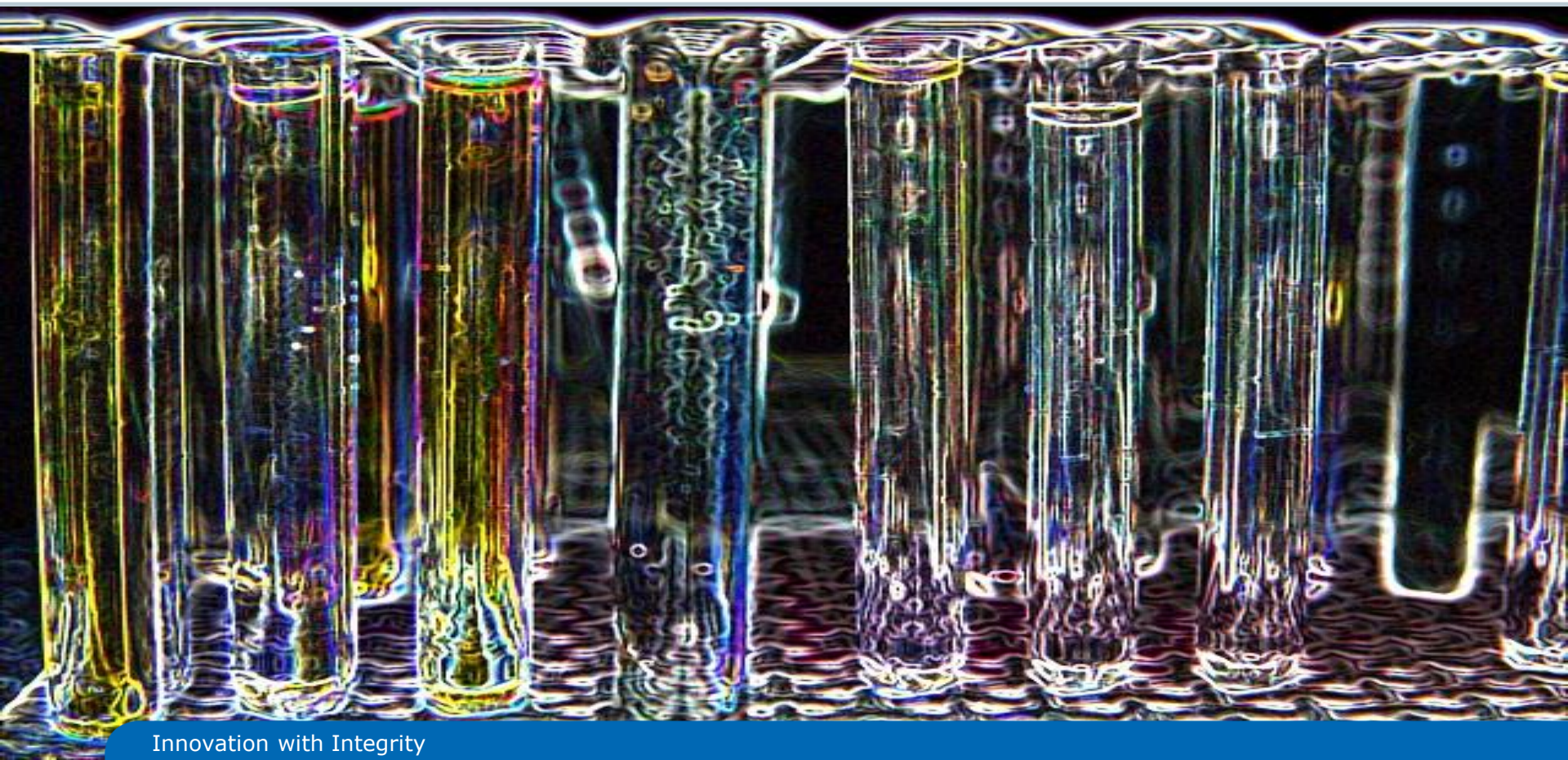
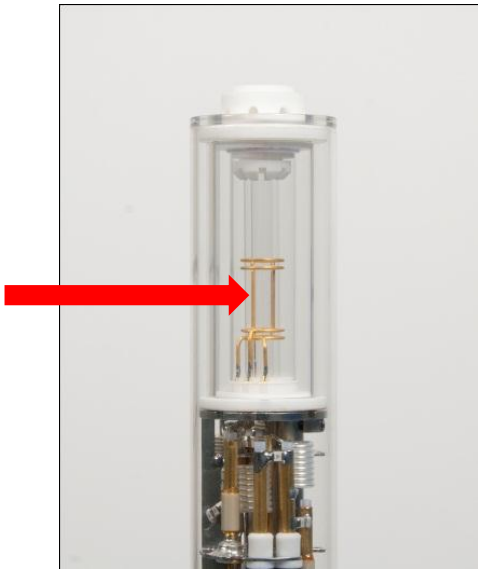
AVANCE III HD

Dr. Detlef Moskau, Bruker Biospin Switzerland



AVANCE III HD – NMR Thermometer
Where to measure sample temperature?

