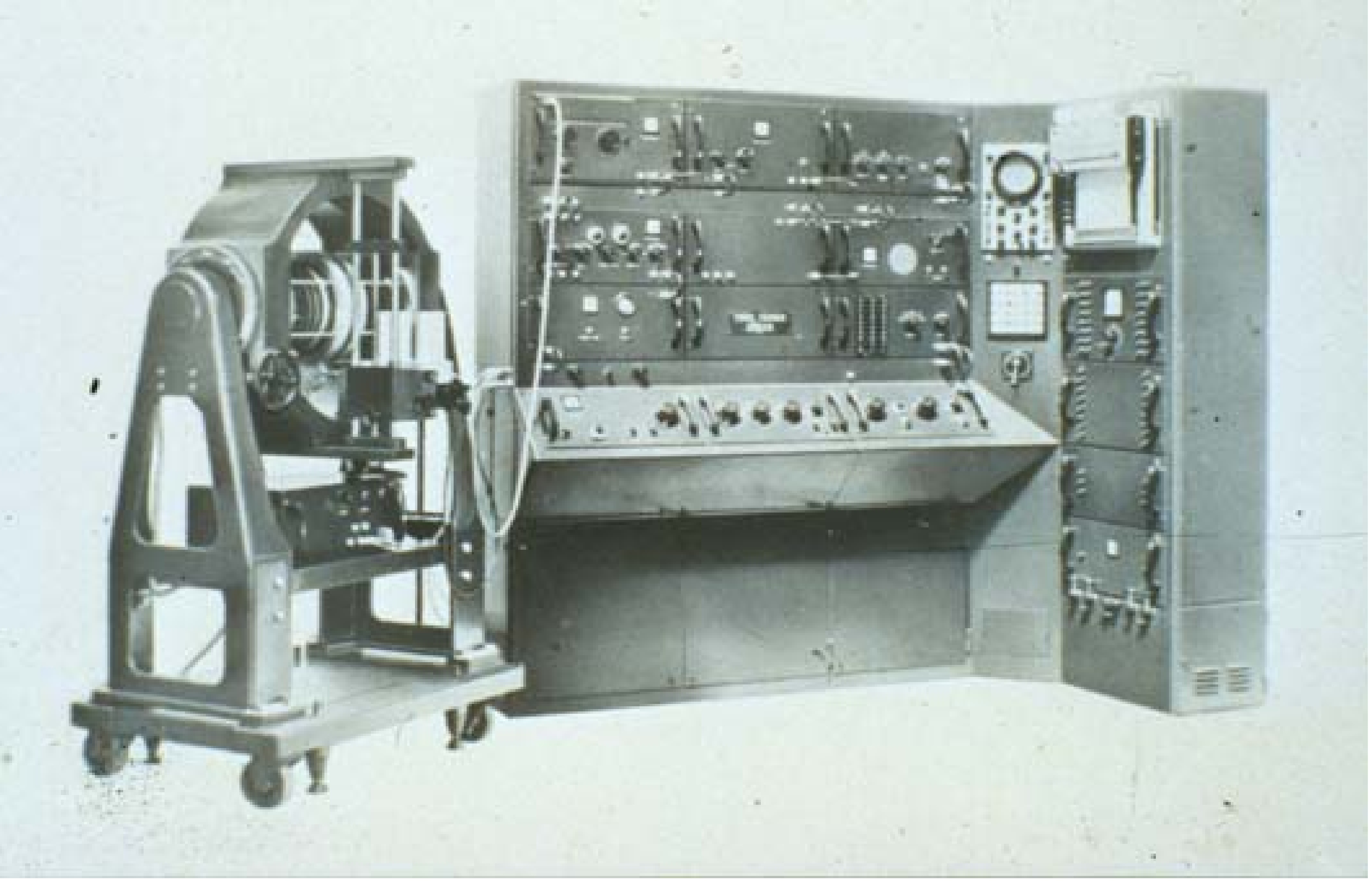
NMR now and then
-
Sensitivity, Magnets, Technology

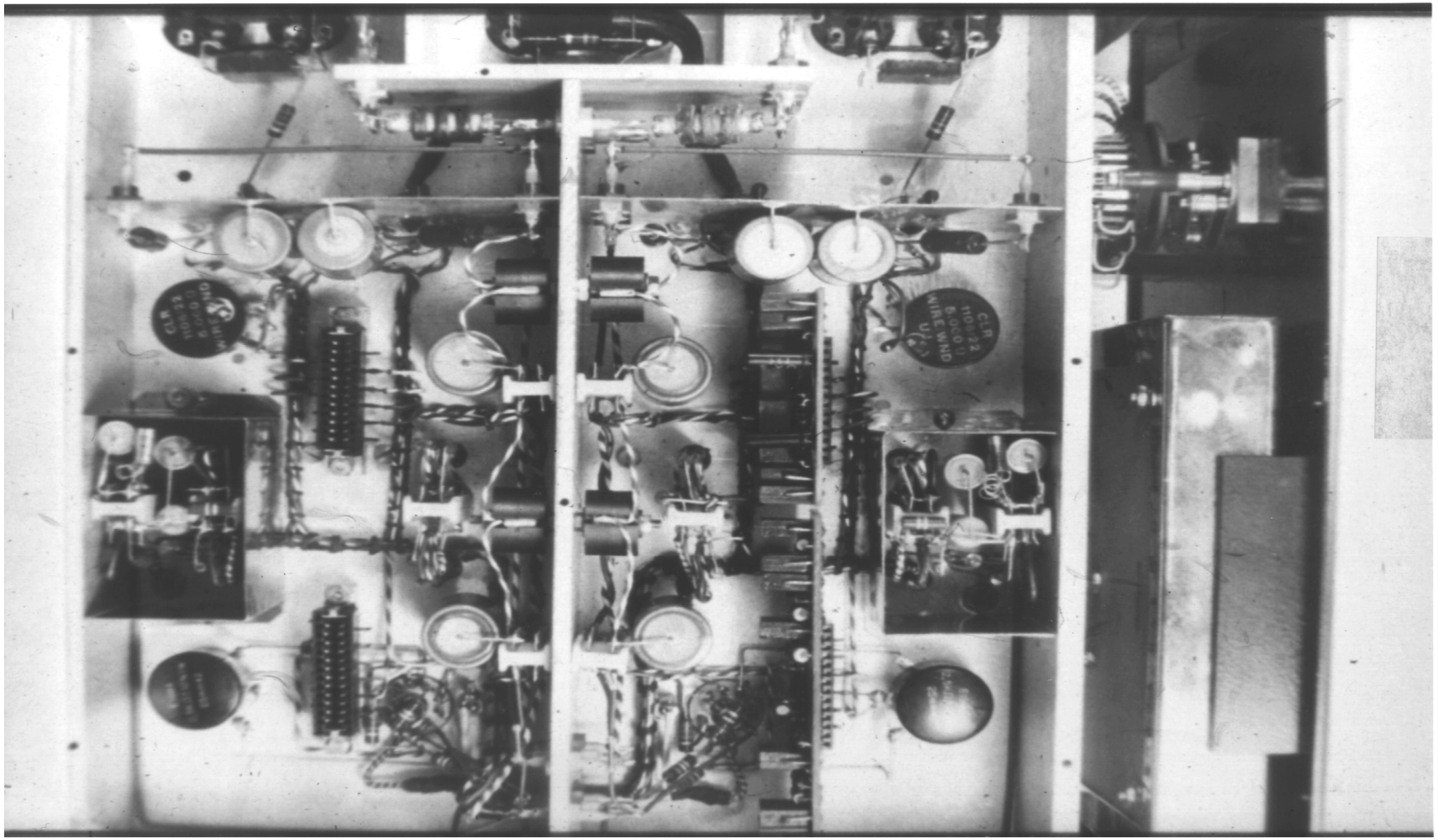
Rainer Kerssebaum, Bruker BioSpin, Germany

1960: KIS 25



Trüb Täuber in cooperation with ETH Zurich

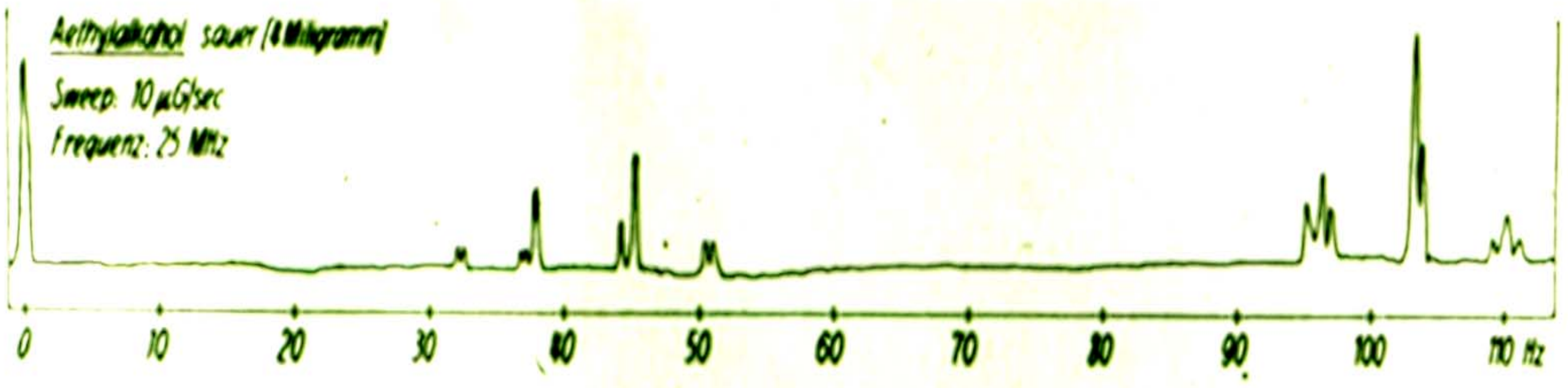
NMR with Tubes



KIS 25 Spectrometer

Highest Resolution 1960

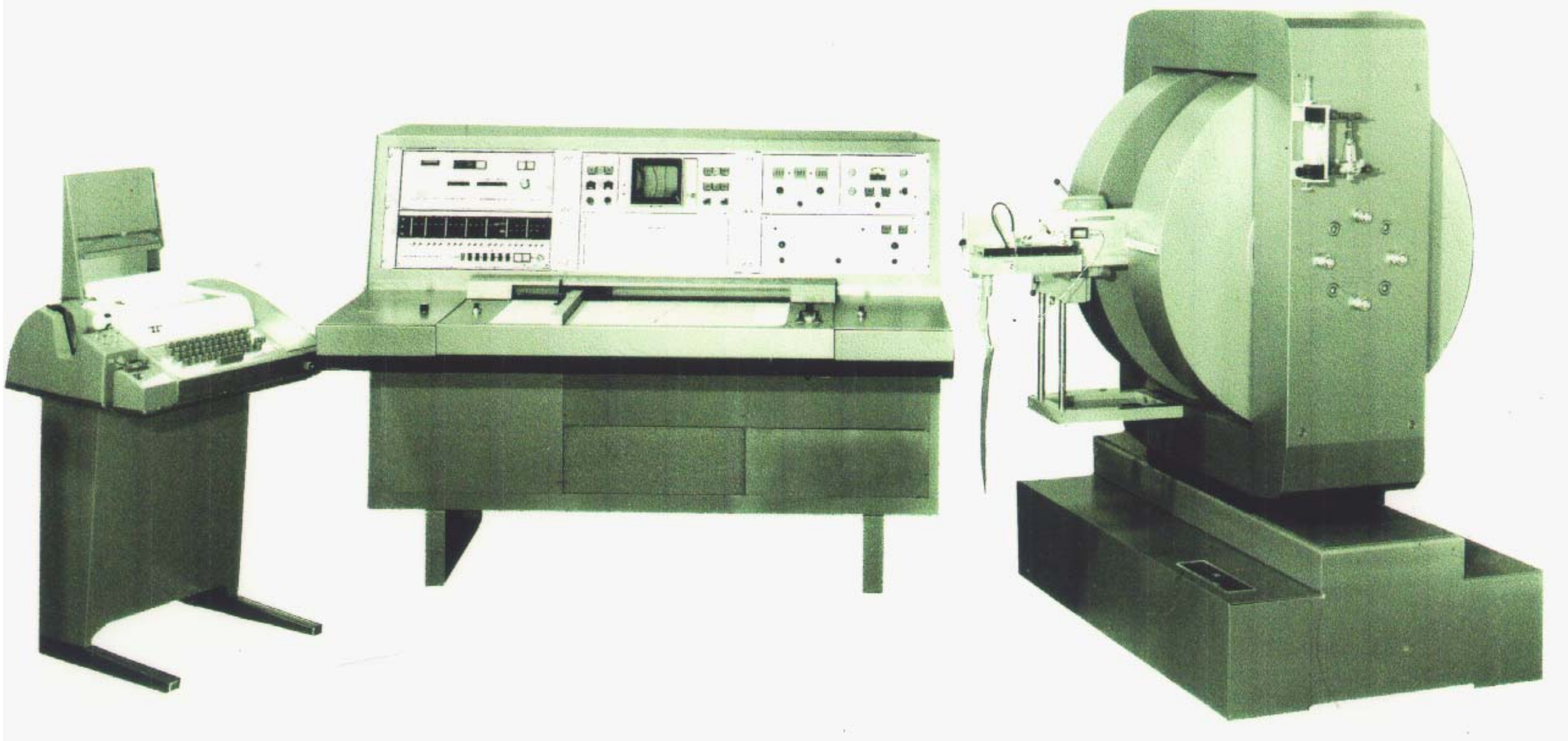
12 h measuring time, for stability reason at night



