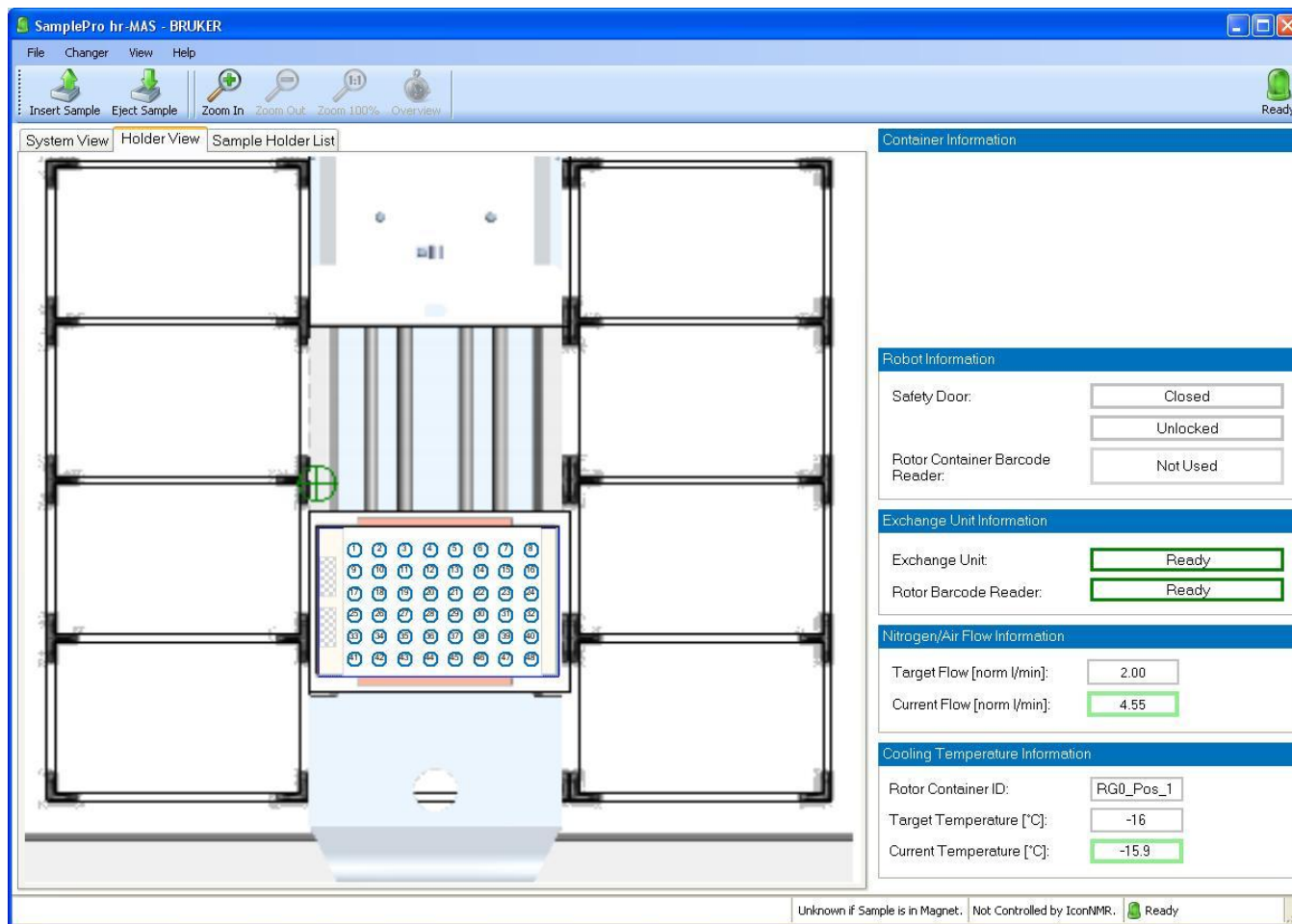


SamplePro-HR-MAS™ : Control Software



View:
Complete system

SamplePro-HR-MAS™ : Control Software