High Resolution

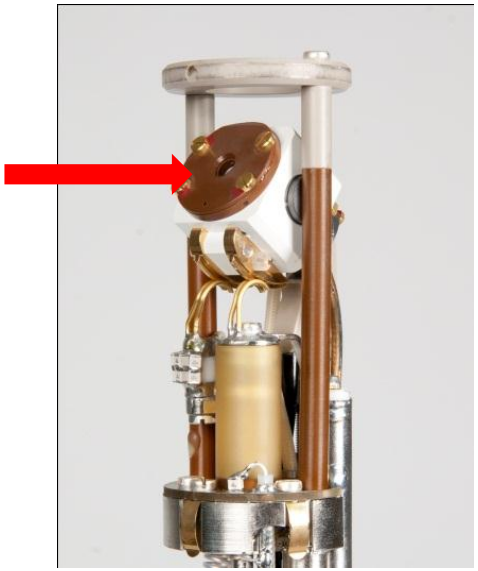


H_2O: ~ 0.01ppm / K
@600MHz = 6Hz / K

RF Loading 0 to ~2K

0 – 12 Hz shifts

HR-MAS



Other heating effects

MAS spinning speed

up to 5 - 20K

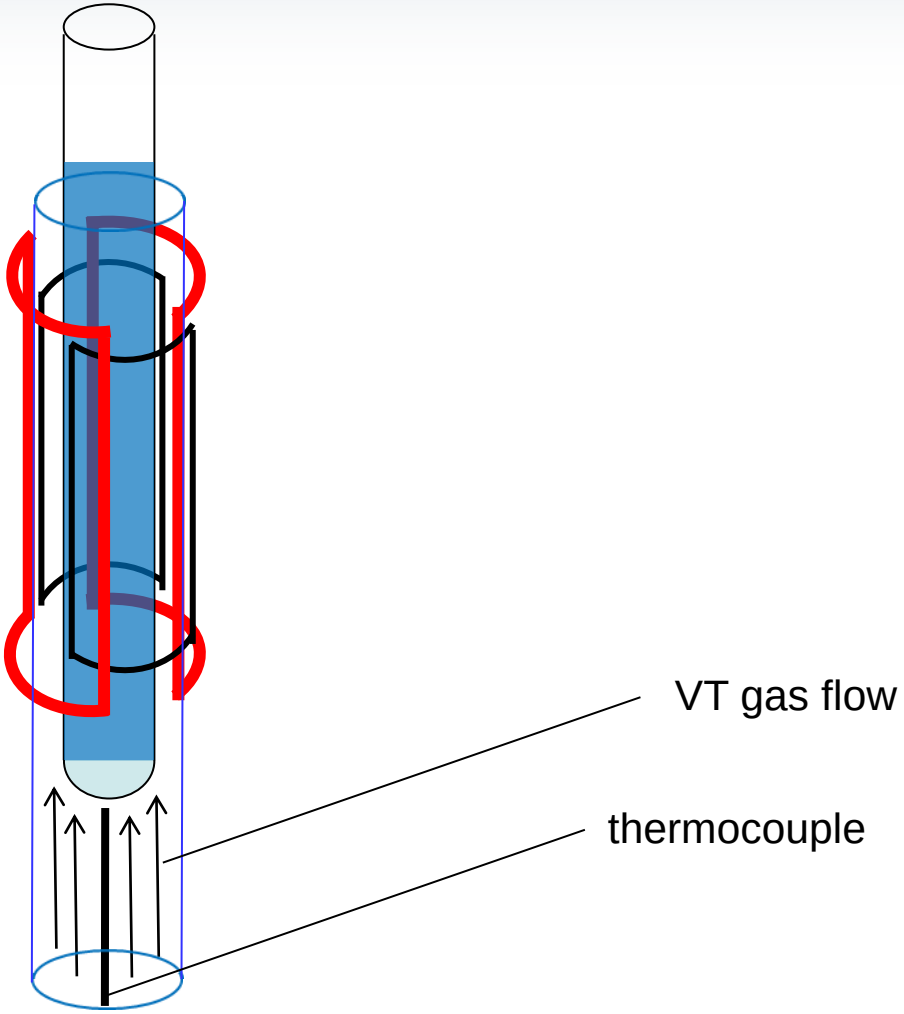
AVANCE III HD – NMR Thermometer
Where to measure sample temperature?

RT probe:

- Sample & RF coil are in VT gas flow
- ~roughly at same temperature

CryoProbe:

- Sample in VT gas flow
- RF coil in vacuum at
  ~15-25 K (He-cooled ***CryoProbe***)
  ~90 K (liq. nitrogen cooled ***Prodigy***)



AVANCE III HD – NMR Thermometer
Motivation

Precise / constant / repetitive temperature is essential:

- Dynamics
- Kinetics
- Diffusion / DOSY

- Compare chemical shifts / coupling patterns:
  - experiments recorded at different spectrometers
  - experiments recorded many weeks/month later

- Remove internal sample heating effects (rf load /MAS)

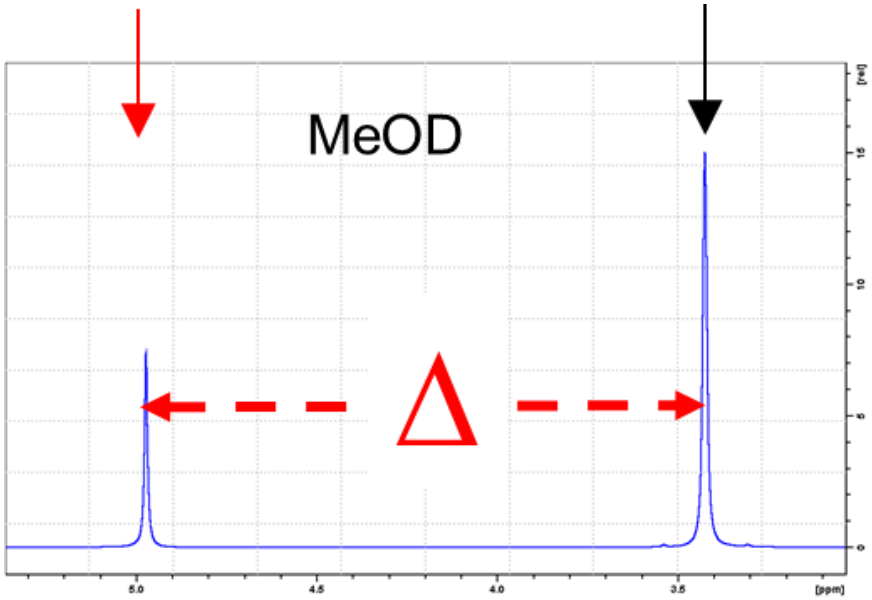
AVANCE III HD – NMR Thermometer
Motivation