Ethyl alcohol slightly acid

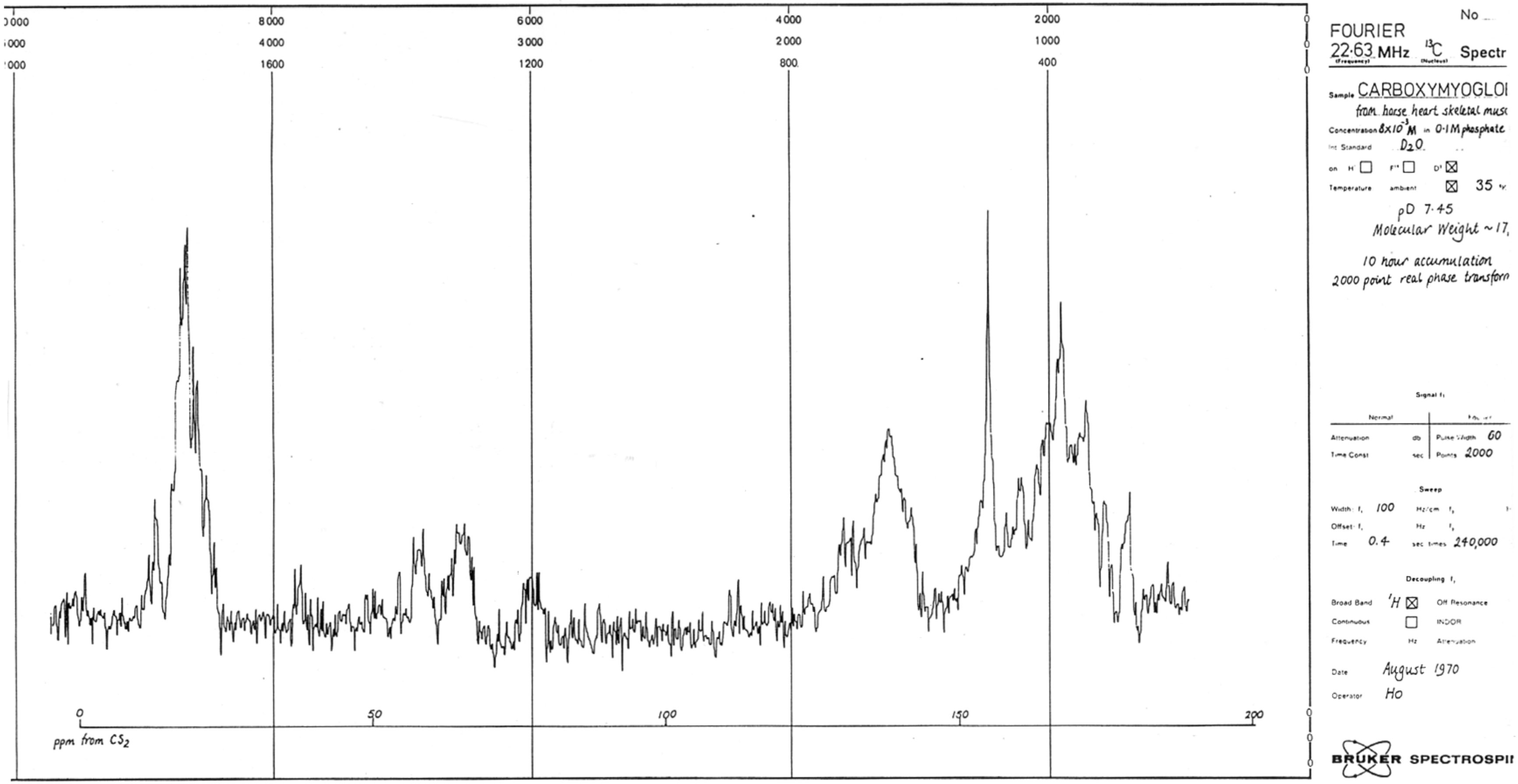
Courtesy of Prof. Richard Ernst

1972: WH90 - First FT only System



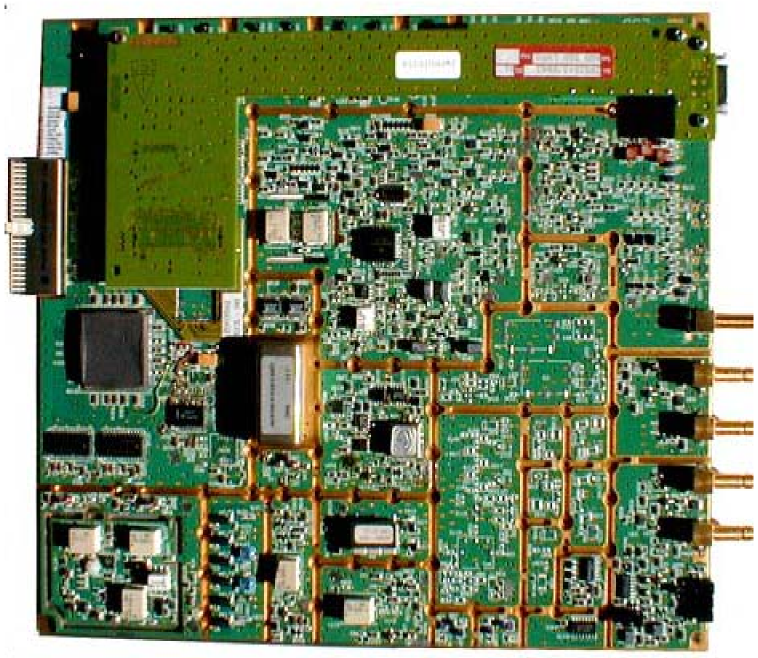
Ancestor of all modern Spectrometers

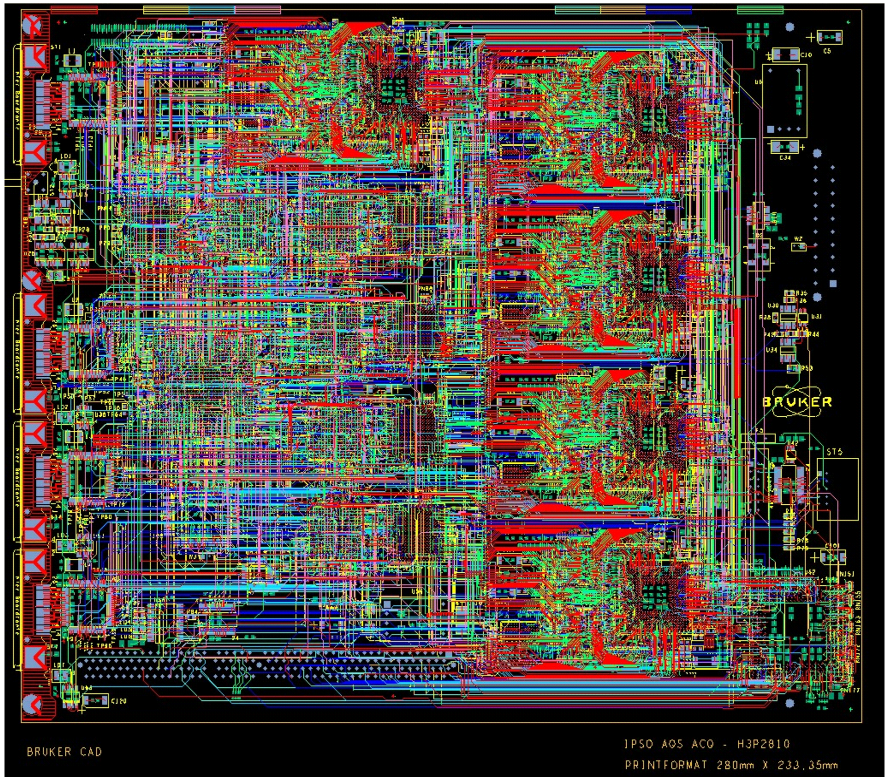
^{13}C on Myoglobin



Bruker BioSpin

Modern electronics: SGU, IPSO





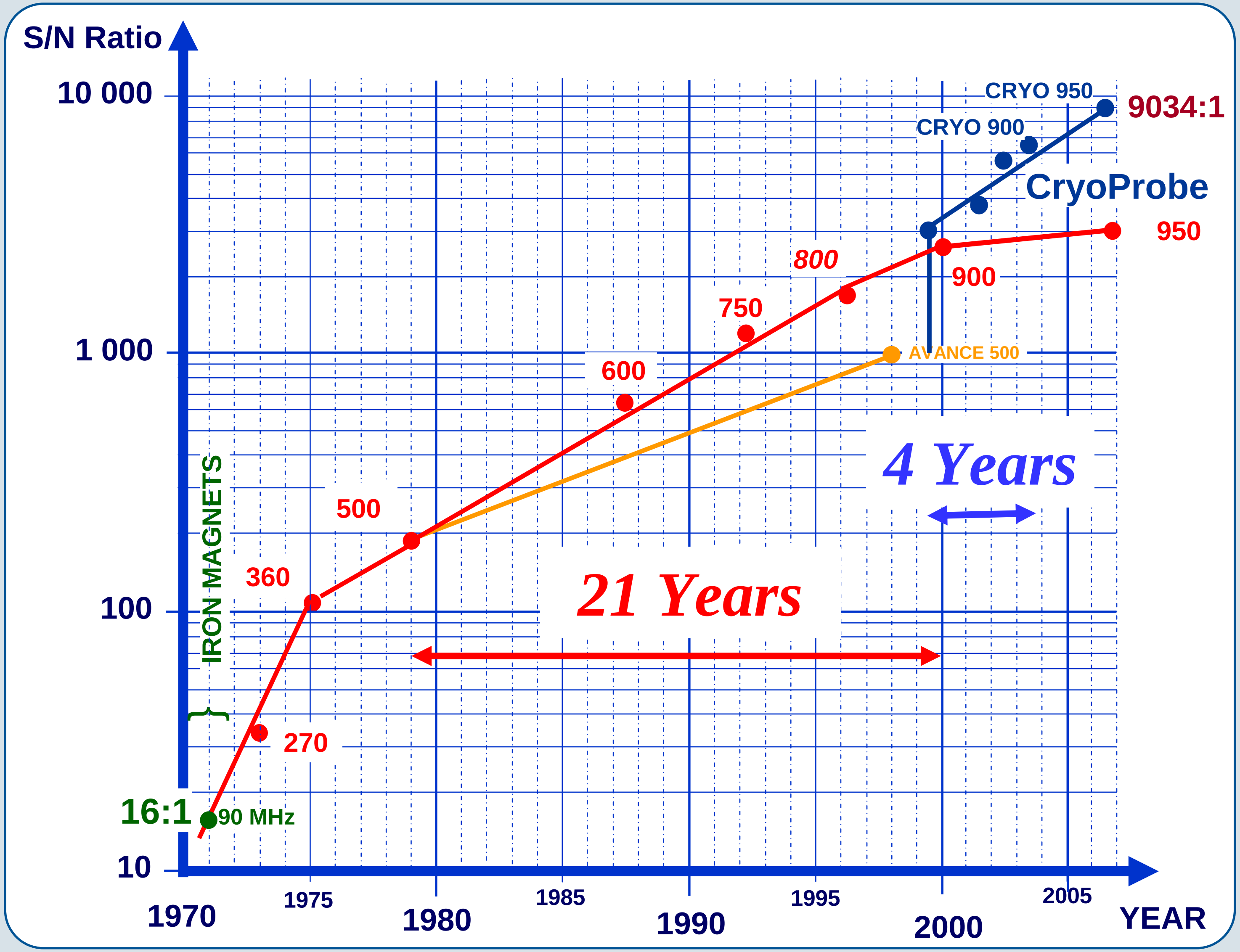
Cutting edge Micro-electronics replace aging Synthesizer Technology

Over 3000 Components on (22 x 27) cm²

Board with:

Layers	**16**
BGA's	**30**
Connection	**10000**
VIA's	**13000**
Pins	**14000**

NMR Sensitivity 0.1% EB 5mm tube

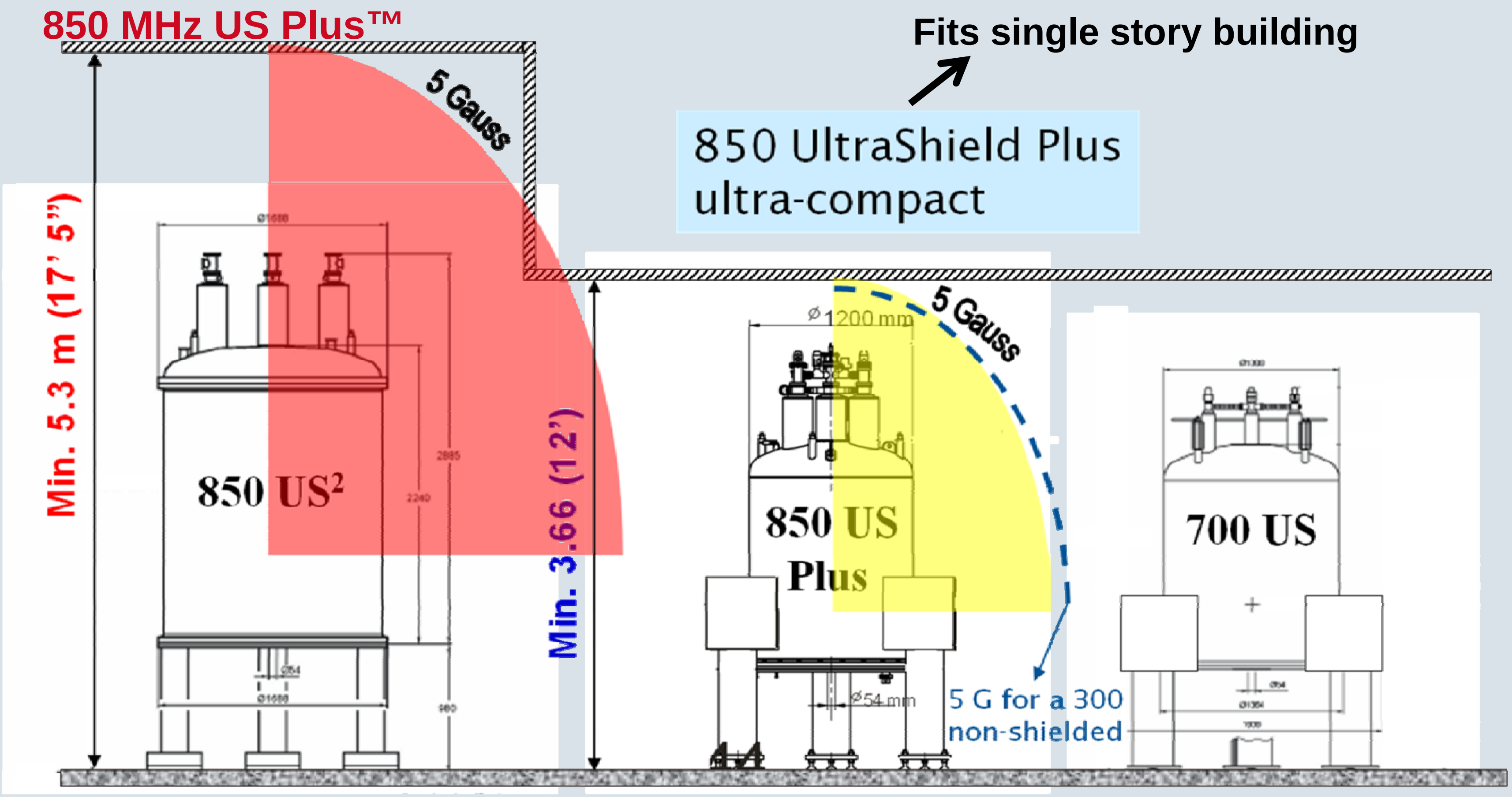


Bruker UHF Magnet Milestones

- 1992 First 750 MHz NMR magnet
- 1995 First 800 MHz NMR magnet
- 1998 First 750 MHz wide bore magnet
- 2001 First 900 MHz NMR magnet
- 2004 First 850 MHz WB shielded magnet
- 2004 First 900 MHz shielded magnet
- 2006 First 800 MHz compact shielded mag
- 2006 First 950 MHz shielded magnet
- 2009 First 900 MHz WB shielded magnet
- 2009 First 850 MHz compact shielded mag
- 2009 First 1000 MHz NMR Magnet

Highest Field Compact NMR Magnet

Recent Bruker Innovations



- Compact size and small stray field improve siting flexibility
- Outstanding stability and high-resolution NMR performance

Highest Field Wide Bore NMR Magnet

Recent Bruker Innovations

900 MHz 89 mm US²

- Expands the highest field available on actively-shielded wide bore magnets to 900 MHz
- Combines UltraStabilized™ sub-cooling and UltraShield™ active shielding technologies
- Improved protection against external perturbations for outstanding stability and high-resolution NMR performance

Avance 1000 - World's First 1 GHz NMR Spectrometer

- World's first, standard-bore, high homogeneity 1 GHz NMR magnet
- Persistent superconducting magnet
- UltraStabilized™ sub-cooling technology
- Magnetic field strength of 23.5 Tesla
- Proton NMR frequency of 1000 MHz
- Standard bore size of 54 mm
- The high field strength and high field stability in combination with the first 1 GHz 5mm triple-resonance CryoProbe™ enables unique 1 GHz NMR applications

Bruker BioSpin

First AVANCE 1000 installation in Lyon

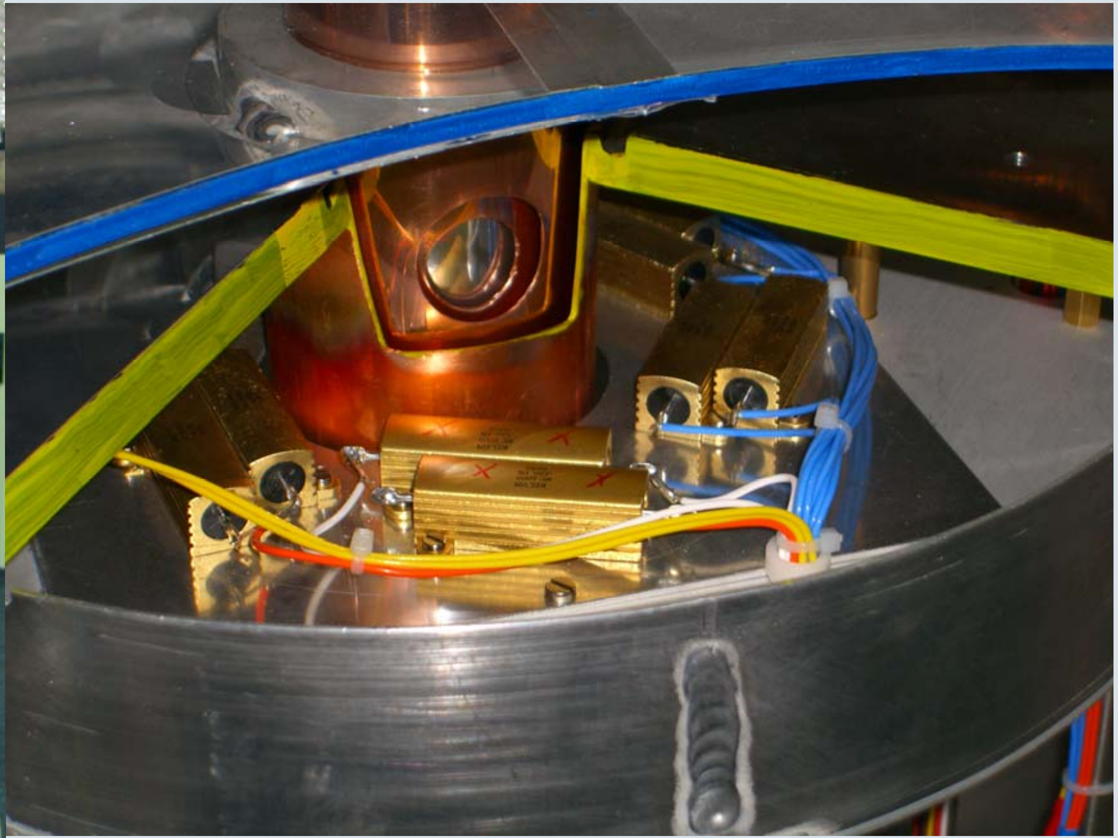
- **The first 1 GHz system is installed at the new ‘Centre de RMN à Très Hauts Champs’ in Lyon, France**

800 MHz Magnet Delivery

NMR by crane

NMR by AIR MAIL

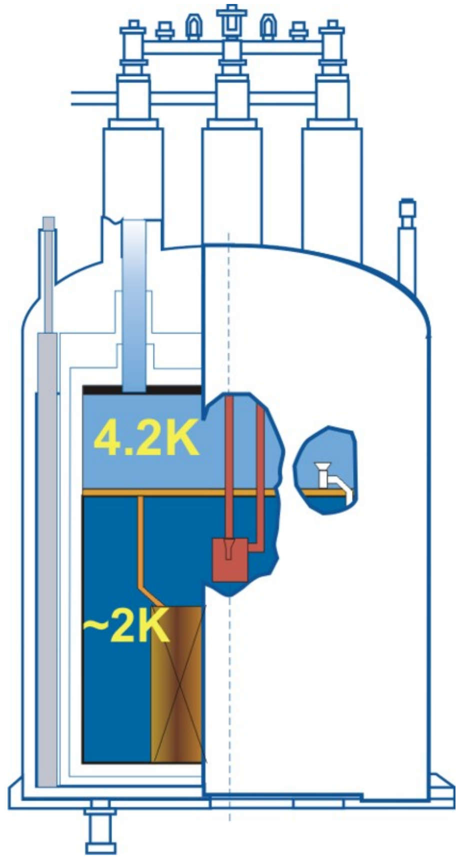
Magnet insight



Long Term Commitment to UHF NMR

(UHF = ultra high field)

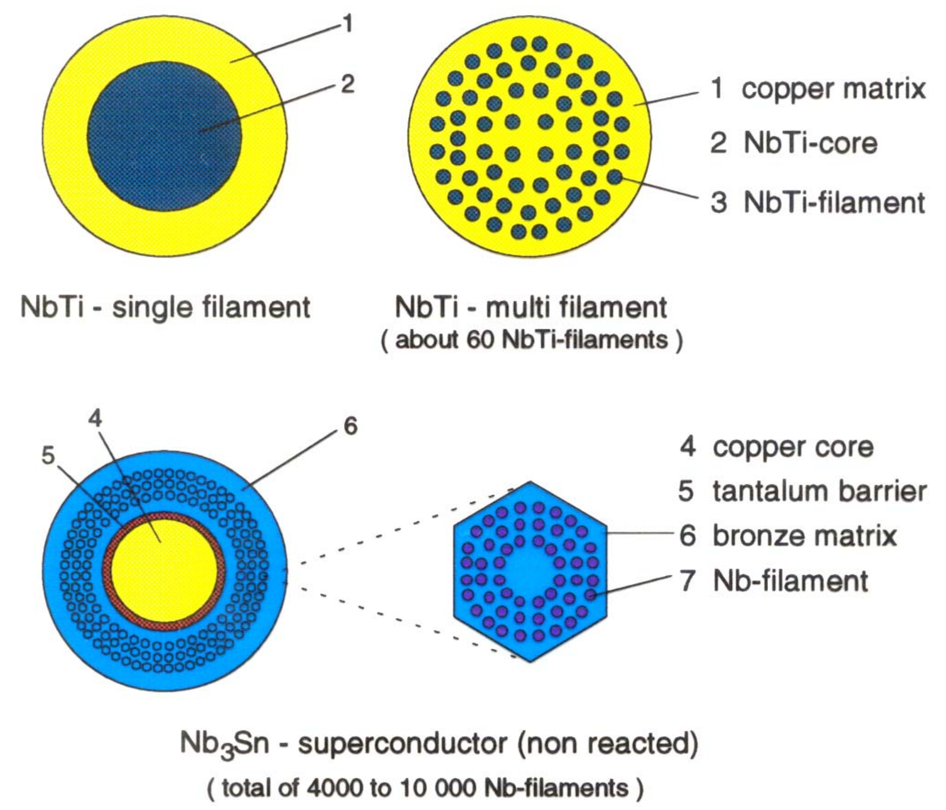
- **Subcooled NMR magnets (2K) improve the properties of the superconducting wires.**
- **Reduction of temperature around the superconductors is essential to achieve stable magnets at ultra-highfield strength.**
- **Extremely high stability of helium bath temperature (thermal barrier between 2K and 4 K reservoir)**
- **Very low field drift**



UltraStabilized™ technology delivering unique performance, stability and safety

SC Magnets

Cross-Sectional View of typical Superconducting Wires for NMR Magnets



Bruker BioSpin

Superconducting Wire

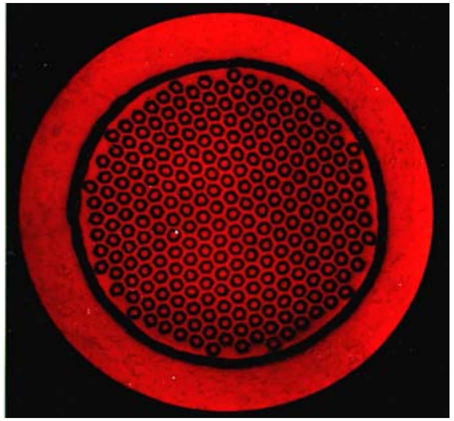
Filament Dimensions

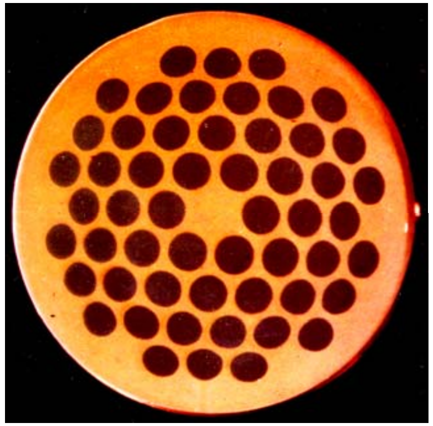
cm

Nb_3Sn

NbTi

~16000 Filaments





54 Single Wires

NMR Sensitivity

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Induced Signal Voltage to Noise Voltage

