Advanced NMR Training Course: February 27th, 2004

National Program for Genomic Medicine High-Field NMR Core Facility,
The Genomic Research Center, Academia Sinica

Course Topic: Cryogenic Probes

by

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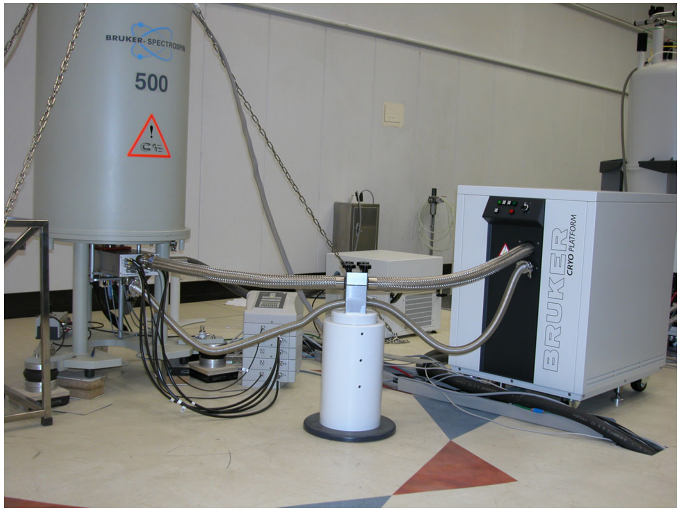
500 MHz CryoProbe System in Academia Sinica

Overview



•5 mm TXI Z-gradient CryoProbe

Bottom part of the probe

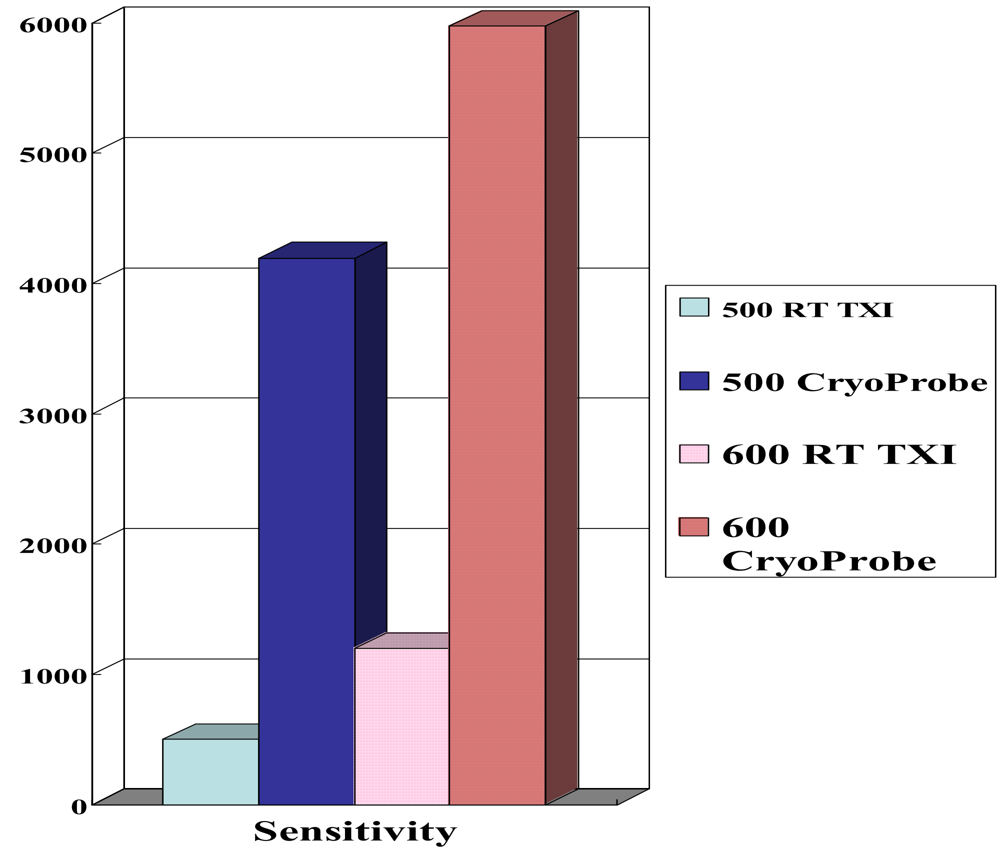
Laptop for monitoring the CryoProbe system