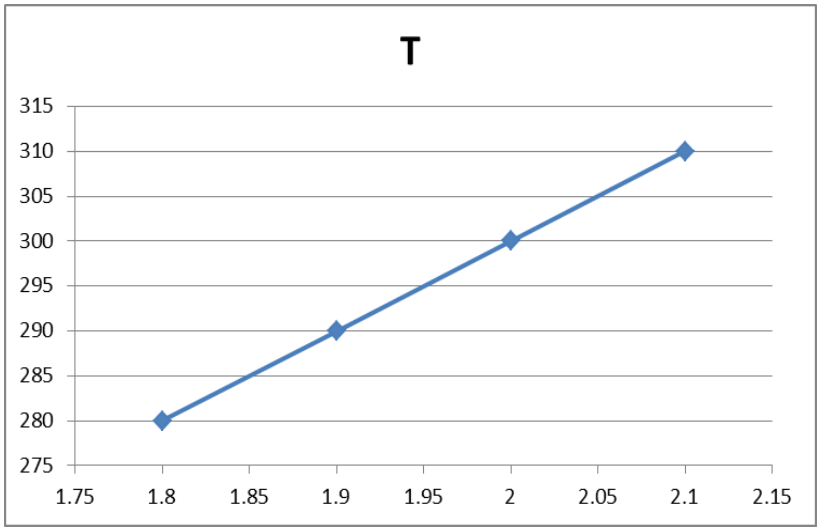
Minimizing the effect of rf-heating in multidimensional NMR experiments

A.C. Wang, A. Bax, Journal of Biomolecular NMR, 3 (1993) 715-720

"As a consequence, when different experiments are recorded at the same set temperature, the actual temperatures can be substantially different. The resulting changes in chemical shift are frequently nonuniform and can seriously hamper the effectiveness of automated resonance-assignment procedures"

AVANCE III HD – NMR Thermometer
The principle





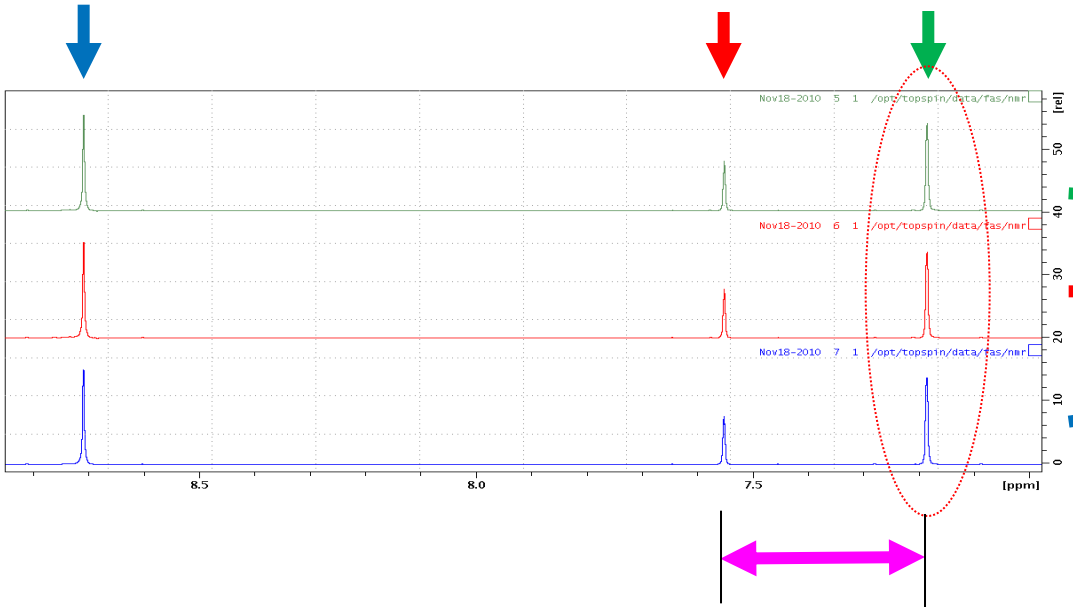
1. Measure distance

2. Calculate temperature

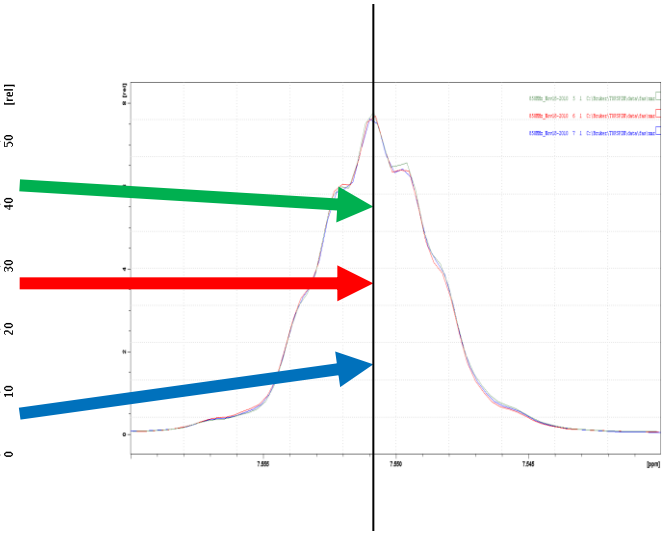
AVANCE III HD – NMR Thermometer
Ability to easy lock on complex ^{2}H solvents

Lock on multi-peak solvents ..