Coil Design

$$\frac{S}{N} \propto \frac{U_I}{U_N} \propto \frac{\omega \cdot M_0 \cdot V \cdot (B_1/I_{coil})}{\sqrt{4 \cdot k \cdot \Delta f \cdot R \cdot T}}$$

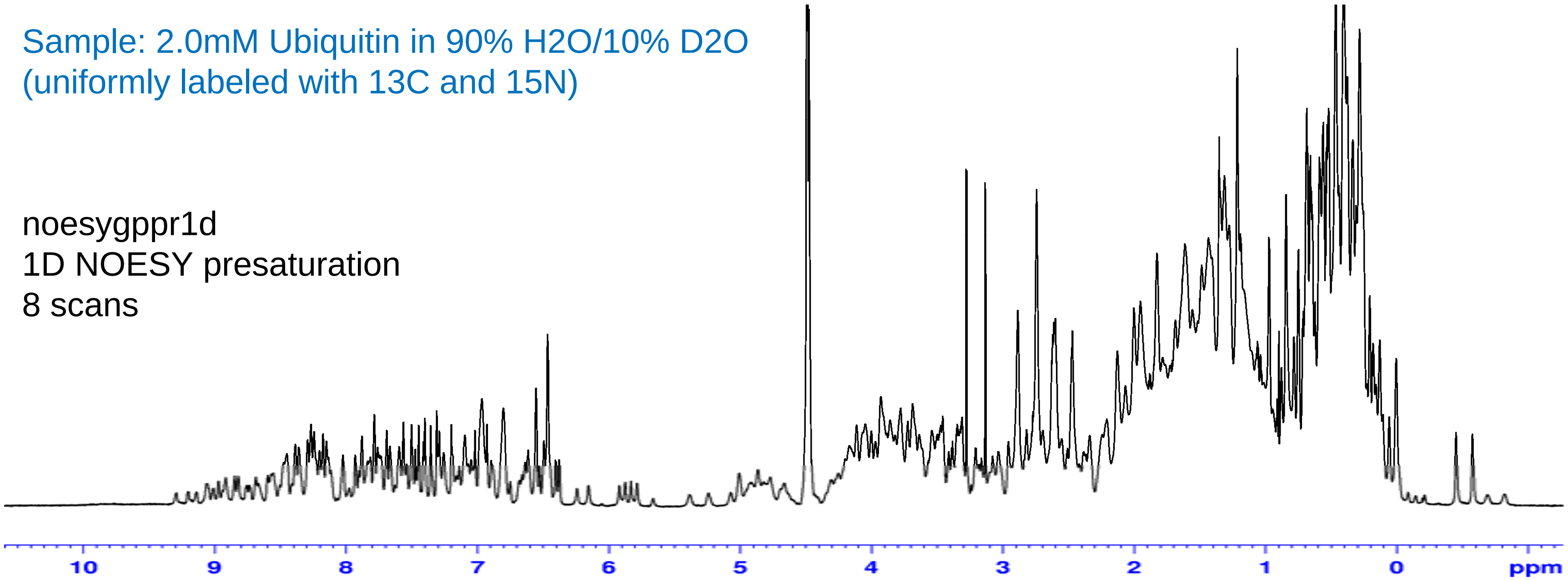
Coil Resistance

Temperature

1 GHz NMR Applications with Cryoprobe

Sample: 2.0mM Ubiquitin in 90% H_2O/10% D_2O
(uniformly labeled with 13C and 15N)

noesygppr1d
1D NOESY presaturation
8 scans



1ppm @ 600 MHz: 600 Hz

1ppm @ 1000 MHz: 1000 Hz

^{1}H-^{15}N HSQC Spectrum

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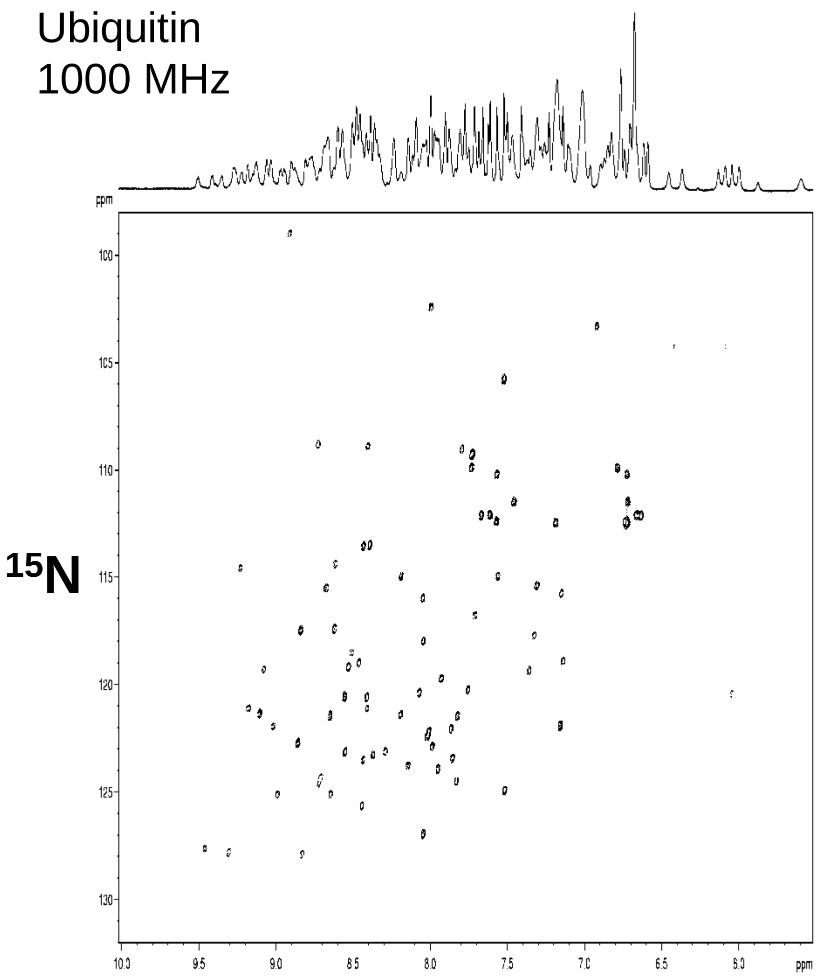
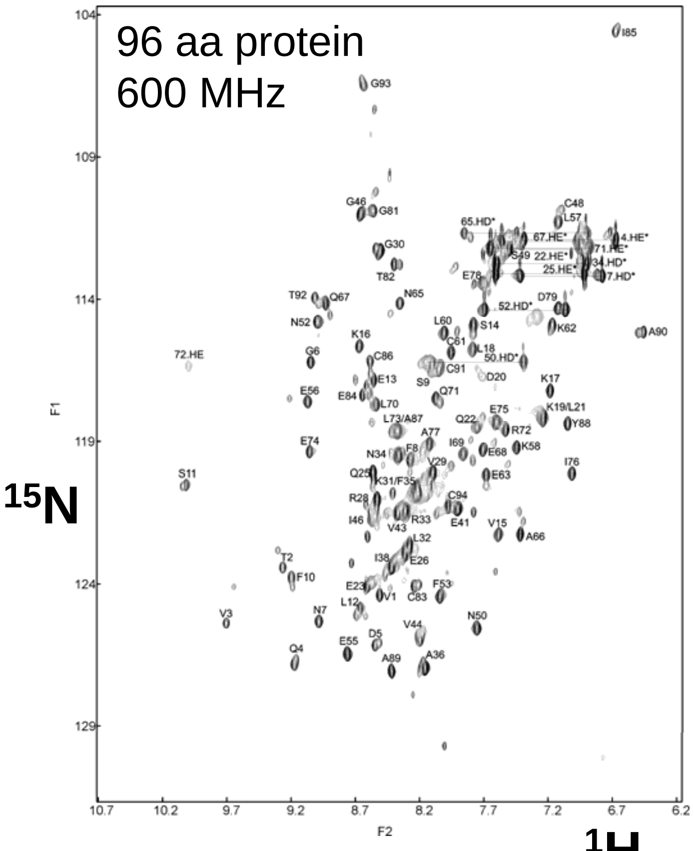
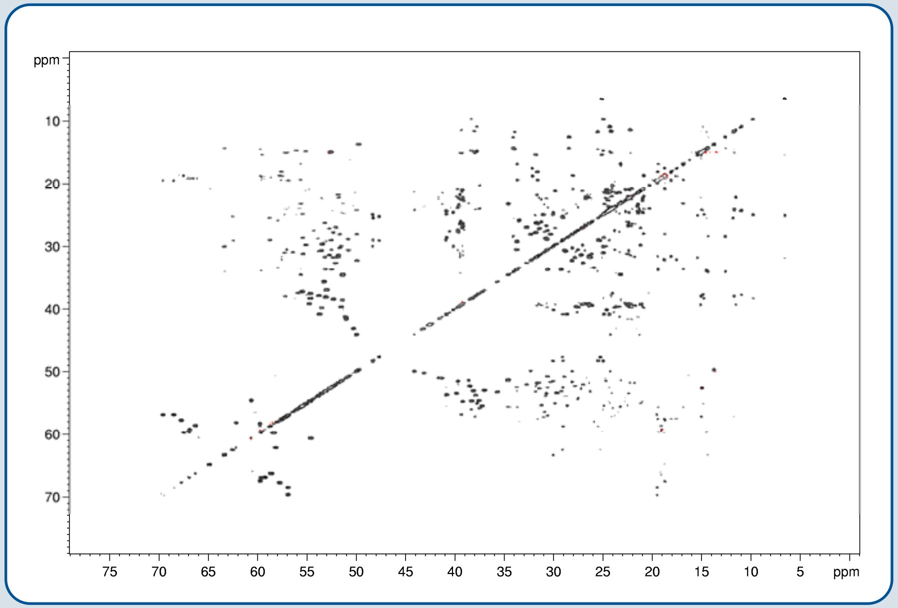