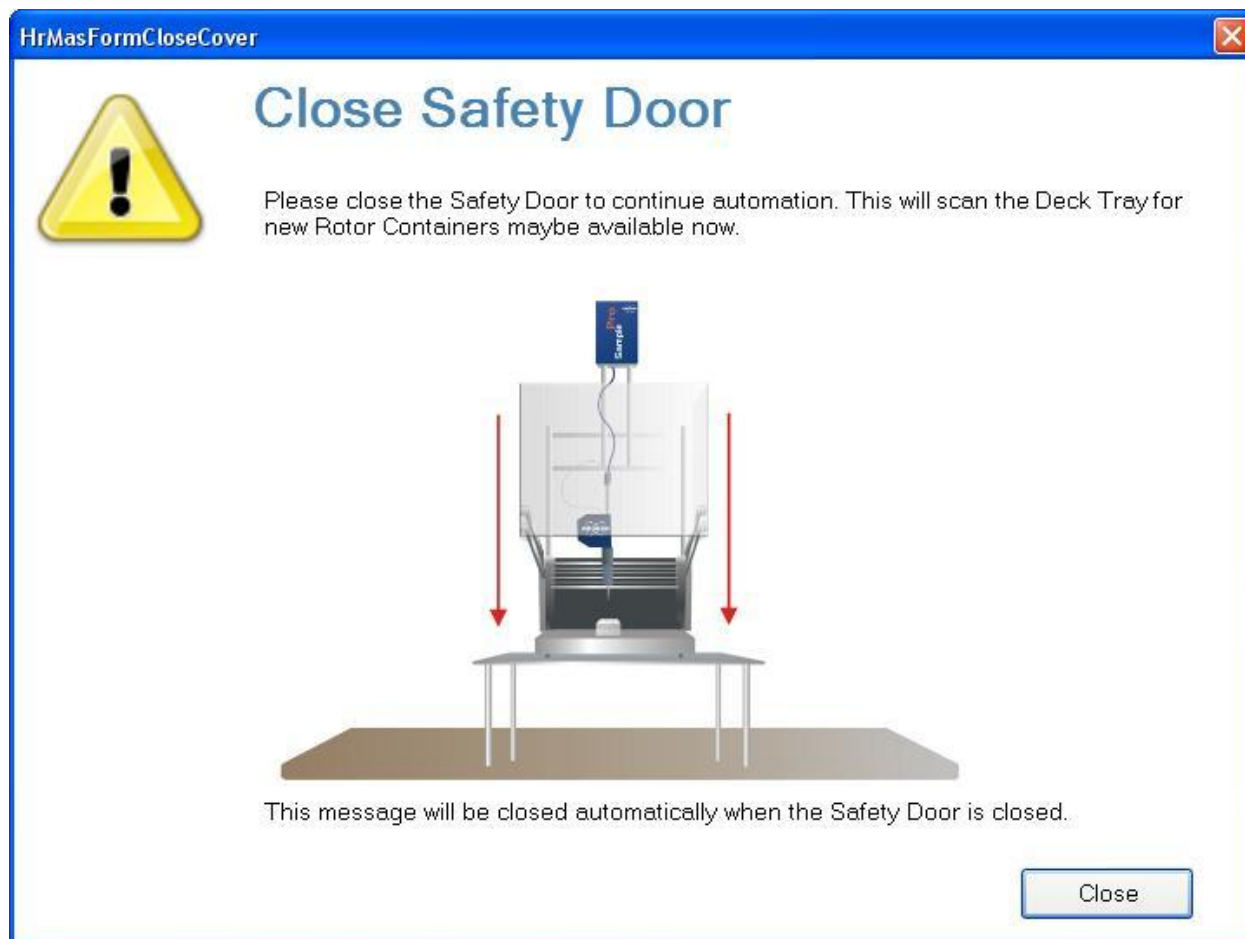


View:

Holders
(in wellplate)

Control Software

SamplePro-HR-MAS™



View:

Warning
Safety cover open

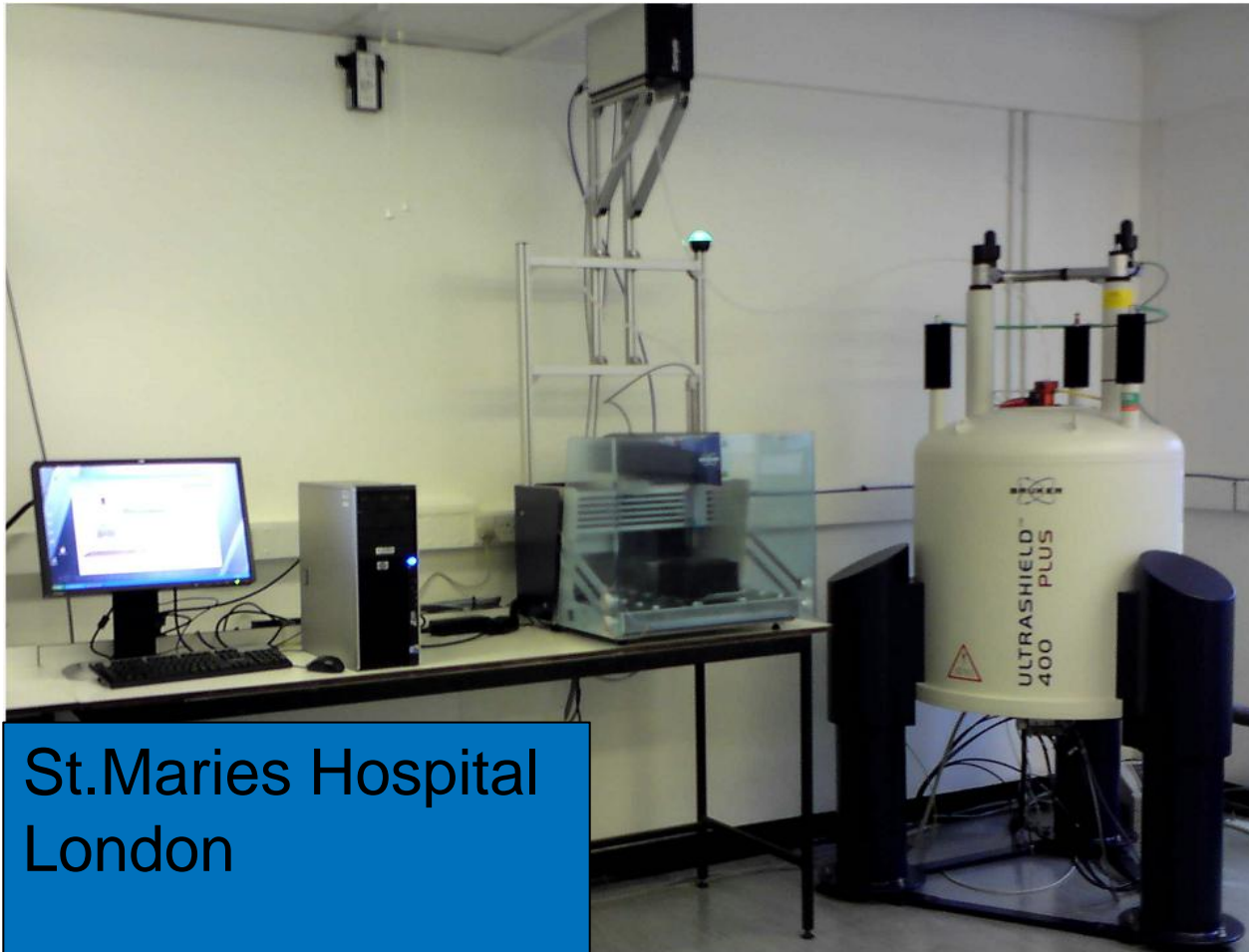
When closed
Check for new
Container occurs.