.. also under full automation

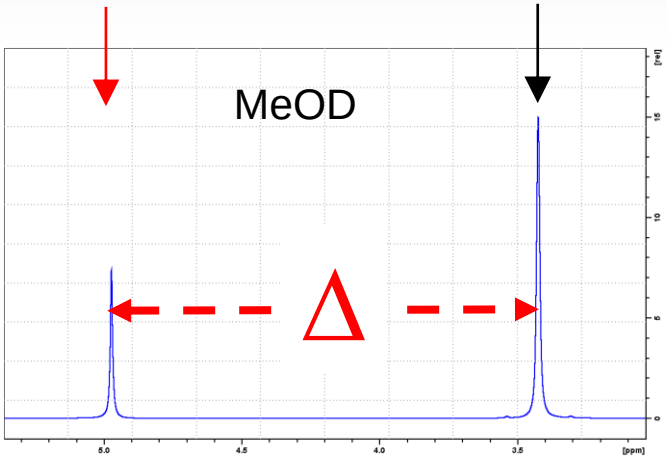


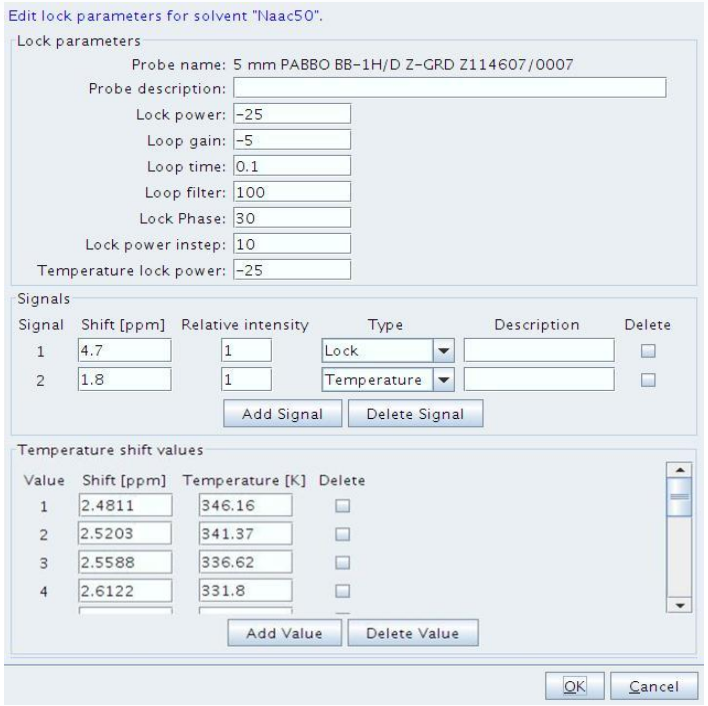
.. 50Hz !



1H spectra of pyridine-d5 99.5% deuterated, locked on different 2H signals, 5mm TCI850

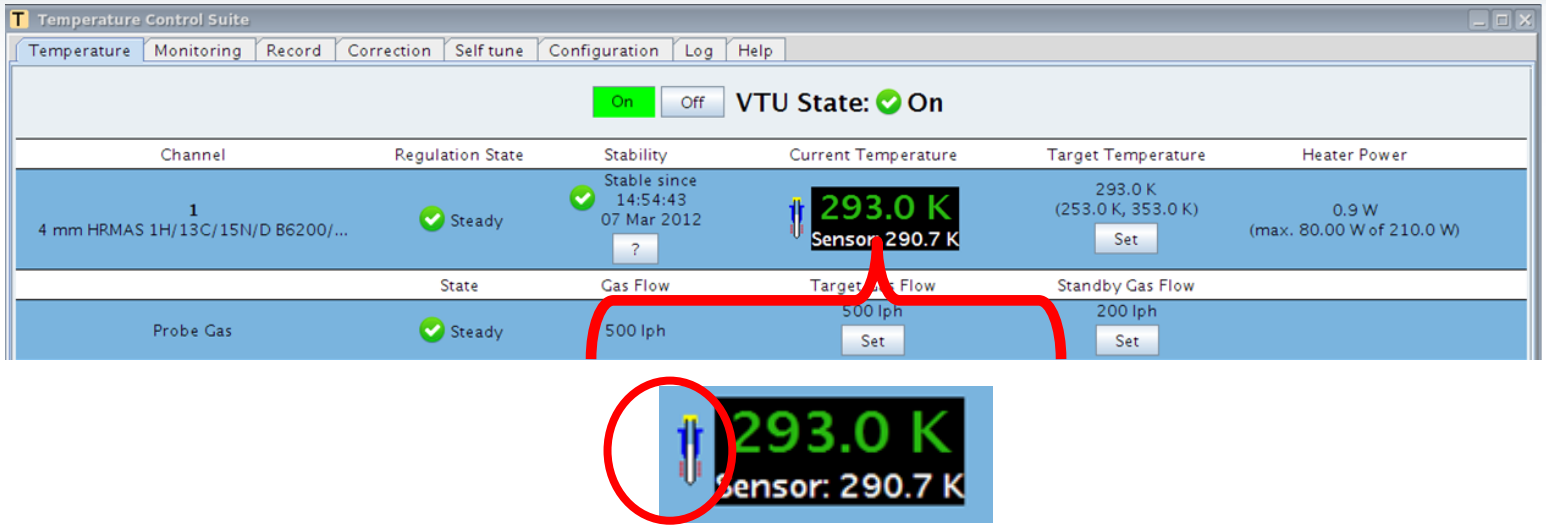
AVANCE III HD – NMR Thermometer
Measure the temperature with the spins





Sample with thermo-sensitive ^{2}H compound

AVANCE III HD – NMR Thermometer
Topspin User Interface



Benefits NMR Thermometer:

- **Measure**
- **Maintain**
- **Monitor**

} **temperature within the sample**

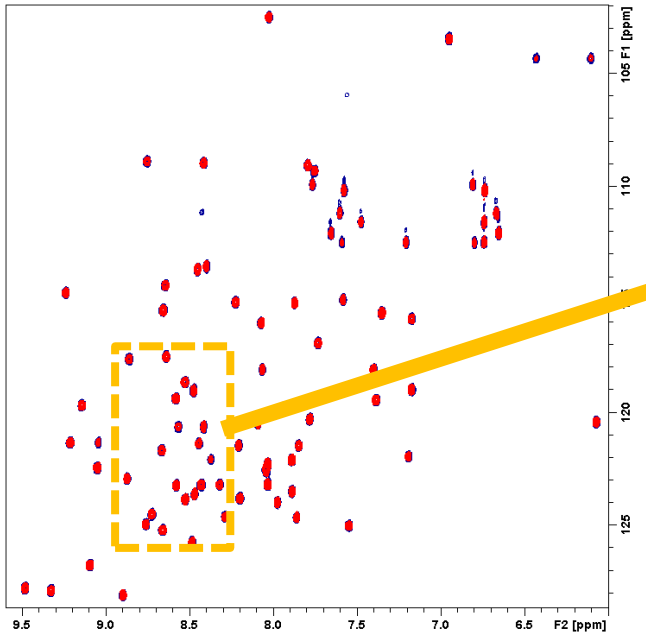
AVANCE III HD – NMR Thermometer

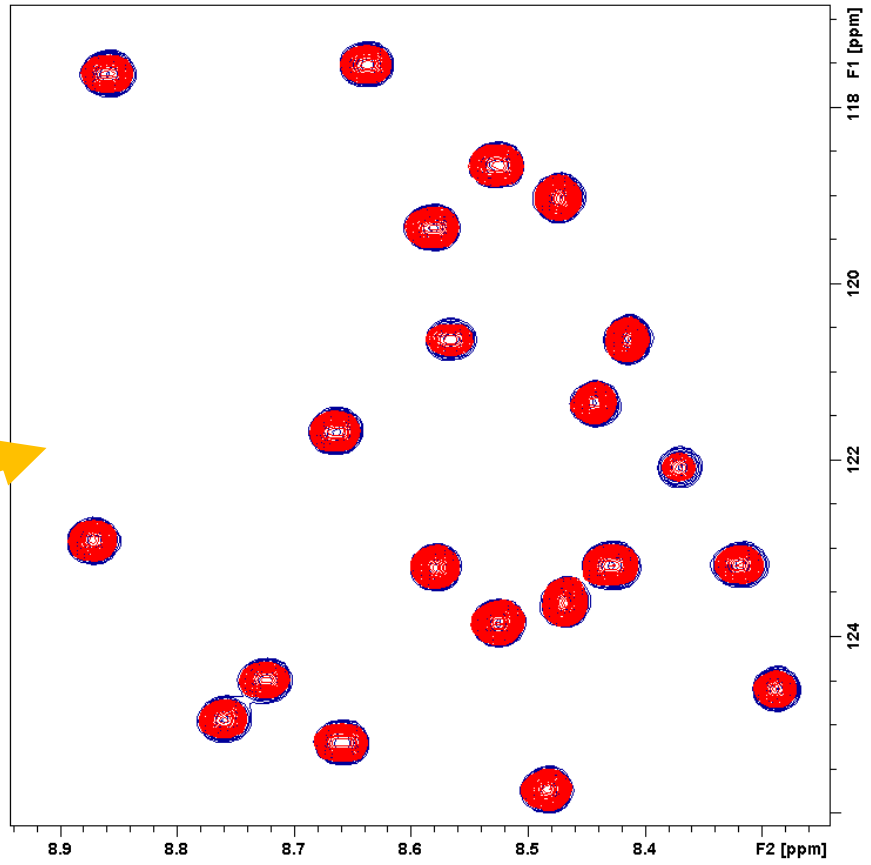
Monitoring temperature

AVANCE III HD – NMR Thermometer
Same temperature on instruments

600 MHz RT TXI / 800 MHz TCI CryoProbe

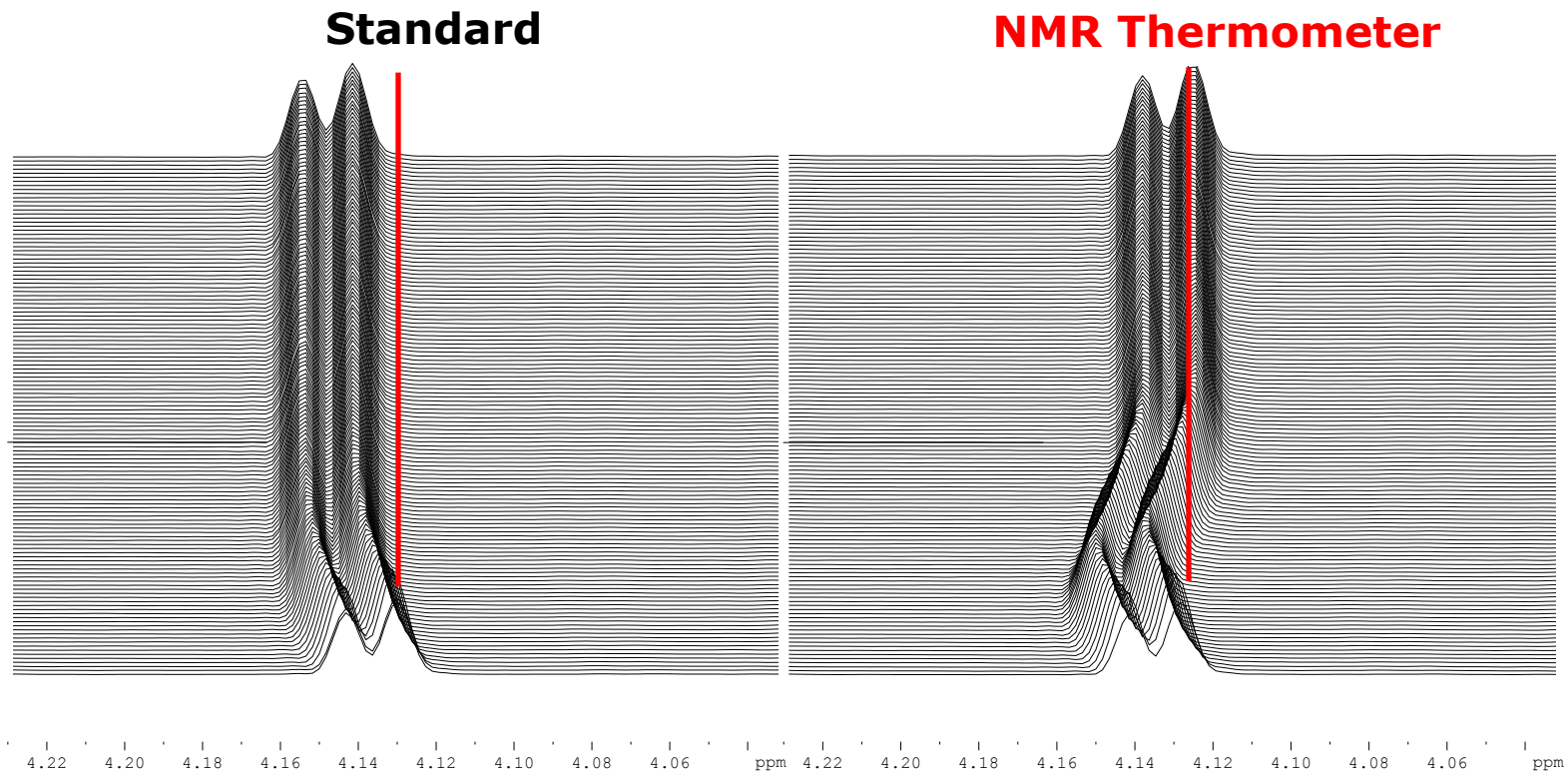