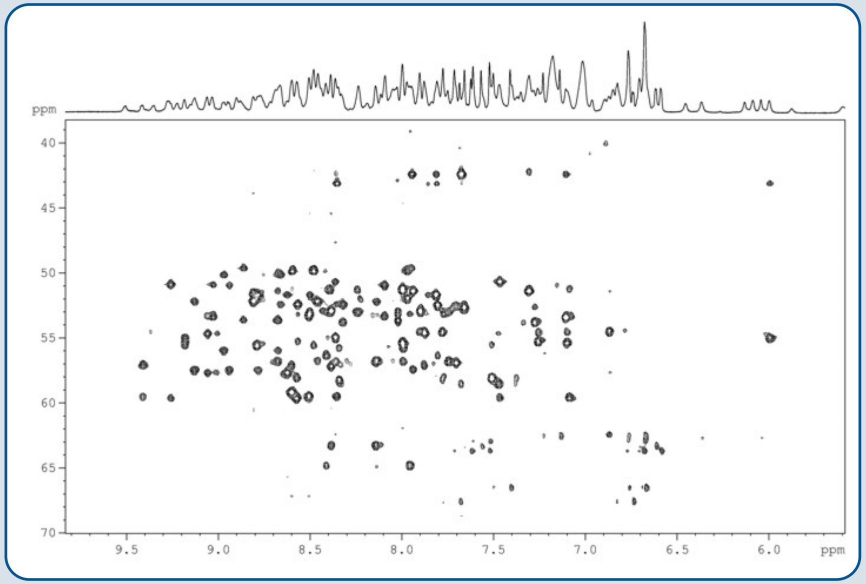


1 GHz NMR Applications with CryoProbe

- 2D carbon detected CC-TOCSY
- 12 ms mixing time
- NS=4
- exp. time: 1 hour



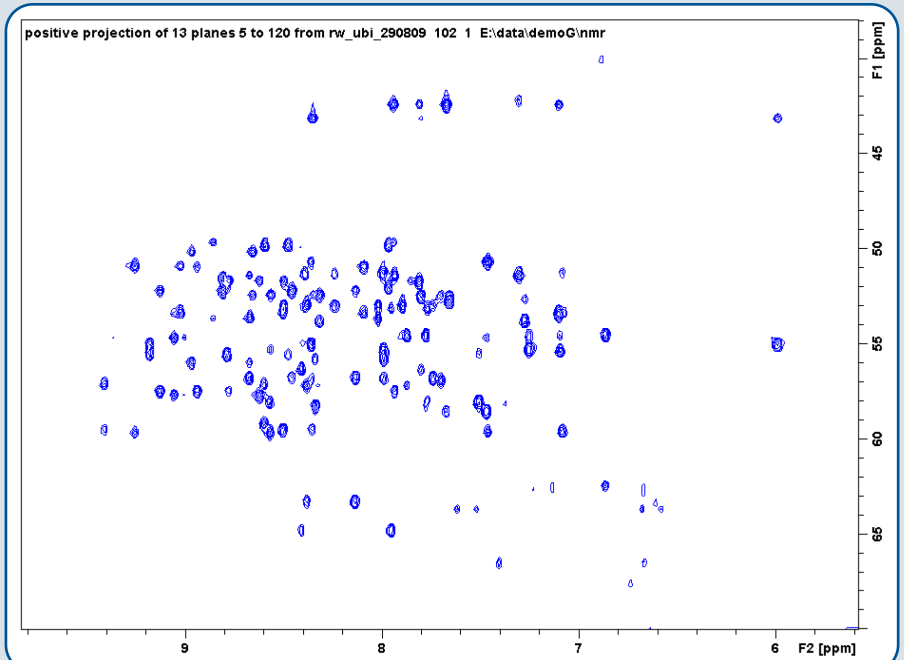
- TROSY-HNCA
- NS=4

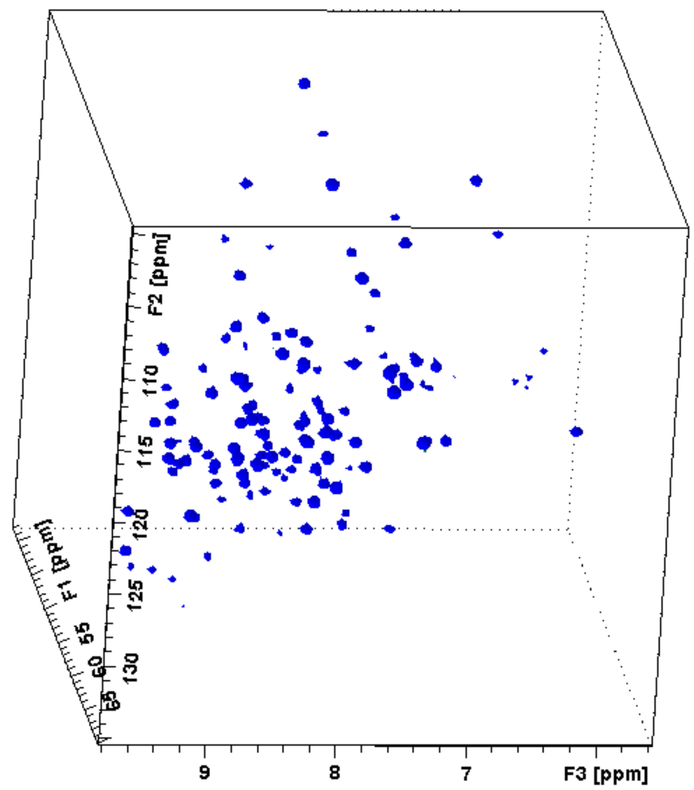


Sample: 2.0mM Ubiquitin in 90% H_2O/10% D_2O (uniformly labeled with 13C and 15N)

1 GHz NMR, CryoProbe and Fast Acquisition

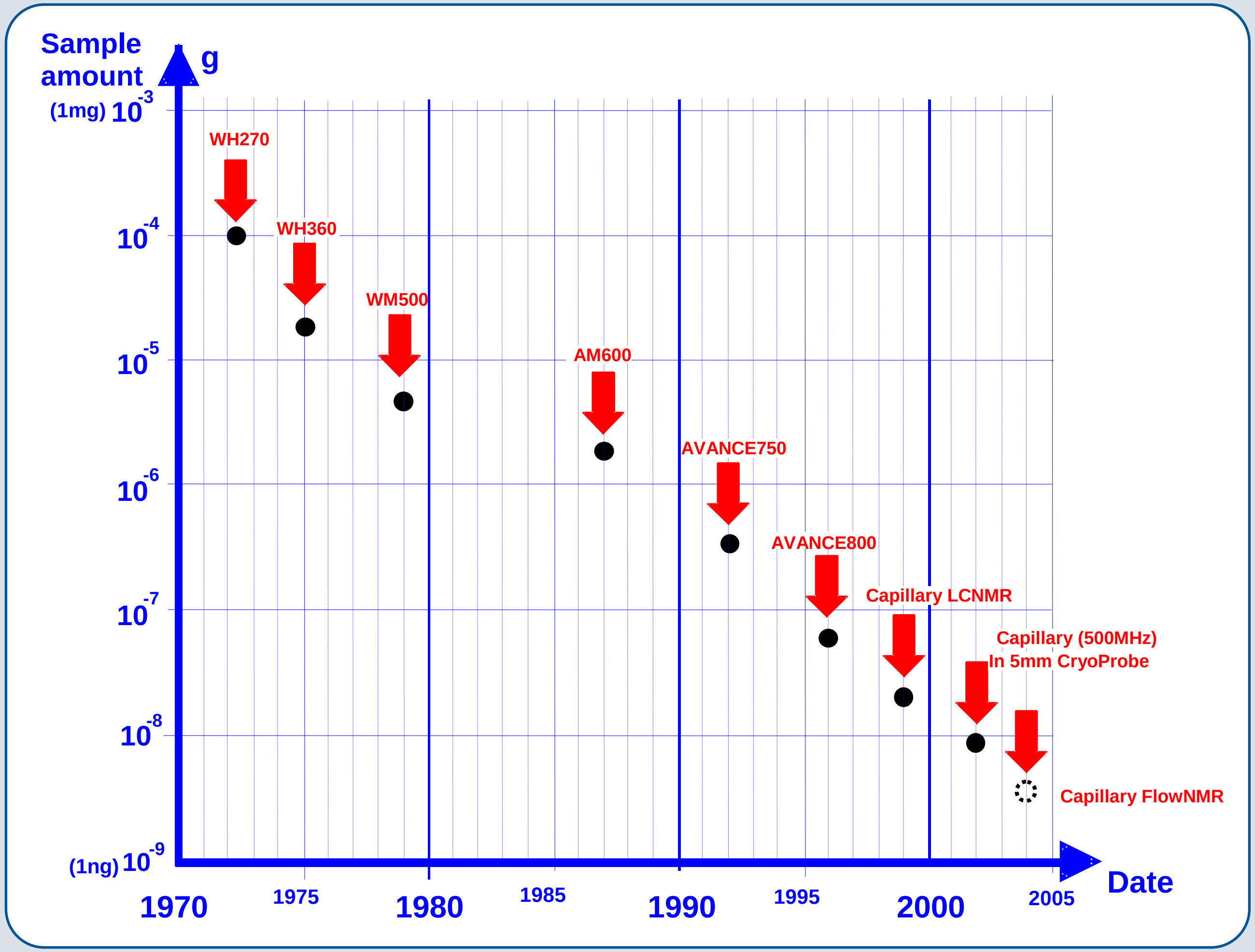
- **3D TROSY-HNCA**
- **Non-uniform sampling mode (10% of regular sampled data points actually recorded)**
- **Exp. time: 80 minutes !**





Sample: 2.0mM Ubiquitin in 90% H_2O/10% D_2O (uniformly labeled with 13C and 15N)

Detection Limit ^{1}H NMR

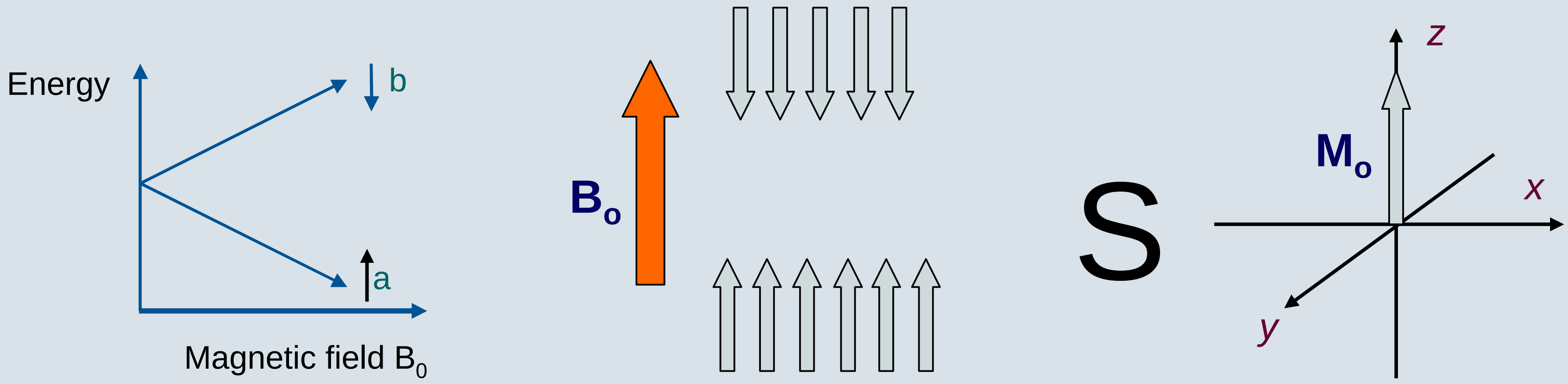


Approximate Detection Limit for small to medium sized Molecules (≤ 400 Dalton).

Acquisition time: ~1h

Further ways to enhance sensitivity

changing the thermal population of spin states by polarisation transfer



In equilibrium:
Bulk magnetisation
along Z axis: M_o

⇒ Dynamic Nuclear Polarisation (DNP)

DNP – Sensitivity boost for solid state NMR

263 GHz solids DNP spectrometer

Dynamic Nuclear Polarization (DNP) can be used to increase the sensitivity of NMR experiments by transferring the much higher polarization of unpaired electrons spins to nuclear spins. This polarization transfer is driven by high frequency microwave irradiation. The Bruker BioSpin 263 GHz solids DNP spectrometer consists of five main development areas:

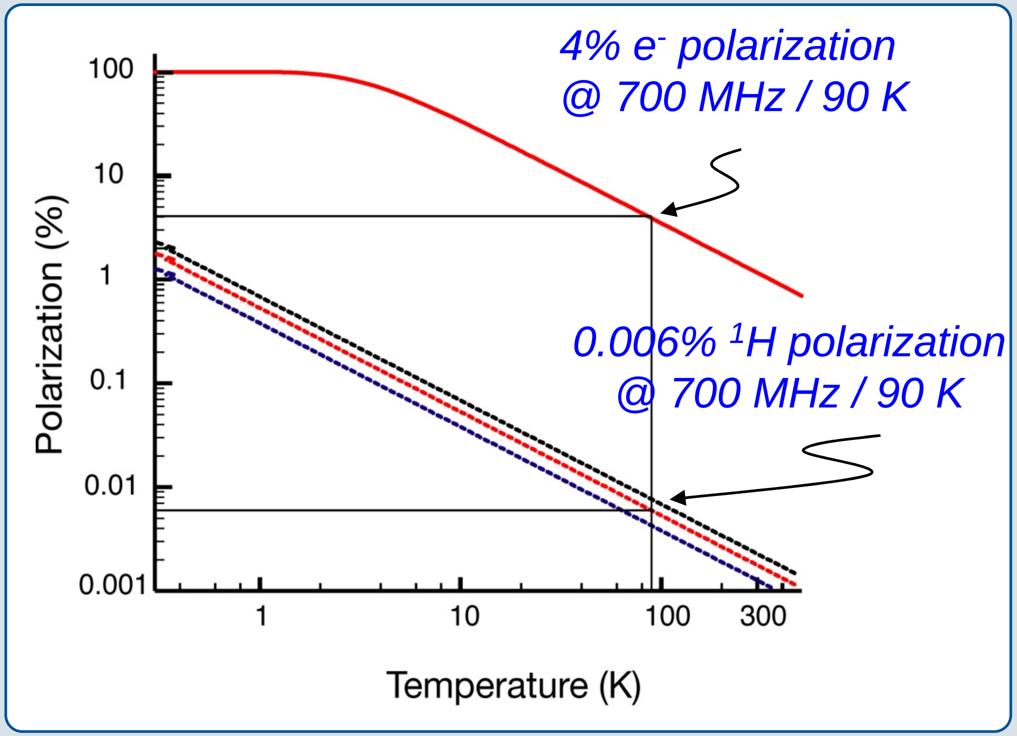
- 25 W 263 GHz gyrotron tube
- 9.7 T gyrotron magnet with superconducting gun coil
- Control system hardware and software, power supplies and cooling networks
- 263 GHz microwave transmission line from gyrotron to NMR sample
- Low temperature MAS probe with built-in waveguide and cold gas supply