Installation at St-Maries Hospital London

SamplePro-HR-MAS™



Installed customer system

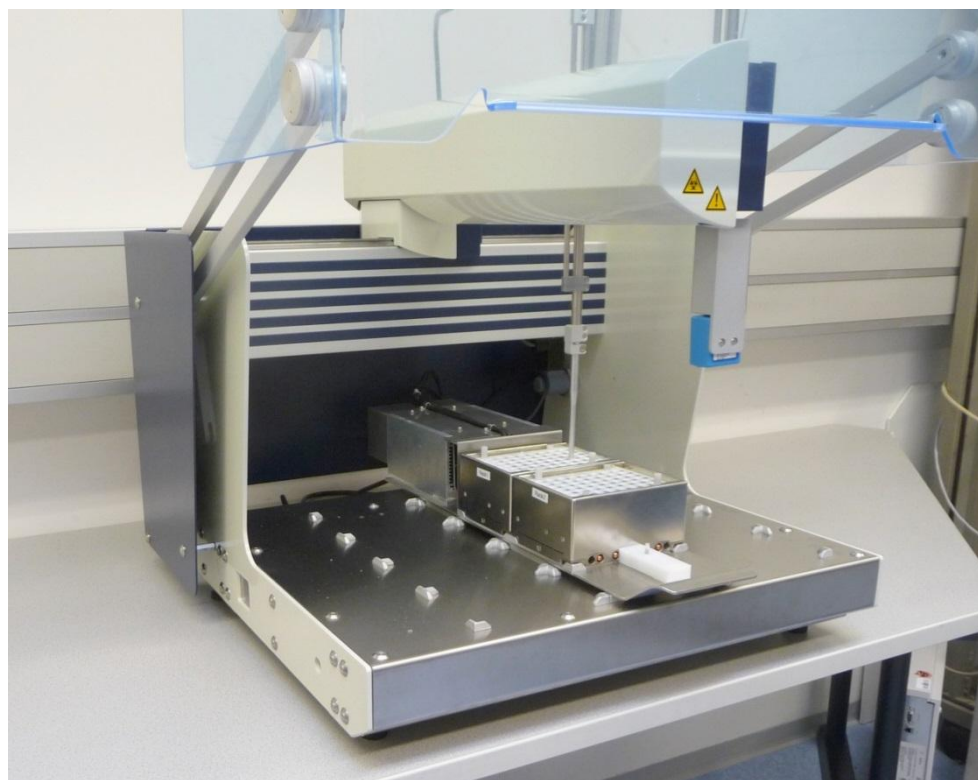
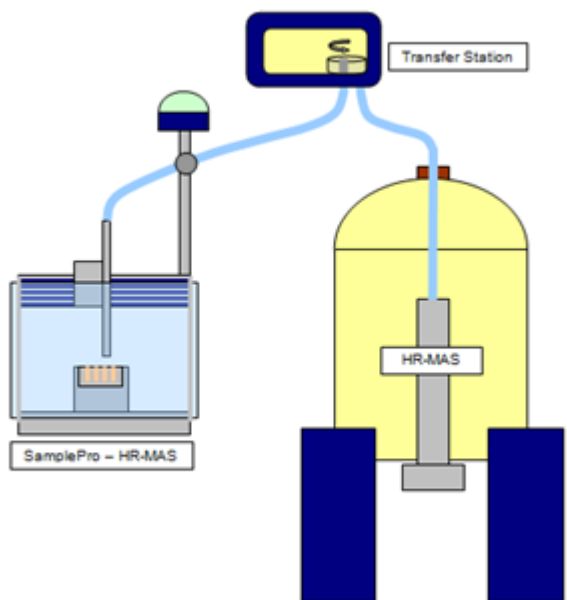