Identical chemical shifts





HSQC spectra of 0.5mM ubiquitin in D_2O and ~50mM NaAc-d3

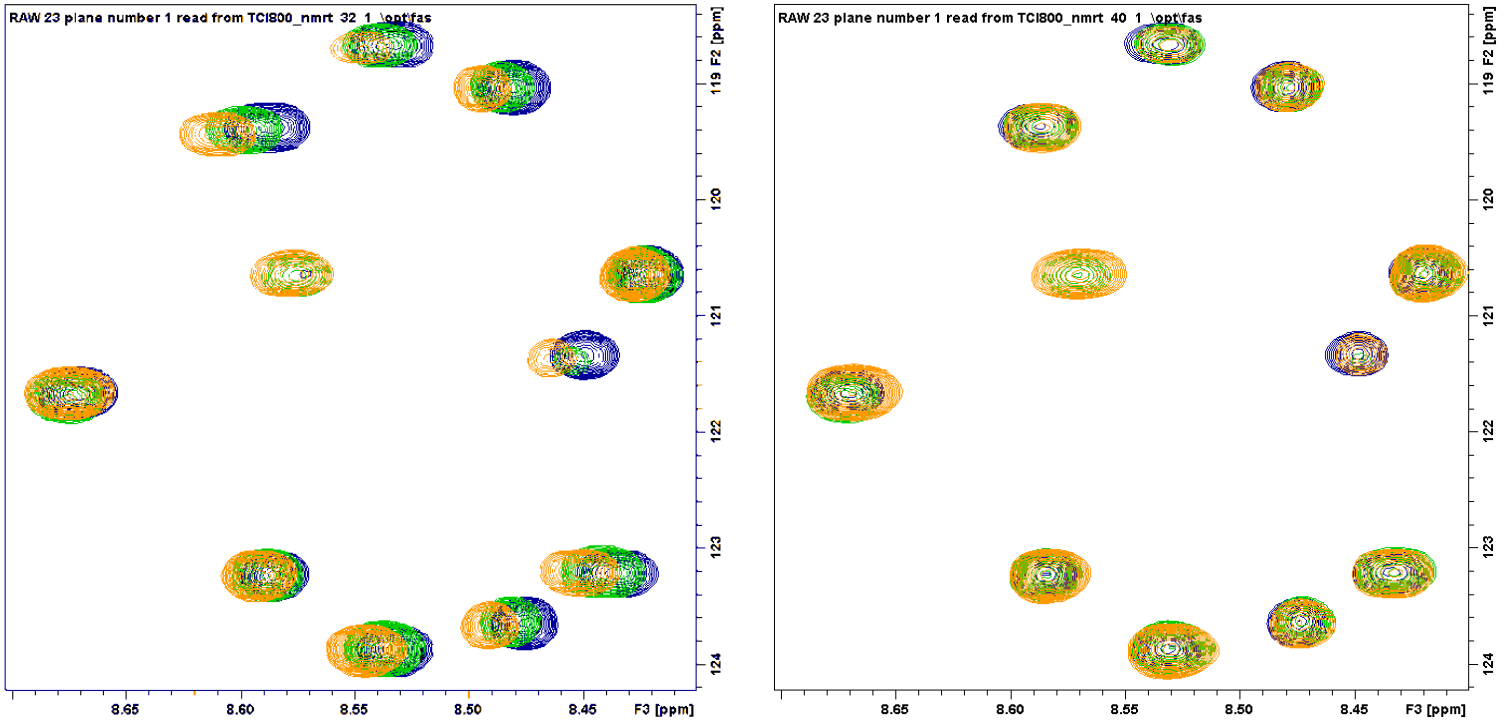
AVANCE III HD – NMR Thermometer
Maintaining the sample temperature



Spectra of 10 mM raffinose in D_2O with ~50 mM NaAc-d3

AVANCE III HD – NMR Thermometer
RF load effects

NOESY-HSQC TOCSY-HSQC ^{15}N CPMG-HSQC (T_2-determination)