Dynamic Nuclear Polarization – DNP

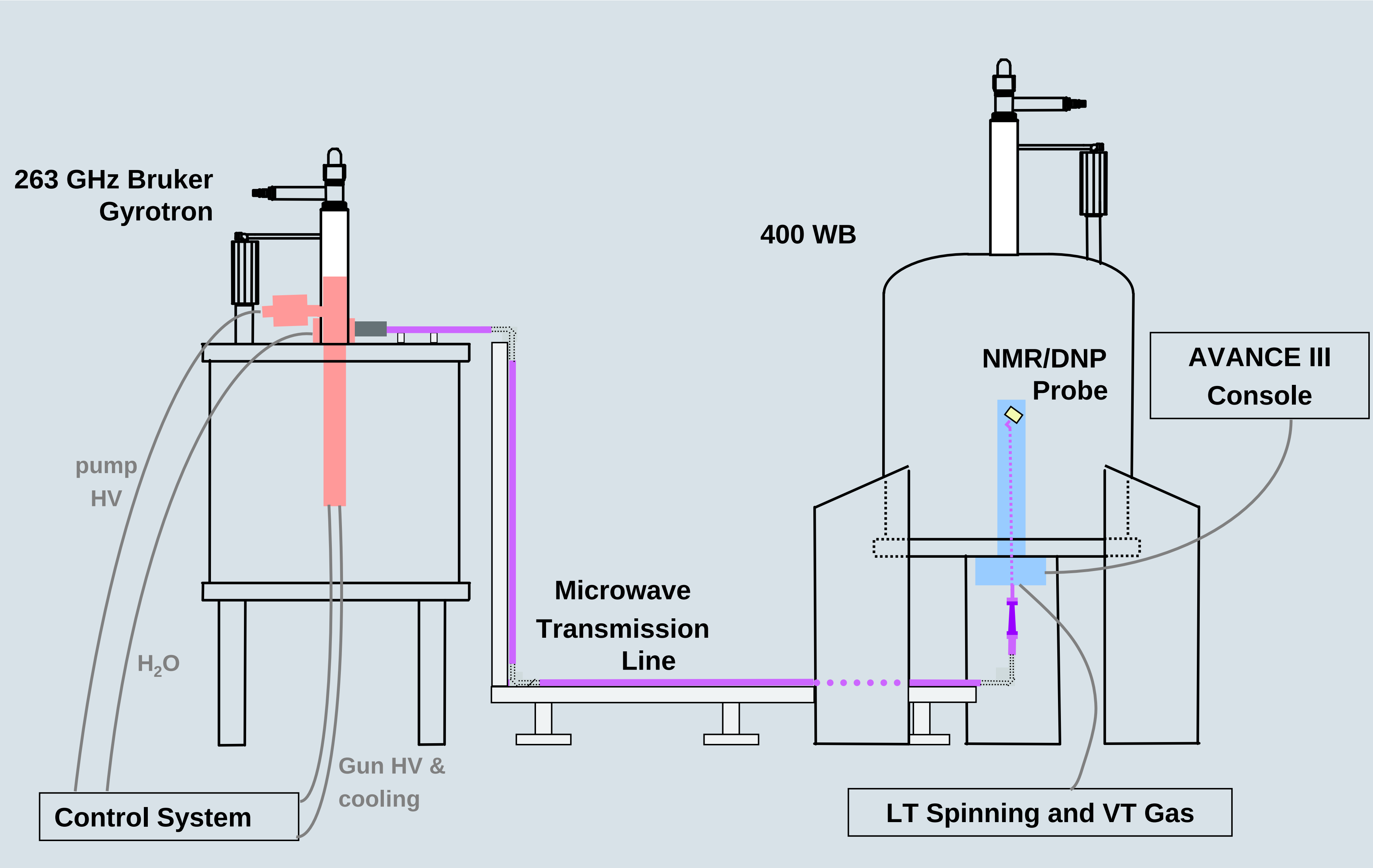
- Enhance nuclear polarization by transfer from electrons
- Principles known since 50 years
- Potential gains:

$$\gamma_e / \gamma_N$$

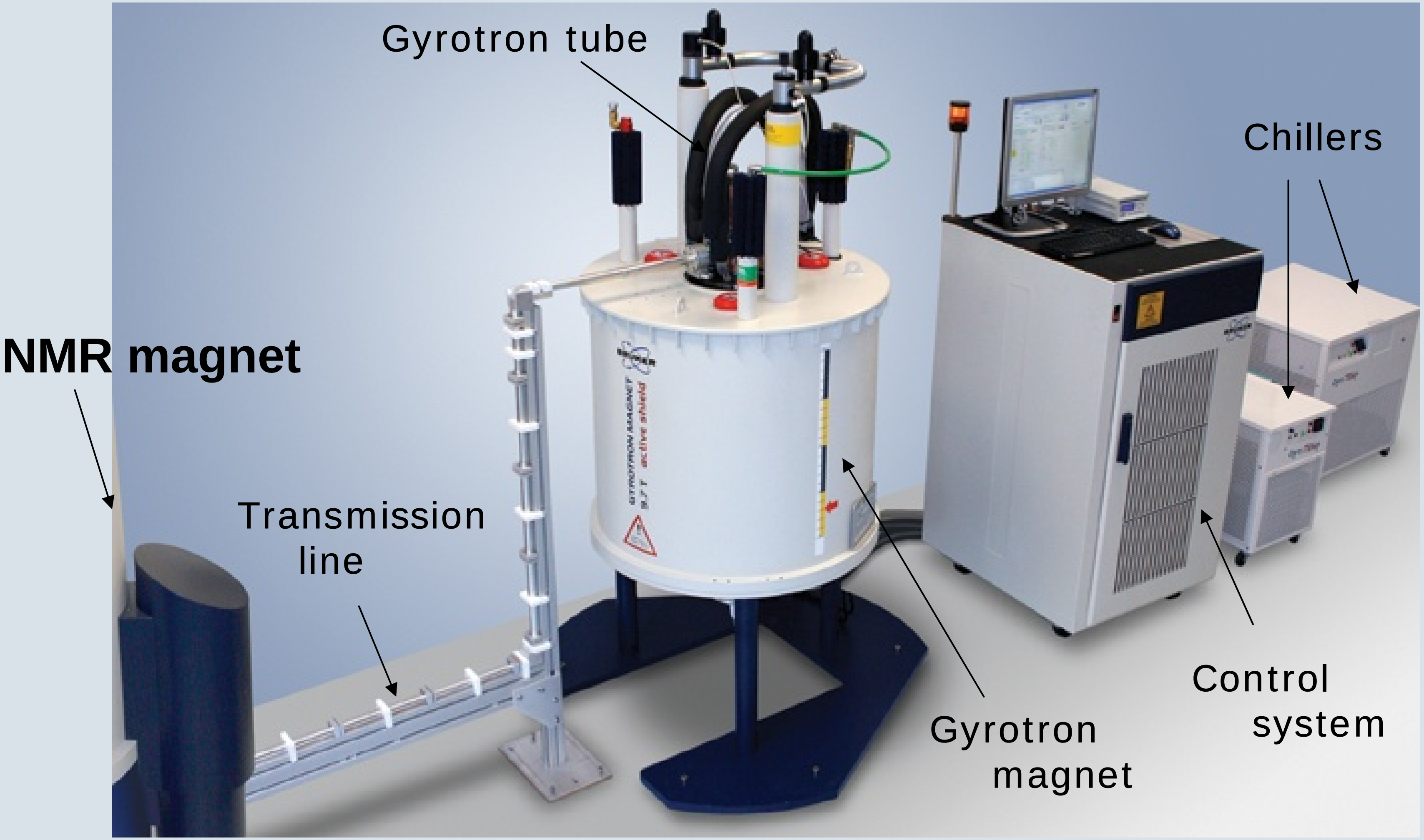
- For protons: ~660
- For carbons: ~2600
- Ingredients:
  - Unpaired electron spin
    - *Internal or additive*
  - µ-waves !



Solid State DNP Spectrometer Components



263 GHz Gyrotron Accessory

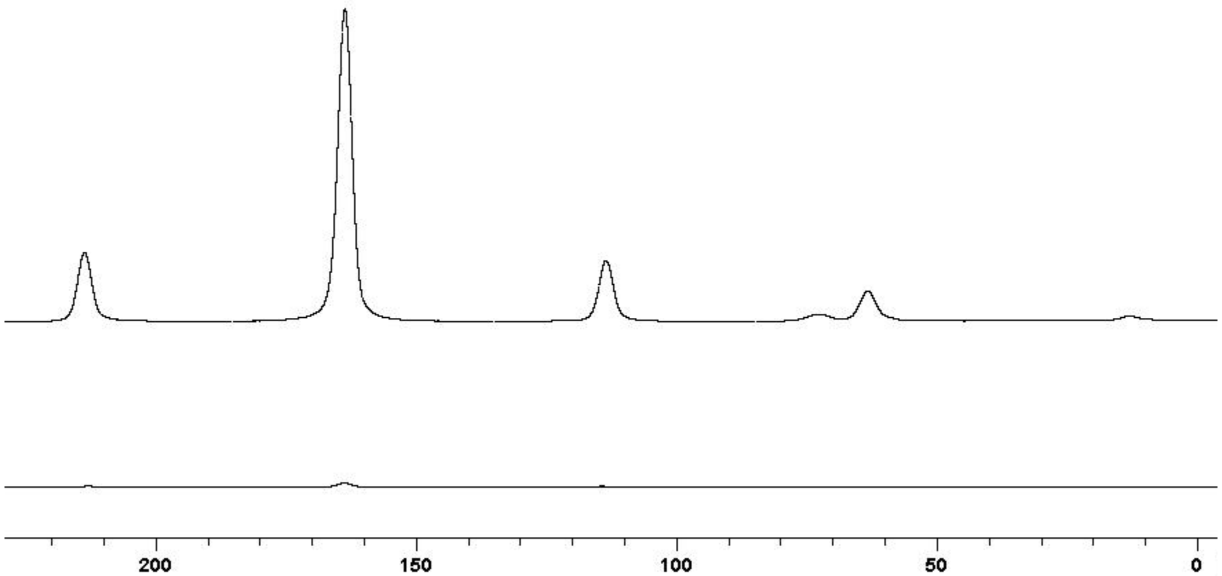


DNP Spectrometer in Billerica operational since July 2008

263 GHz DNP-NMR Spectrometer at FMP/Berlin

Operational since February 2009

263 GHz DNP Experiment at 105 K



^{13}C Chemical Shift (ppm)

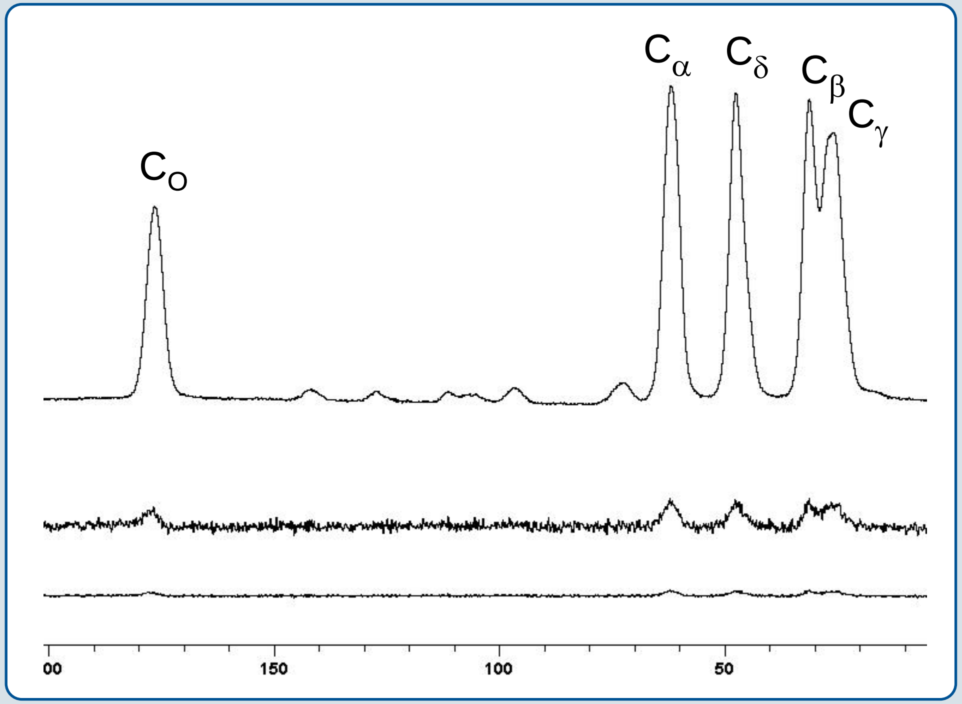
DNP signal enhancement = 80

20 mM TOTAPOL in frozen glycerol/water with 2 M ^{13}C Urea, 5 kHz MAS

microwaves on

microwaves off

DNP-Enhanced CPMAS of ^{13}C-Proline



^{13}C Chemical Shift (ppm)

DNP signal enhancement = 70

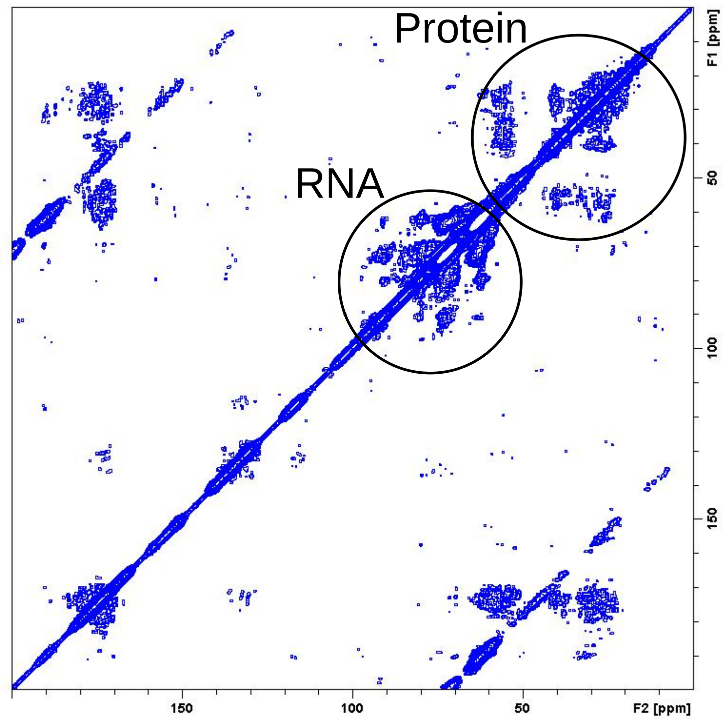
microwaves on

microwaves off x 5

microwaves off

- **25 ml sample, 1.5 mg U-^{13}C-^{15}N Proline, 8 kHz MAS, CPMAS with 100 kHz Spinal 64 decoupling, 110 K sample temperature**
- **8 seconds acquisition time for both microwaves on and off spectra**
- **Glycerol/Water, 20 mM TOTAPOL**