CryoProbe^{TM} Systems in Academia Sinica

•^{1}H Coil and preamplifier are cooled to 30 k and 77 k respectively.

•Low resistance and low thermal noise: sensitivity increases 3-4 fold relative to conventional room temperature probeheads.

•5 mm TXI Z-gradient CryoProbe.



•Signal-to-noise: Measurement of 0.1% ethylbenzene in $CDCl_3$ (200 Hz noise region)

Sensitivity of a Cryogenic Probe

- $S/N \sim 1/(T_cR_c + T_a[R_c + R_s] + T_sR_s)^{1/2}$

- T_c: temperature of the coil
- R_c: resistance of the coil
- T_s: temperature of the sample
- R_s: resistance added to the coil by the sample
- T_a: noise temperature of the preamplifier

- For a room temperature probe: T_c~298 k, T_a~298 k
- For a cryogenic probe: T_c~30 k, T_a~77 k

- Sensitivity is enhanced by the lowered temperature, and lowered resistance.

Sensitivity Evaluations of the 500 MHz TXI CryoProbe

The S/N from 0.1% ethylbenzene in $CDCl_3$ looks very promising; however, how about the sensitivity for aqueous samples?

Sensitivity measurements for the CryoProbe:

(1). Simple 1D spectra of aqueous samples.

(2). 2D homonuclear spectra in D_2O.

(3). 2D homonuclear spectra in H_2O (water is harder to suppress on a cryogenic probe.).

(4). 2D ^{1}H, ^{15}N-HSQC of proteins in H_2O.

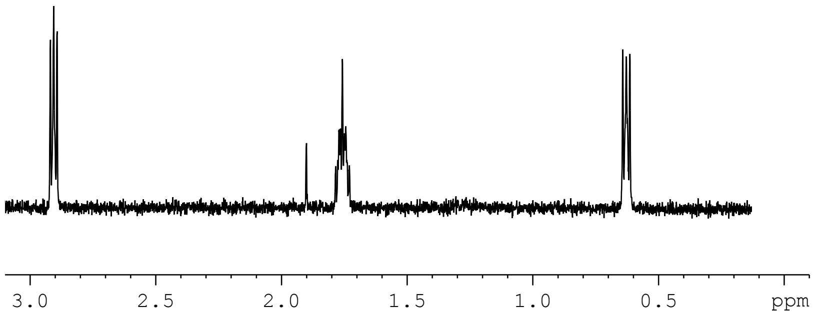
(4). Effect of conducting salts on the sensitivity of a CryoProbe.

Sensitivity Comparison in D_2O: Simple 1D spectra.

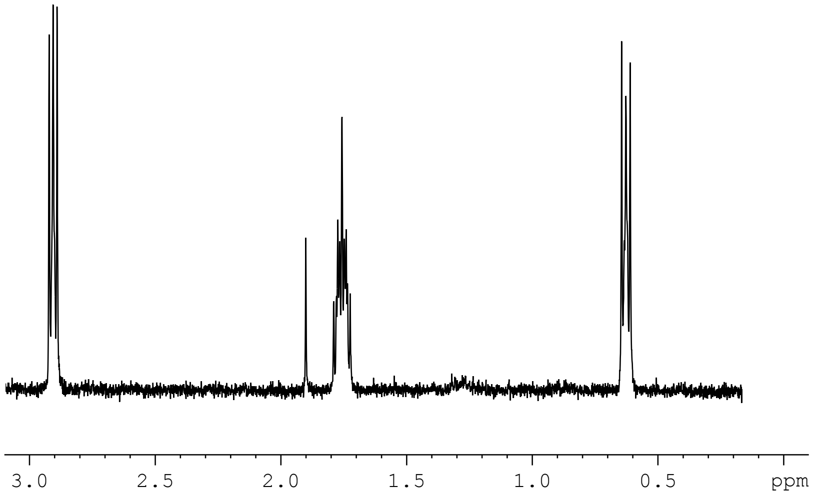
1D spectrum of 2 mM Sucrose in D_2O:

S/N ratio (500 CryoProbe): S/N ratio (600 MHz RT probe)=2.3:1

600 MHz RT TXI probe



500 MHz TXI CryoProbe

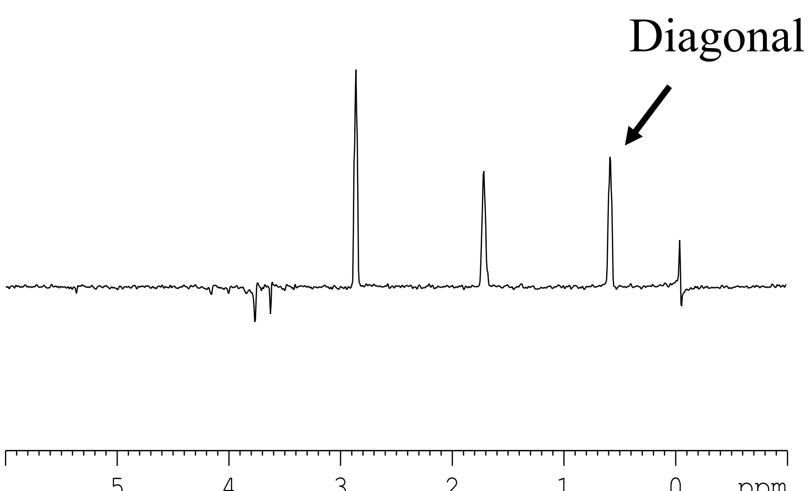