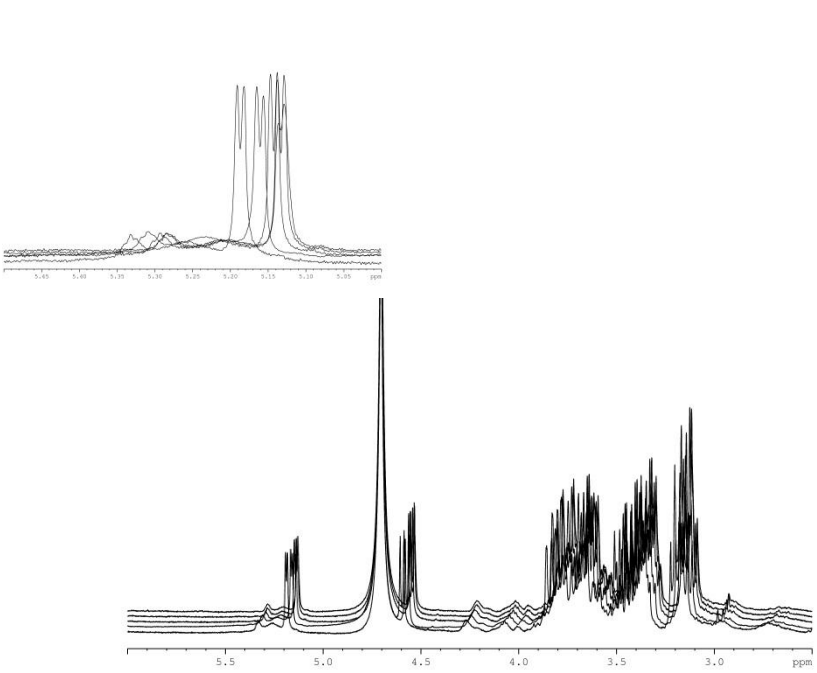
Standard **NMR Thermometer**

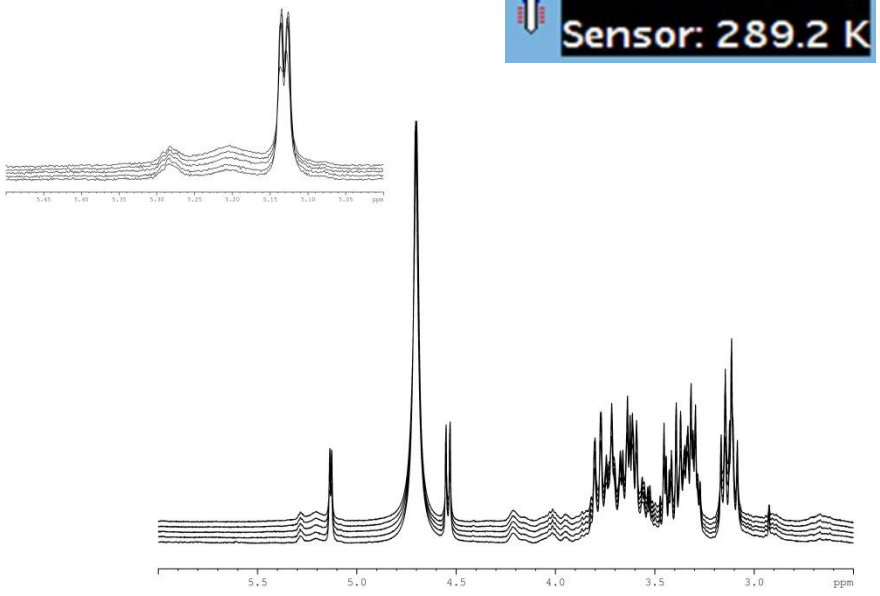
Spectra of 0.5 mM ubiquitin in D_2O with ~50 mM NaAc-d_3, Avance-III HD 800 MHz

AVANCE III HD – NMR Thermometer Example on Tissue NMR

Unique: Constant temperature with HR-MAS applications!

MAS spinning 1, 2, 4, 6 and 8kHz





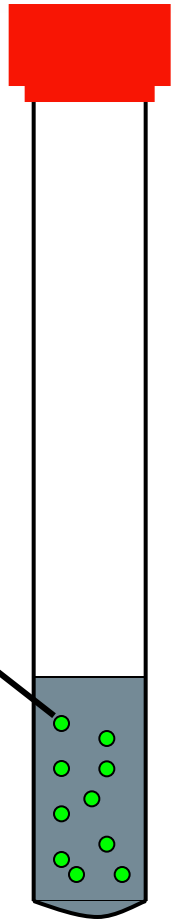
400 MHz ^{1}H liver spectrum, ~500mM NaAc-d3 in D_2O added

Possible setups

Combined tube

NMR-T. compound
in separate tube
(inside or outside)

NMR-T.
compound
dissolved

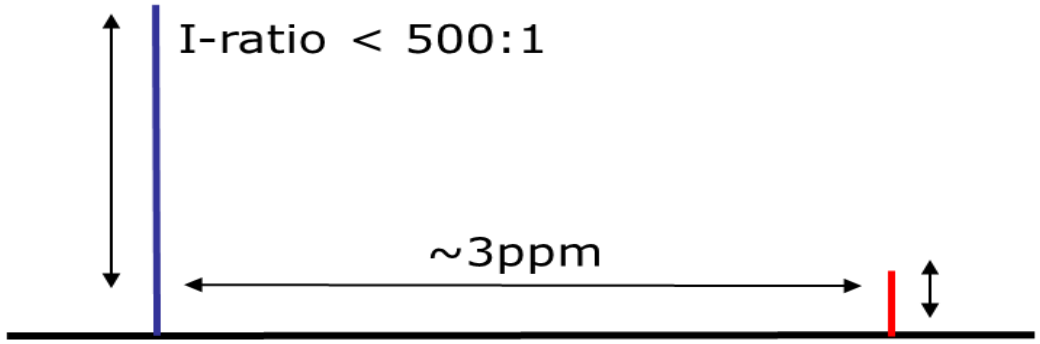


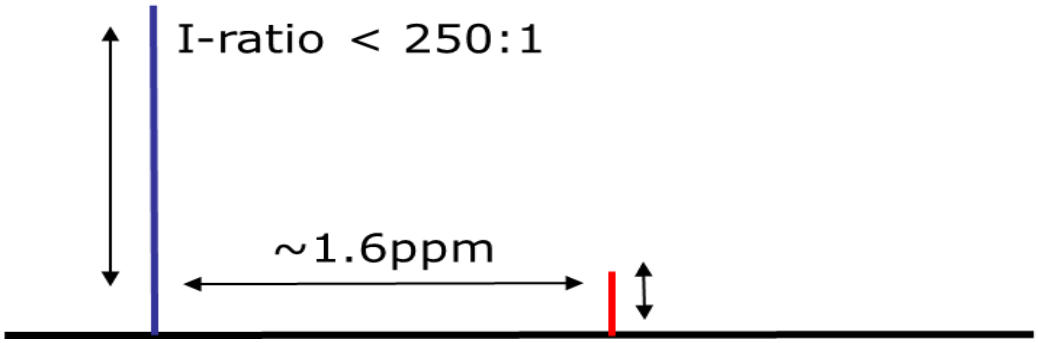
AVANCE III HD – NMR Thermometer
Thermometer compounds

- Large number of deuterons: the larger the number of chemically equivalent deuterons, the lower is the required concentration.
- Possibly one additional ^{2}H signal.
- Moderate or no salt effect
- The compound should not interact with the sample or change essential structural properties of the sample.
- Acceptance. Difference compounds are commonly added to protein solutions.
- Price and availability.