DNP Experiment: Barnase Ribosome Complex

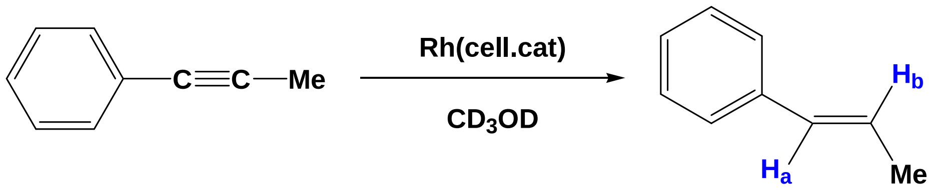


- ^1H driven spin diffusion ^{13}C-^{13}C correlation experiment on Barnase ribosome nascent chain complex with 10 mM TOTAPOL (nascent chain ^{13}C labelled)

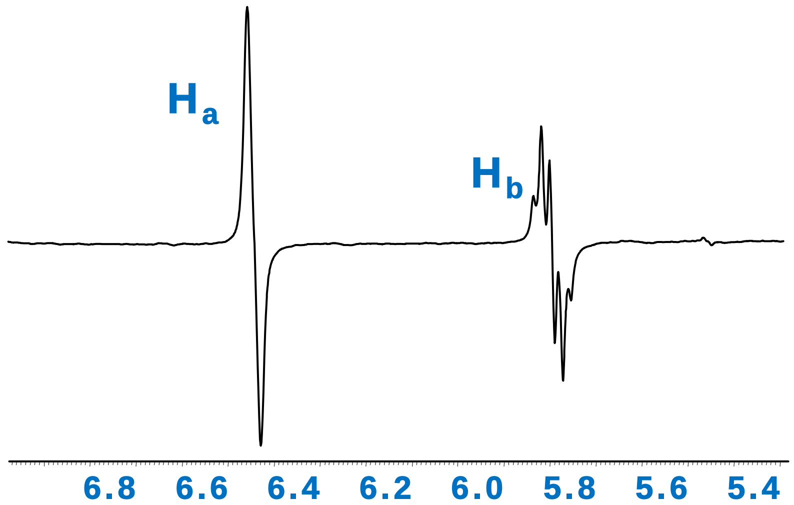
Sample courtesy of Hartmut Oschkinat, Leibnizinstitut für Molekulare Pharmakologie, Berlin

Para Hydrogen Induced Polarisation (PHIP)

- **Para hydrogen has anti-parallel spins > NMR invisible**
- **Reaction with para hydrogen yields hyperpolarised molecule => NMR visible if the two protons have different chemical shift**
- **Enhanced absorption and emission signals**



Catalytic hydrogenation reaction

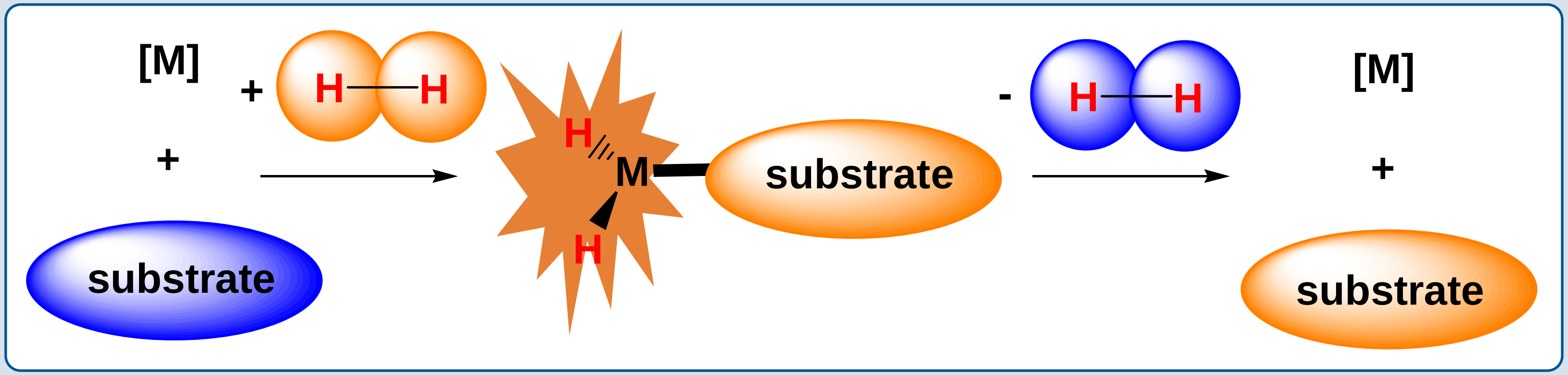


R. Eisenberg Acc. Chem. Res. 1991, 24, 110. (PHIP)
D. P.Weitekamp et al. J. Am. Chem. Soc. 1987, 109, 5541. (PASADENA)
J. Bargon et al. Prog. Nuc. Mag. Res. 1997, 31, 293.
S. Duckett et al. Dalton. 2004, 2601.

All results courtesy Simon Duckett and Gary Green, University of York

Polarisation Transfer in Low Magnetic Field

- **Temporary association of substrate and pH_2 on polarisation template**
- **Redistribution of magnetisation via J-coupling (strong coupling)**
- **pH_2 polarisation is transfered to all nuclei in coupling network**
- **Substrate remains chemically unchanged**

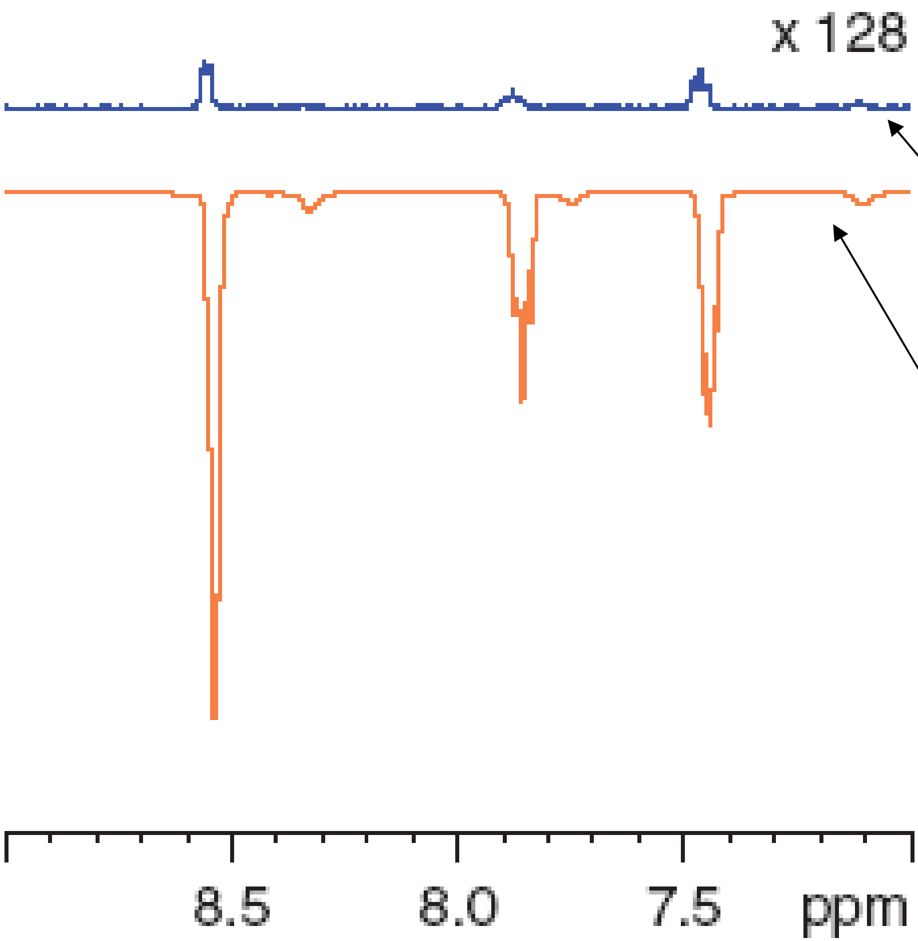


All results courtesy Simon Duckett and Gary Green, University of York

THE UNIVERSITY of York

Bruker BioSpin

First results with prototype polariser



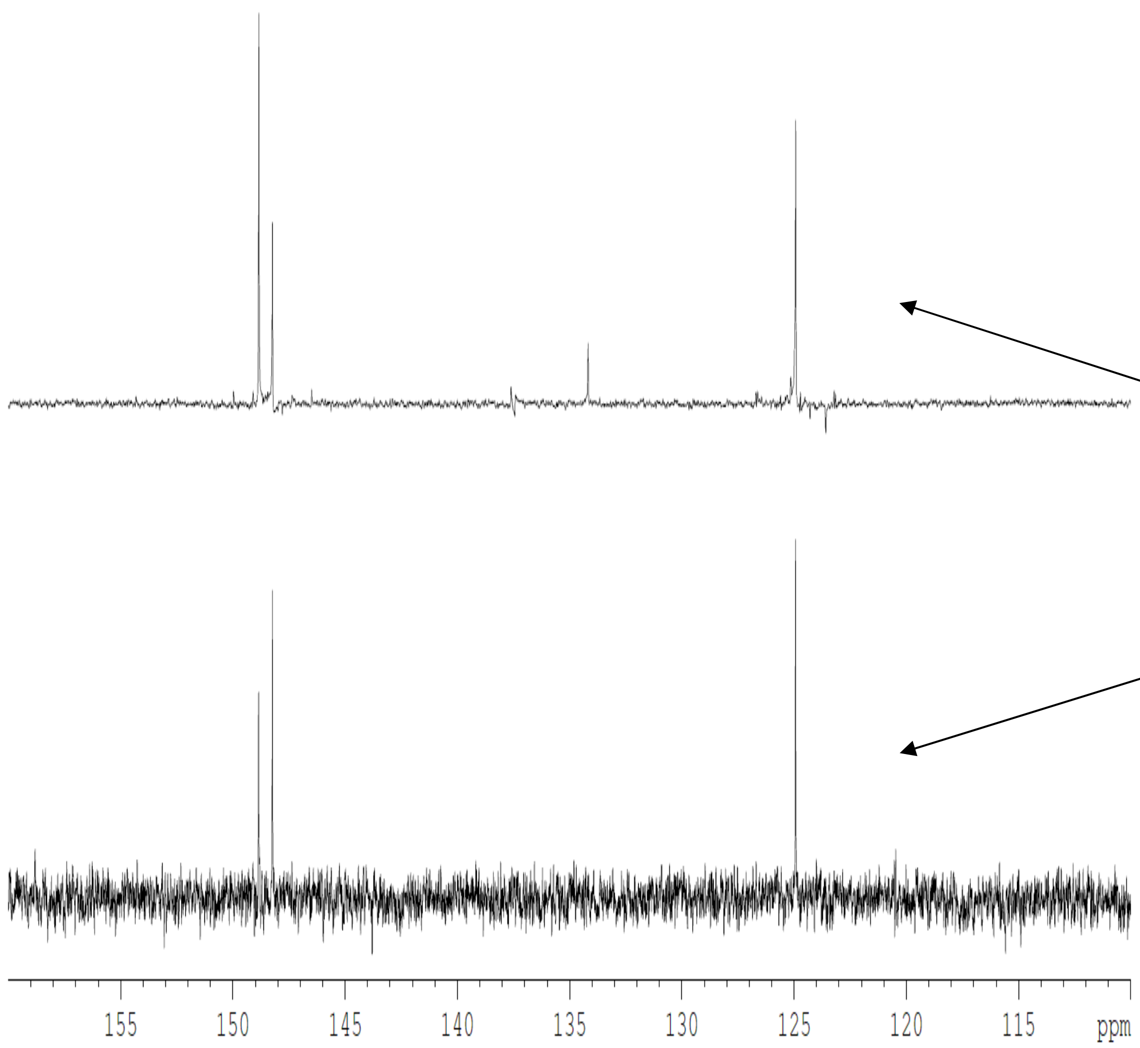
400 MHz (9.4 T)
^{1}H spectra of Pyridine