Sensitivity Comparison in D_2O : 2D TOCSY

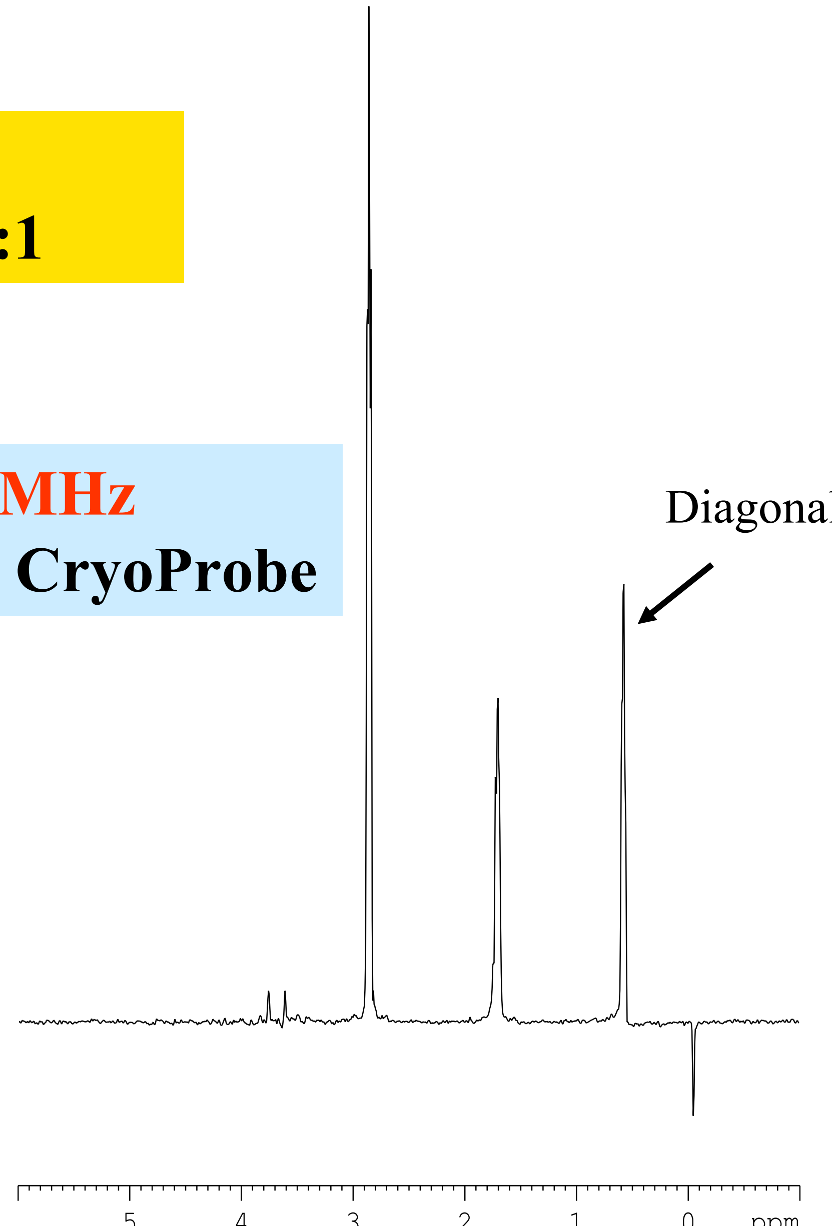
S/N ratio (500 CryoProbe):
S/N ratio (600 MHz RT probe)=2.3:1