AVANCE III HD – NMR Thermometer
Dynamic range

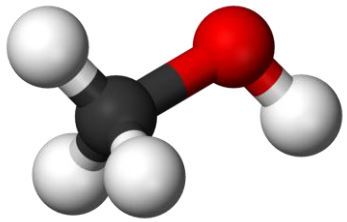
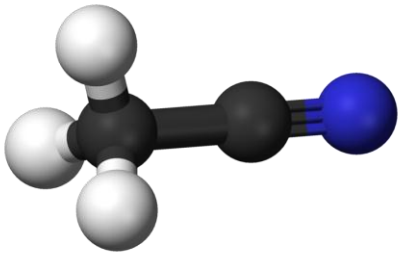
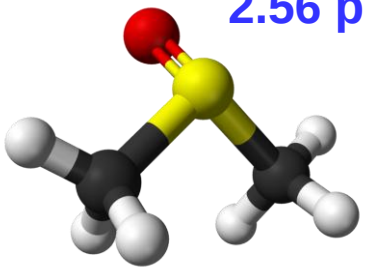
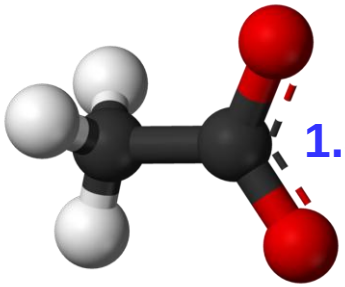
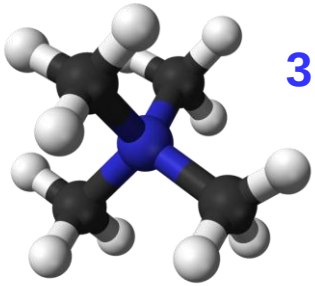
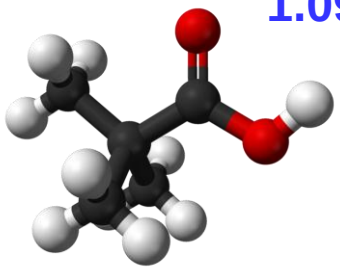
Concentration ratio of compound used for field and frequency lock





AVANCE III HD – NMR Thermometer
Here are the spins for Water

Compounds (typical concentrations 10 – 50 mM)

CD_3OD 3.30 ppm 	CD_3CN 1.93 ppm 	CD_3SOCD_3 2.56 ppm 
$CD_3COO^-Na^+$  1.80 ppm	$(CD_3)_4N^+Cl^-$  3.00 ppm	$(CD_3)_3CCO_2^-Na^+$ 1.09 ppm 

AVANCE III HD – NMR Thermometer
Here are the spins for Organic Solvents

Possible solutions for organic solvents

Solvent methanol-d4:

- Directly suitable for NMR Thermometer

Other solvents:

- Any solvent: external thermometer compound like D_2O in capillary.
- Add D_2O to solvents which can be mixed with water, like DMSO and acetone.

AVANCE III HD – NMR Thermometer
Thermometer compounds

T_MeOD :	**methanol-d4**
T_H2O+D2O+NaAc:	**sodiumacetate-d3 in 90% H2O, 10% D2O**
T_ H2O+D2O+Me4NCl:	**tetramethylammoniumchlorid-d12 in 90%, H2O, 10% D2O**
T_ H2O+D2O+Pivalate:	**pivalic acid-d9 sodium salt in 90%, H2O, 10% D2O**

AVANCE III HD – NMR Thermometer

New NMR Thermometer™:

- Accurate sample temperature
- Perfect chemical shifts match
- Measure the temperature with your spins!

Acknowledgement

R&D:

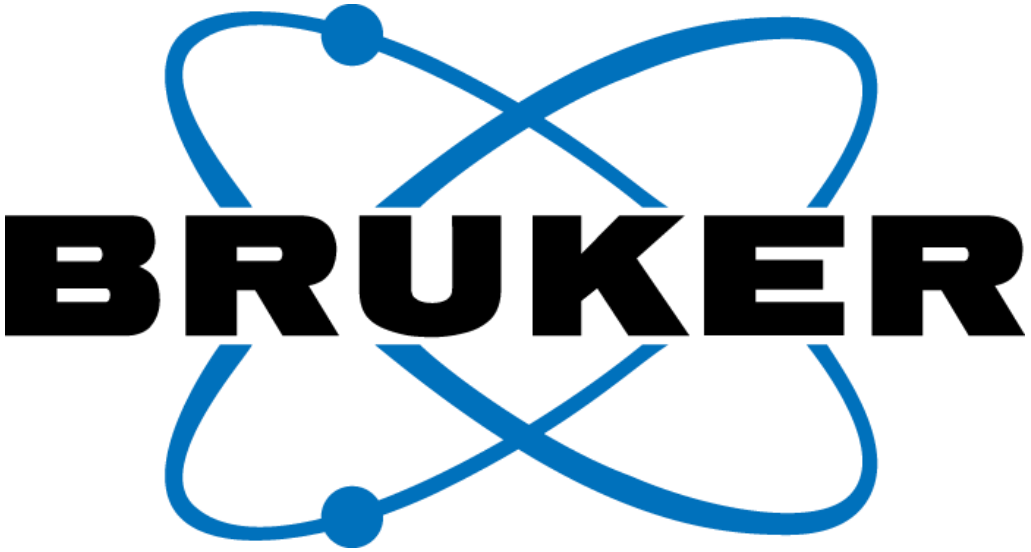
Pietro Lendi, Rolf Hänsel

Application:

Frank Schumann, Clemens Anklin, Wolfgang Bermel, Daniel Matthieu

Gerhard Wagner

Sebastian Hiller



Innovation with Integrity