For direct support
of surgeons in the
operation theatre

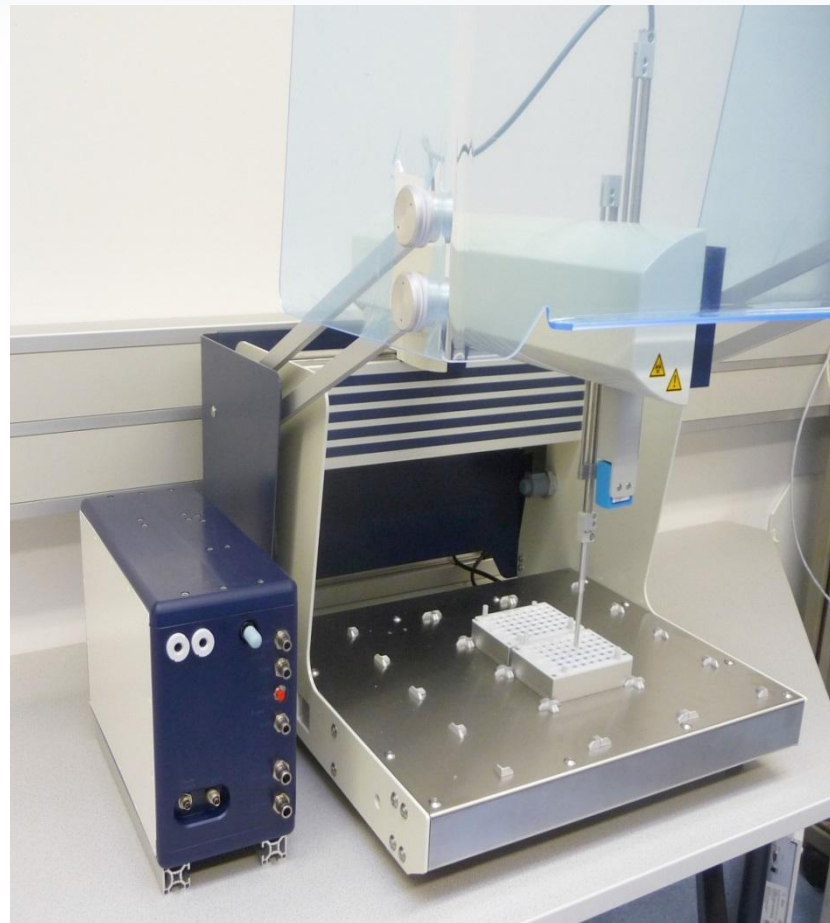
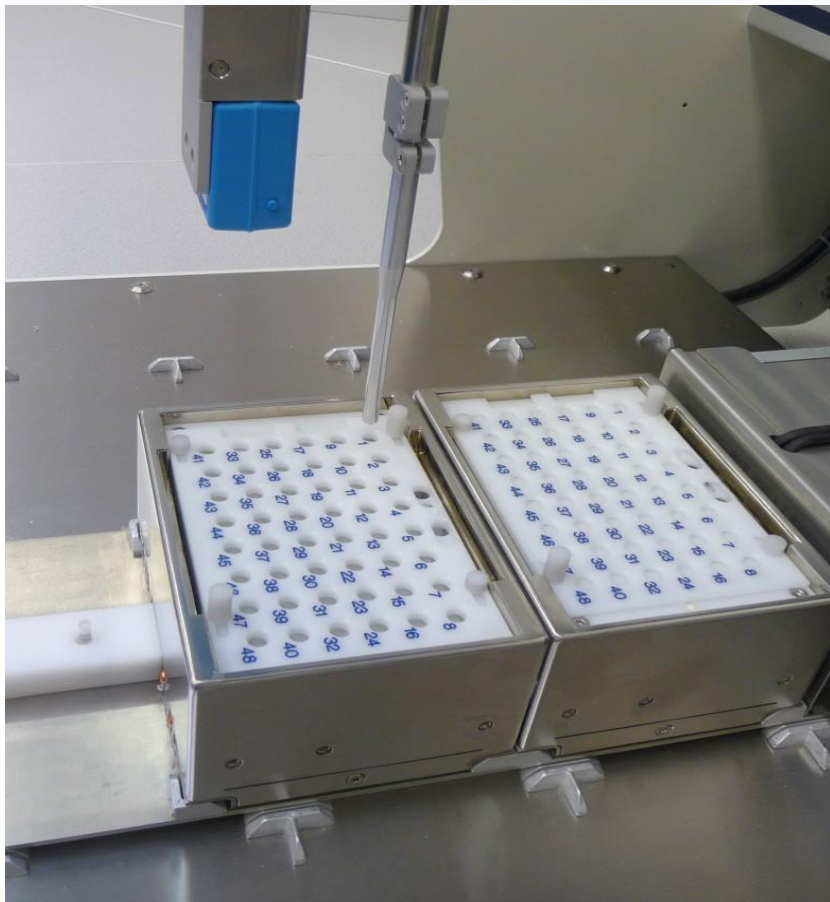
St.Maries Hospital
London