1D row vector of 2D TOCSY in D_2O:

600 MHz
RT TXI probe



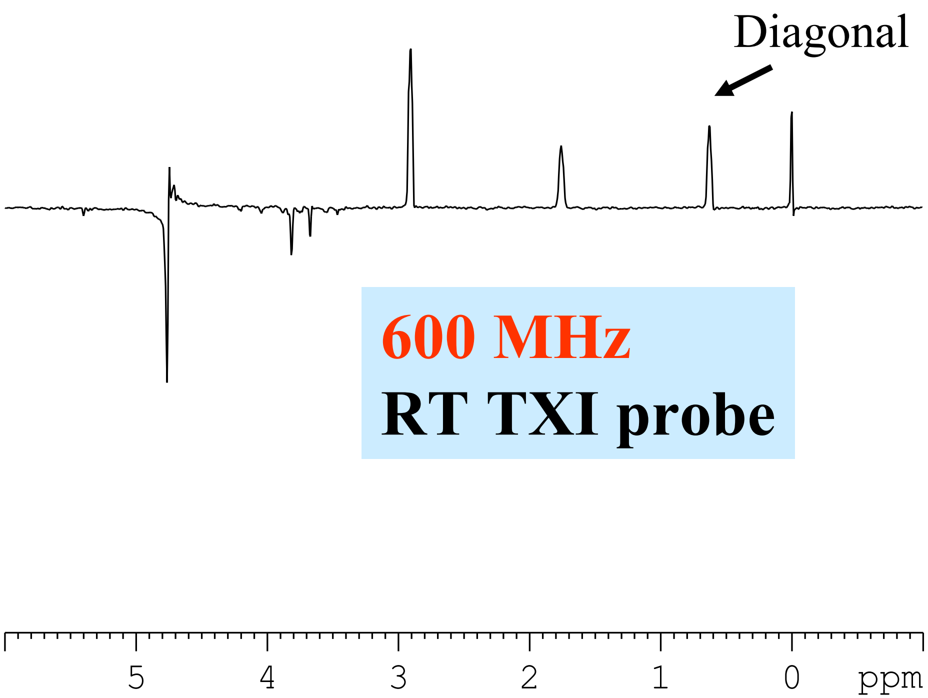
500 MHz
TXI CryoProbe

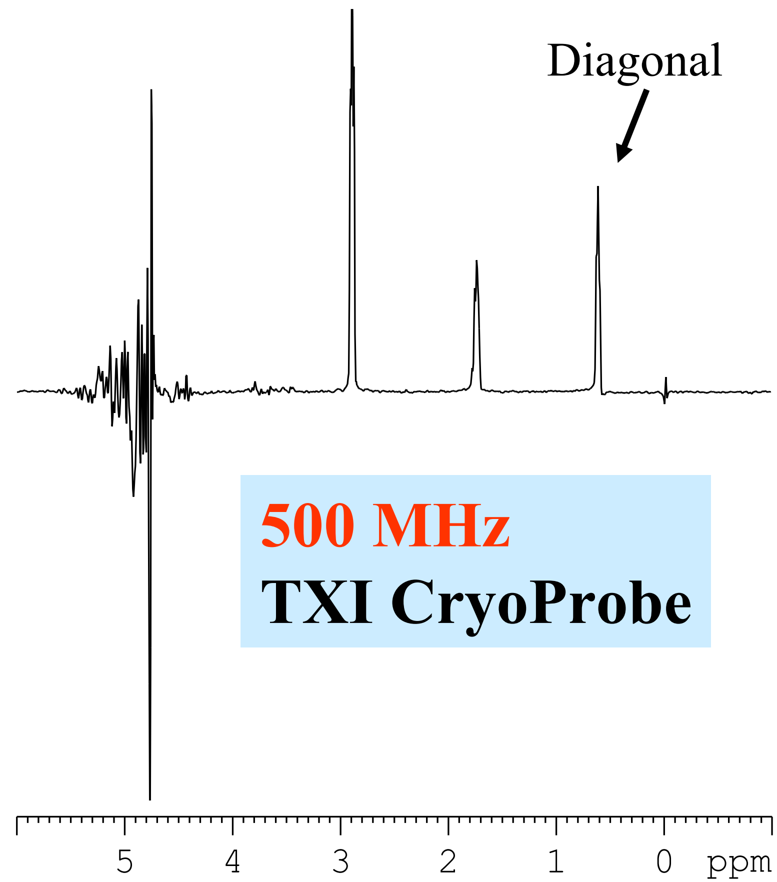


Sensitivity Comparison in H_2O : 2D TOCSY

S/N ratio (500 CryoProbe): S/N ratio (600 MHz RT probe)=2.2:1

1D row vector of 2D TOCSY:





Note: Better water suppression can be obtained by using excitation sculpting on a cryoprobe.