Thermal polarisation

pH_2 hyperpolarised
Single scan
Signal gain ~1000

All results courtesy Simon Duckett and Gary Green, University of York

Polarisation is transfered to X-Nuclei



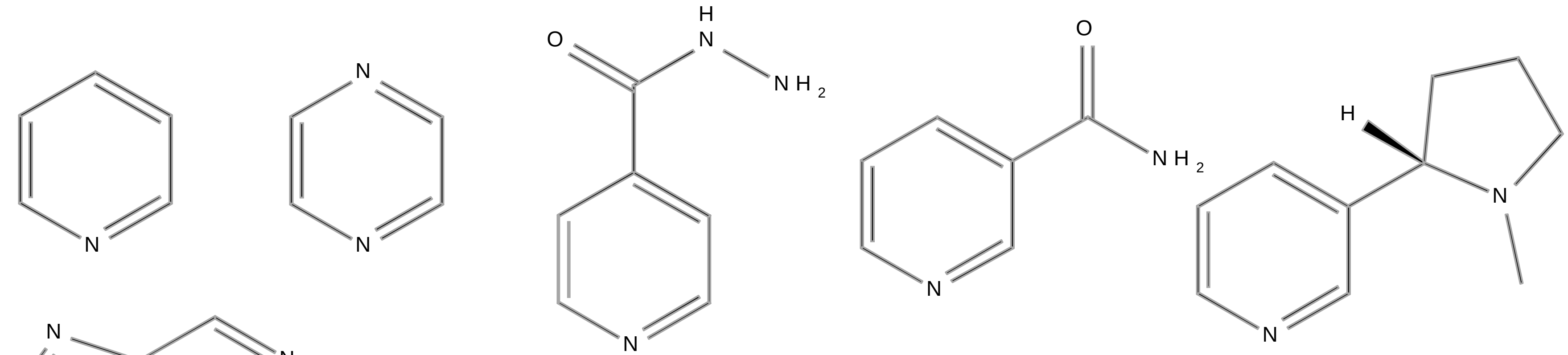
100 MHz (9.4 T)
13C spectra of
4-Methyl Pyridine

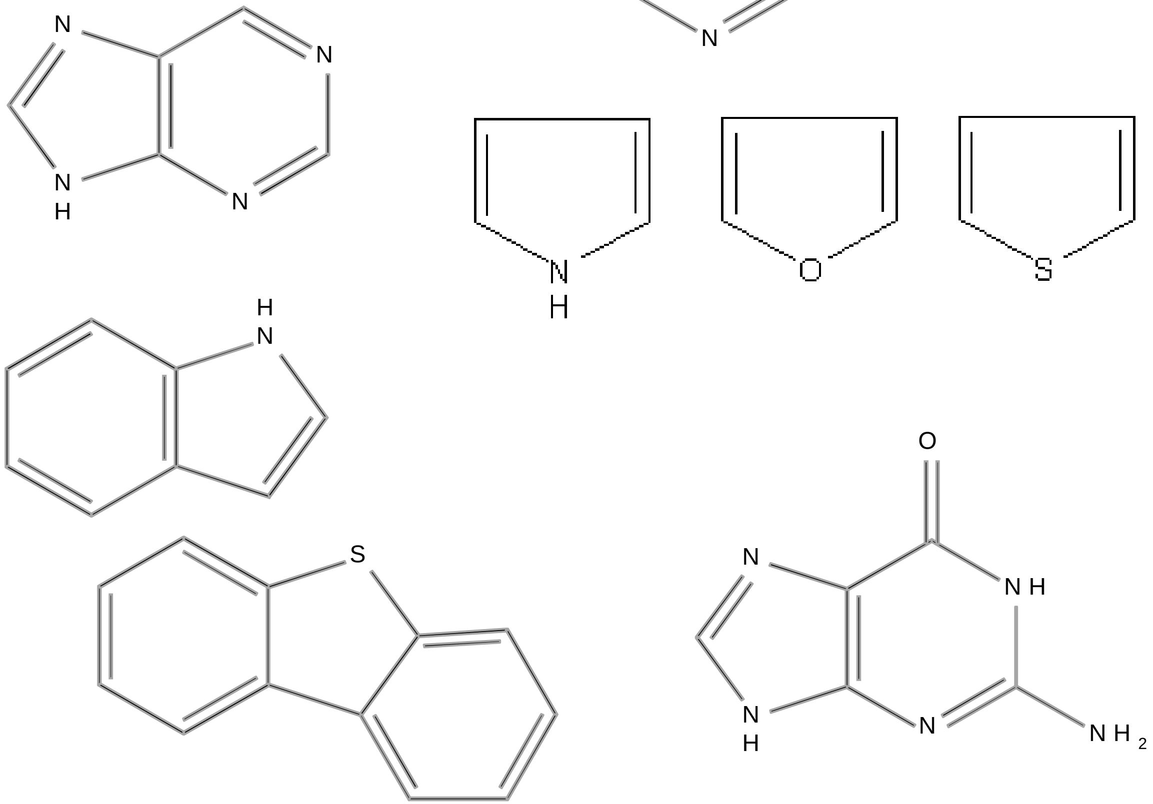
pH_2 hyperpolarised
Single scan
Signal gain ~160

Thermal polarisation
32 averages

All results courtesy Simon Duckett and Gary Green, University of York

What can be Polarised?





Ongoing research:

- **Catalyst**
- **Substrate**
- **Solvent**

Radioactive ... ?

^3H

Tritium CryoProbe

Tritium CryoProbe – ^{3}H Observe

Applications for ^{3}H-NMR

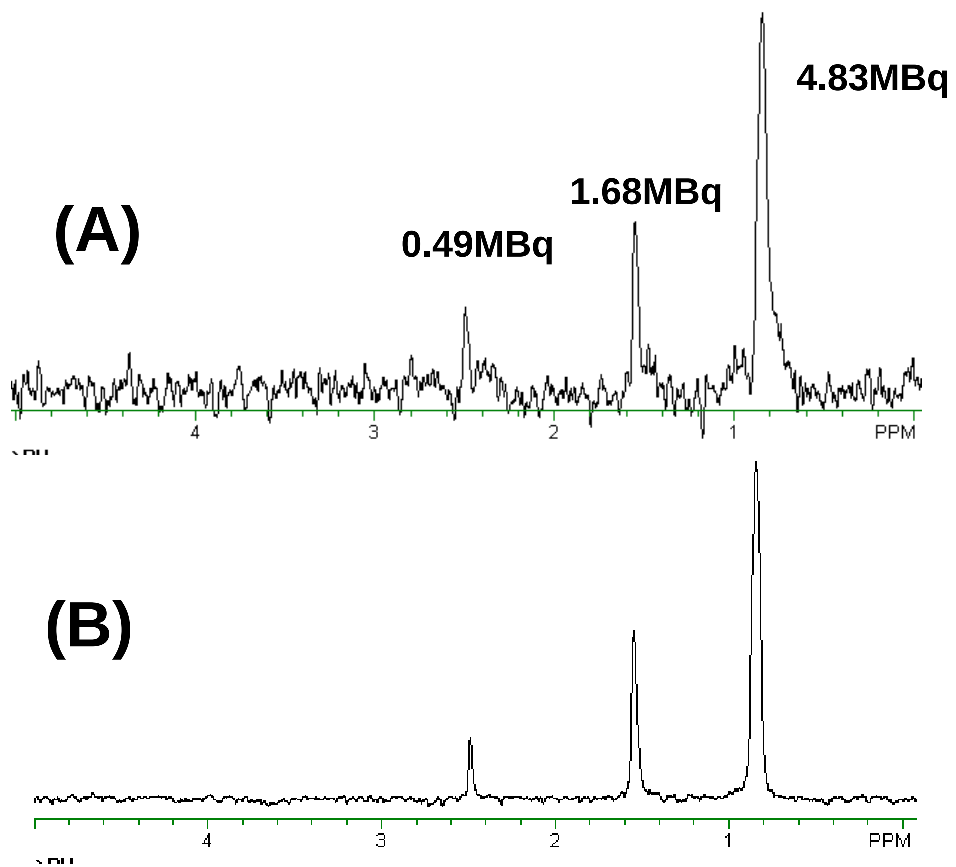
Tritium is a perfect spy to study (bio)chemical reactions

‚Tracking the Molecules of Life'

- **Binding studies of ATP utilizing enzymes**
- **DNA, RNA: conformational dynamics**
- **Glucose metabolism in erythrocytes**
- **Determination of stereochemistry**
- **………………..**

BUT: due to radioactivity only low concentration tritiated samples can be used

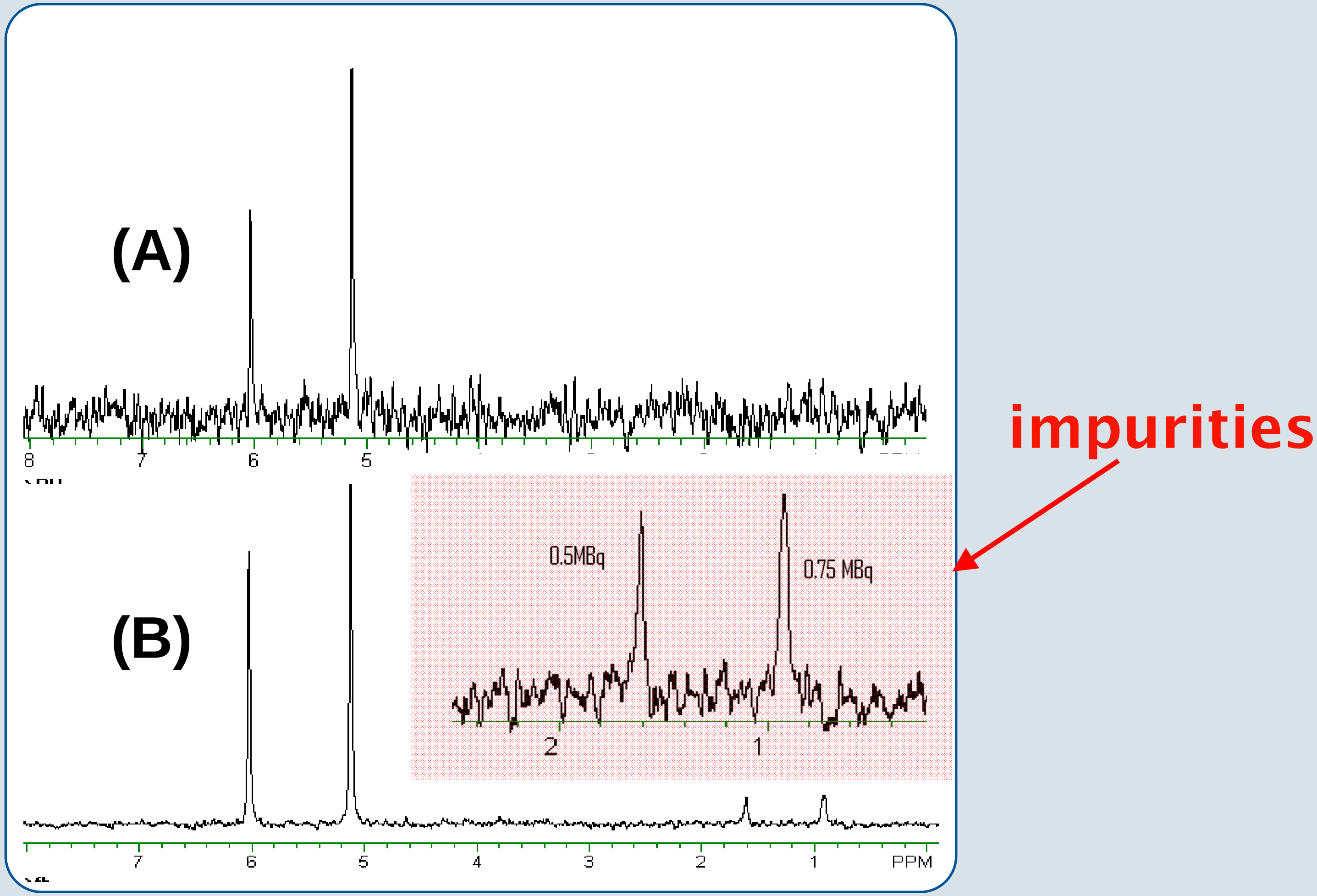
Tritium CryoProbe – ^{3}H Observe



Hydrogenation of a hydrocarbon sample (A) RT probe (B) with CryoProbe

Courtesy of J. P. Bloxsidge, J. R. Jones, E. Alexakis, W. J. S. Lockley, R. N. Garman, D. G. Gillies and Shui-Yu Lu
School of Biological and Molecular Sciences, University of Surrey, Guildford UK

Tritium CryoProbe – ^3H Observe



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School of Biological and Molecular Sciences, University of Surrey, Guildford UK

Tritium CryoProbe – ^{3}H Observe

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Measured values 500 MHz ^{3}H CryoProbe

Sample No	Radioactivity		S/N ratios		Enhancement
	(MBq)	(µCi)	RT probe	^{3}H CryoProbe	
1	165	4459	2230	7350	3.3
2	15	405	170	870	5.1
3	2.5	68	32	147	4.6
4	0.4	11	-	21	-

Detection limit

Courtesy of J. P. Bloxsidge, J. R. Jones, E. Alexakis, W. J. S. Lockley, R. N. Garman, D. G. Gillies and Shui-Yu Lu
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www.bruker-biospin.com