SamplePro – HR-MAS™

- First systems delivered to Hospitals
- Special cooling to -16°C available
- Fast sample Transfer using vacuum
- Barcodes

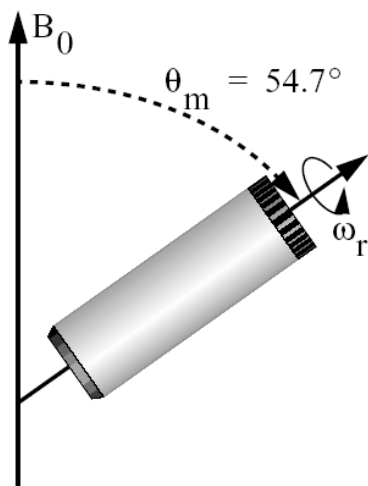


SamplePro – HR-MAS™



Back to NMR-HRMAS configuration

As the mobility is intermediate the configuration of the system is intermediate.



STATOR

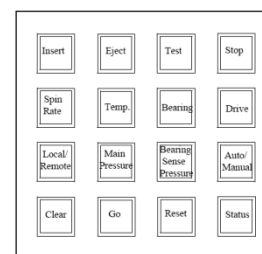
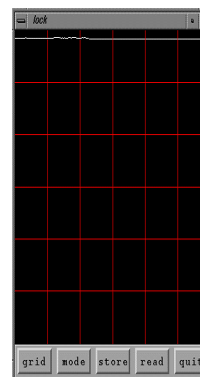


Figure 9.1. Front Panel Keypad

PNEUMATIC
UNIT



LOCK
CHANNEL

BACK TO THE HR-MAS PROBE



Properties of the HR-MAS probe:

- RF coil: Solenoid (different configuration)
- Gradient coil oriented at the magic angle
- MAS. Maximum spinning rate depends on rotor types (diameter, inserts, material,..).
- Sample insertion/ejection without removal of the probe.
- Magic angle adjustment with micrometer screw at probe bottom.
- Optical spin rate counter.



^1H inverse MAS ($^1\text{H}/^{13}\text{C}/^{15}\text{N}/^2\text{H}/\text{grad}$)