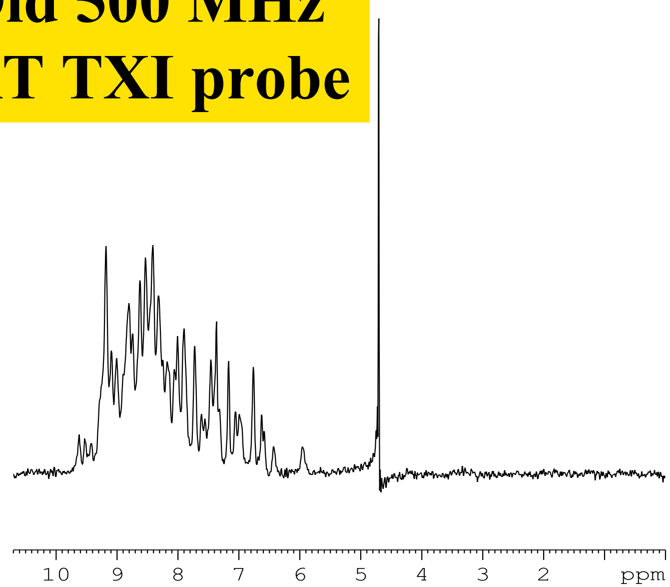
CryoProbe V.S. Room Temperature Probe

Sensitivity improvement: CryoProbe/Old RT TXI: 3.1:1

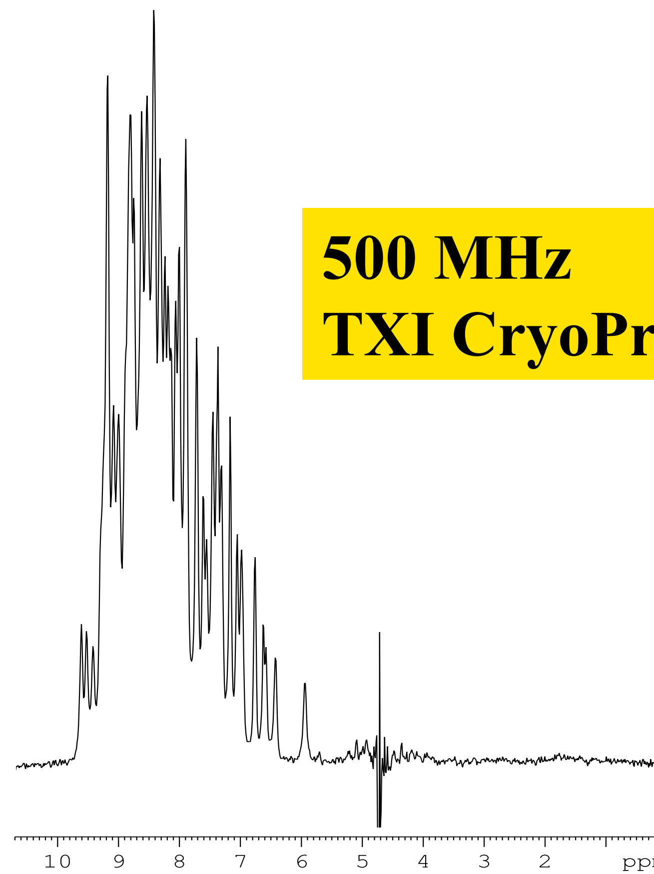
First increment of a ^{1}H, ^{15}N-HSQC (sensitivity enhanced version, pulprog=hsqcetf3gpsi2) of ^{13}C, ^{15}N-LBD in 50 mM phosphate/100 mM NaCl) at pH 7.3, 298 K.

Salty sample:

Old 500 MHz RT TXI probe



500 MHz TXI CryoProbe

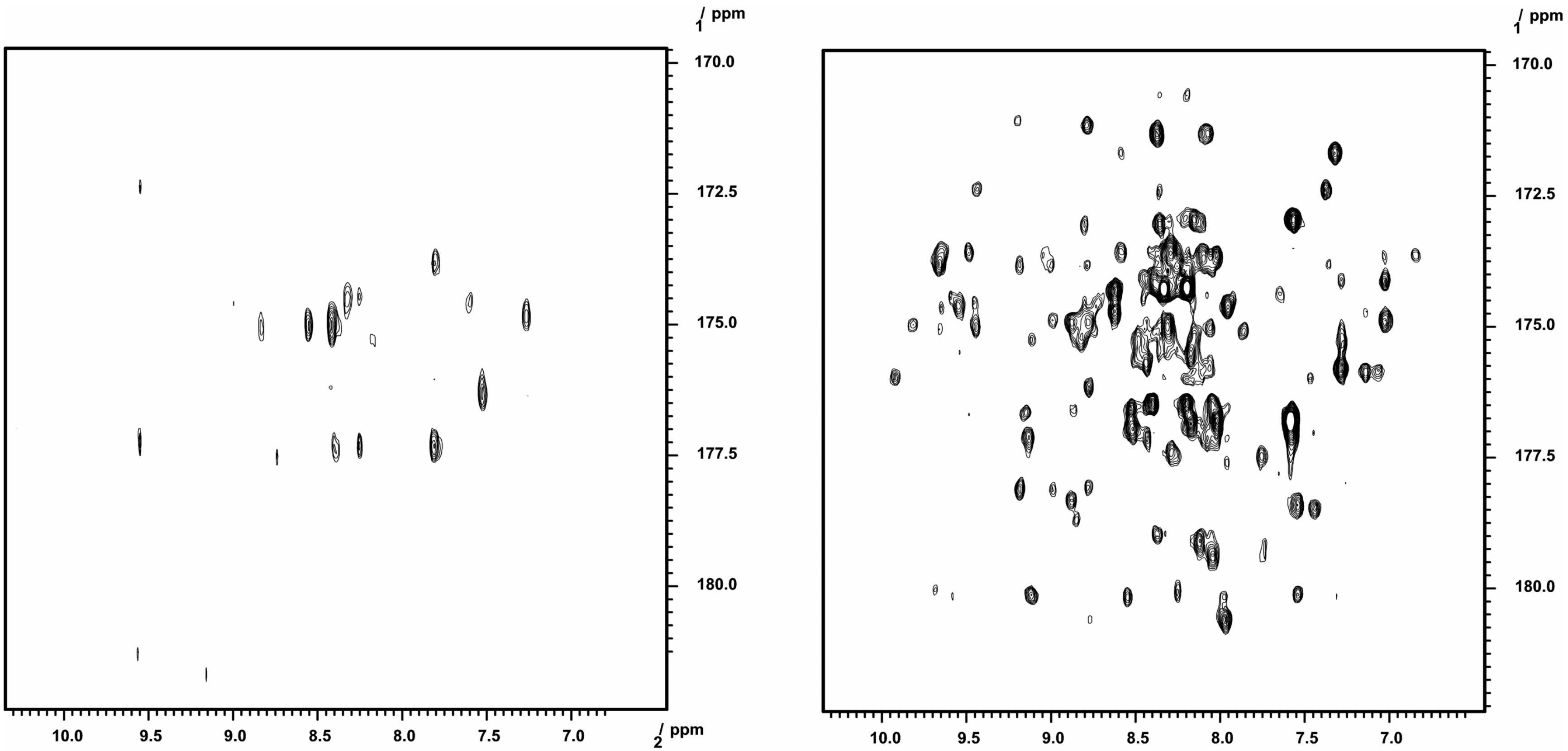


- **Sensitivity enhancement by the CryoProbe is yet to be evaluated for protein sample containing low conducting salts.**

CryoProbe V.S. Room Temperature Probe

600 MHz R.T. Probe (S/N=1200)

500 MHz CryoProbe



Fist 2D ^{1}H, ^{13}C plane of a 3D HN(CO)CA experiment.
1 mM,16 kDa protein. Data from Dr. Lou, Y-C (Dr. Chin-Pan Chen's lab) of IBMS, Academia Sinica.

Sensitivity Gain by CryoProbes

Our 500 MHz ^{1}H {^{13}C,^{15}N} TXI CryoProbe: ^{1}H at cryo-temperature, ^{13}C, ^{15}N at R.T.

- **Experiments that can gain sensitivity: All ^{1}H detection, inverse experiments with proton excitation and detection.**

Our 600 MHz ^{1}H {^{13}C,^{15}N} TCI CryoProbe: ^{1}H and ^{13}C (preamp.) at cryo-temperature, ^{15}N at R.T.