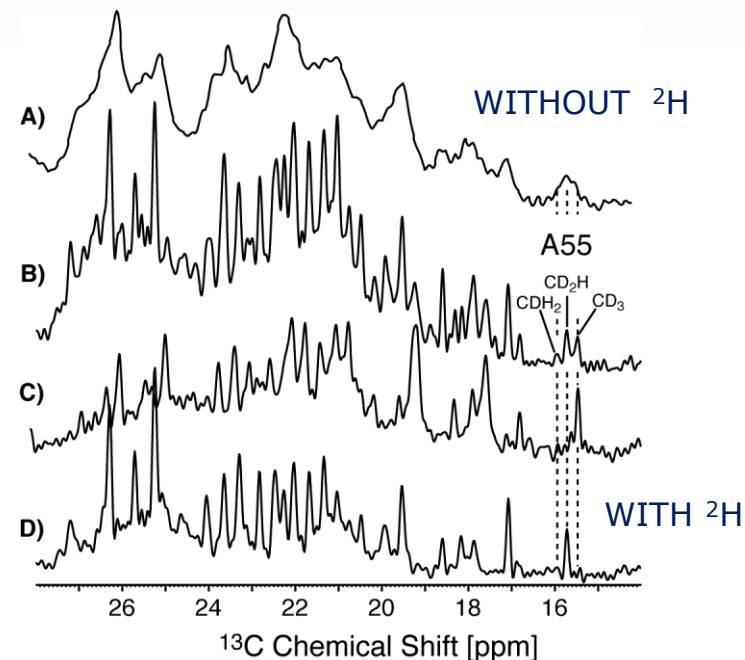
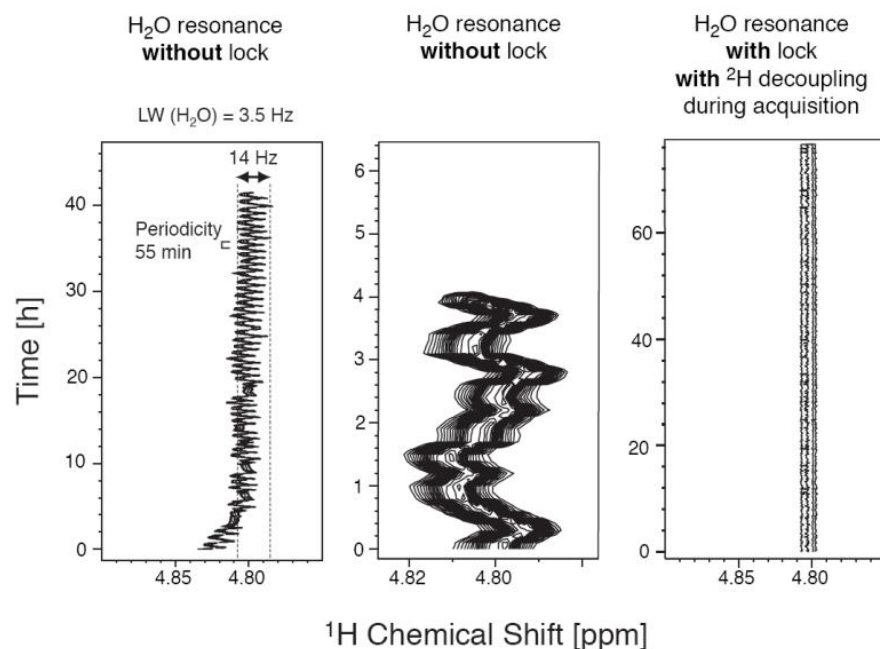


- high-sensitivity ^1H circuit
- 2D and 3D heteronuclear (mainly ^{13}C and ^{15}N) cross correlation experiments
- ^2H lock channel (internal).
- **RF power levels common for solid-state NMR**
- **magnetic RF amplitudes and RF field homogeneities required in experiments with biomolecular solid samples.**
- internal ^2H lock channel can be used for weak ^2H heteronuclear decoupling.
- coil system for pulsed field gradients

^1H inverse MAS ($^1\text{H}/^{13}\text{C}/^{15}\text{N}/^2\text{H}/\text{grad}$)

Internal ^2H lock in MAS probes

RF configuration: $^1\text{H}/^{13}\text{C}/^{15}\text{N} + ^2\text{H}$ lock

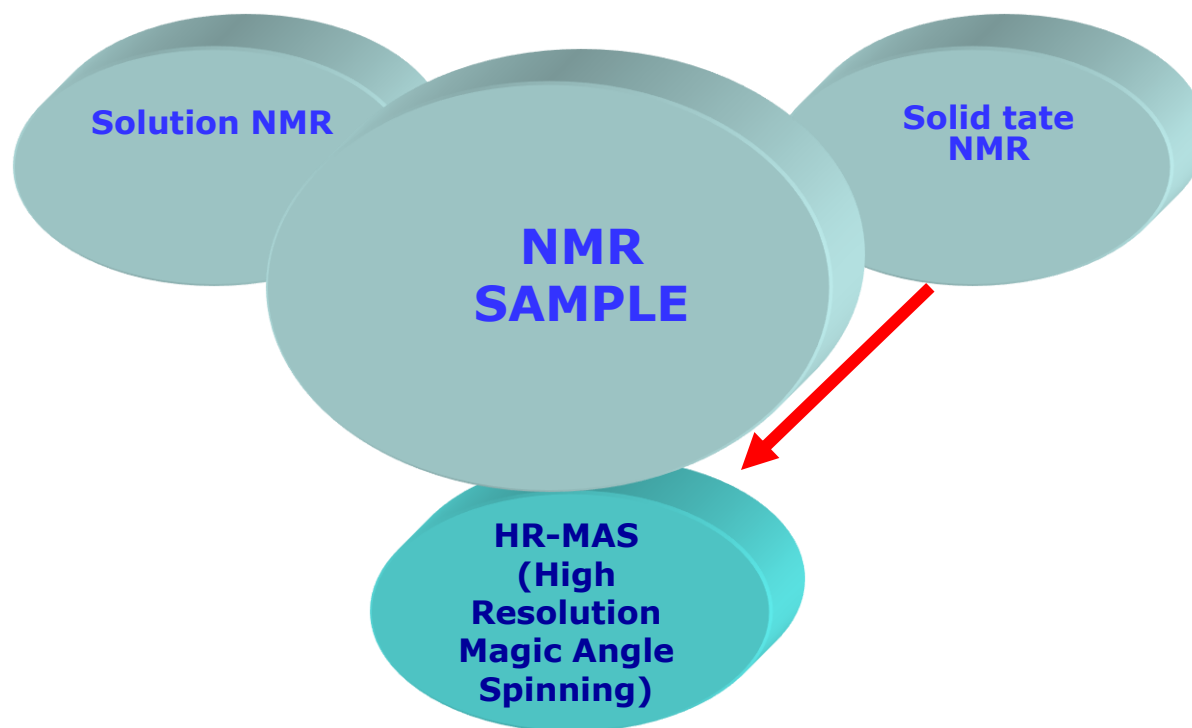


600WB 4mm MAS H/C/N
with orthogonal coils
in MAS stator for ^2H lock

^{13}C -NMR spectra of partially deuterated α -SH3 protein (aliph. region), lockchannel used for weak ^2H -decoupling.
(data courtesy of Bernd Reif, TUM, FMP)

CONCLUSION

High Resolution NMR can be used also for heterogeneous samples, but technology is developing.....



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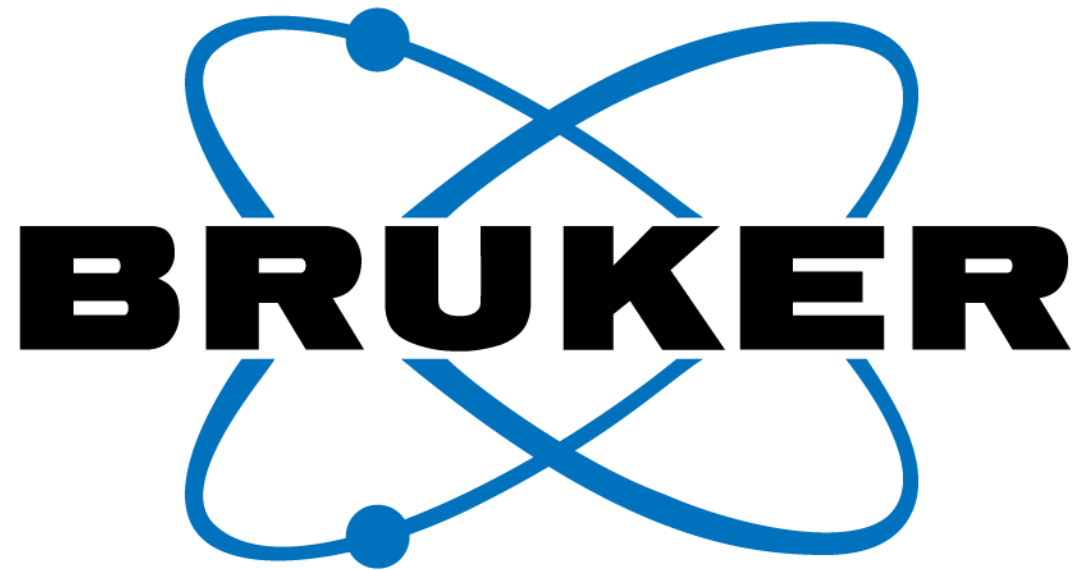


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