- **4-fold sensitivity for *^{1}H AND ^{13}C***

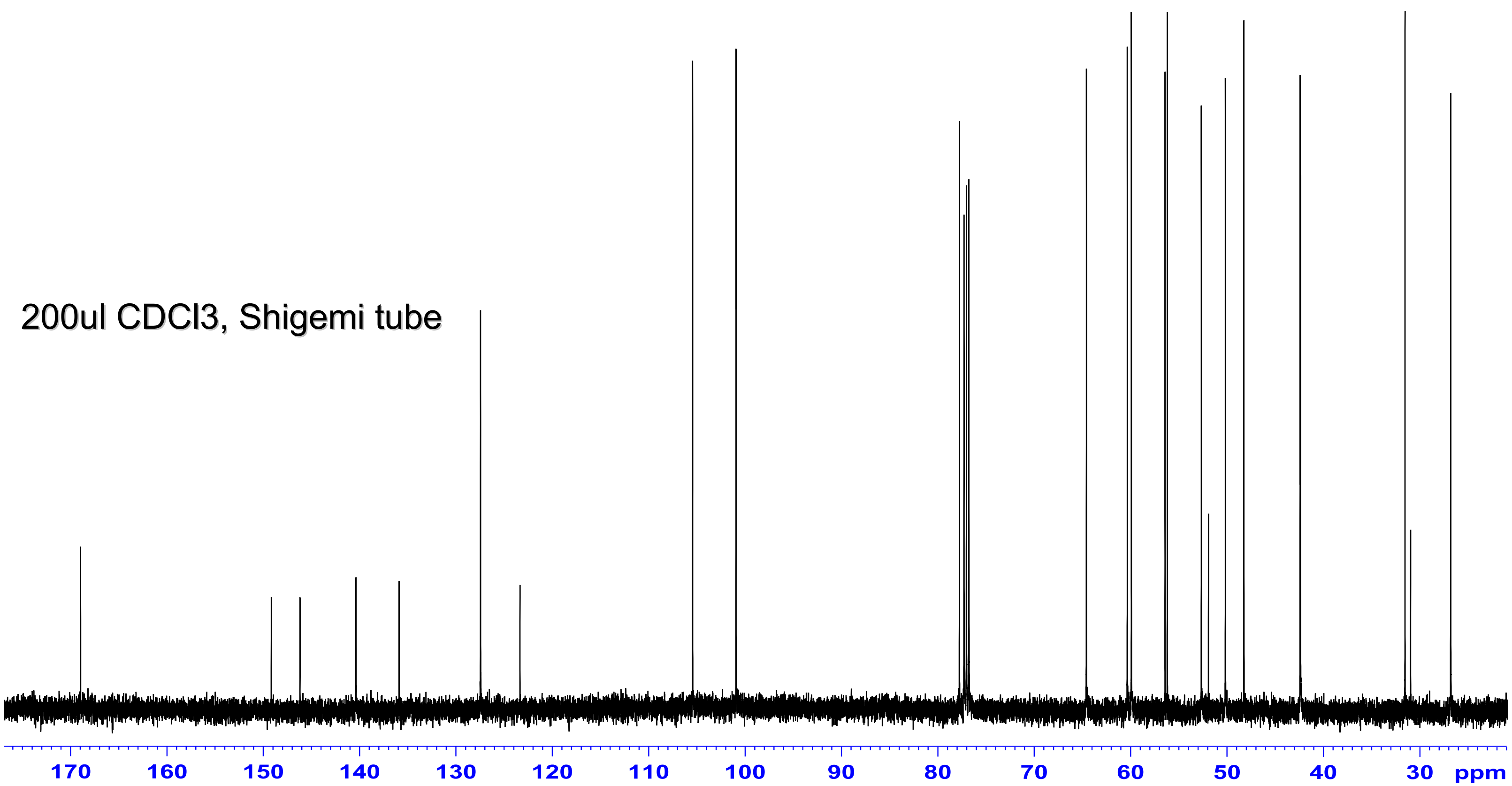
- **Sensitivity gain: All ^{1}H detection, ^{13}C detection (compared to RT-TXI probe), inverse experiments with ^{1}H or ^{13}C excitation and detection.**

5mm TCI CryoProbe™ $^{1}H\{^{13}C, ^{15}N\}$

^{13}C-NMR spectrum of 4mg brucine

NS=256, experiment time 9m 40s

200ul CDCl3, Shigemi tube



Slide from Bruker.

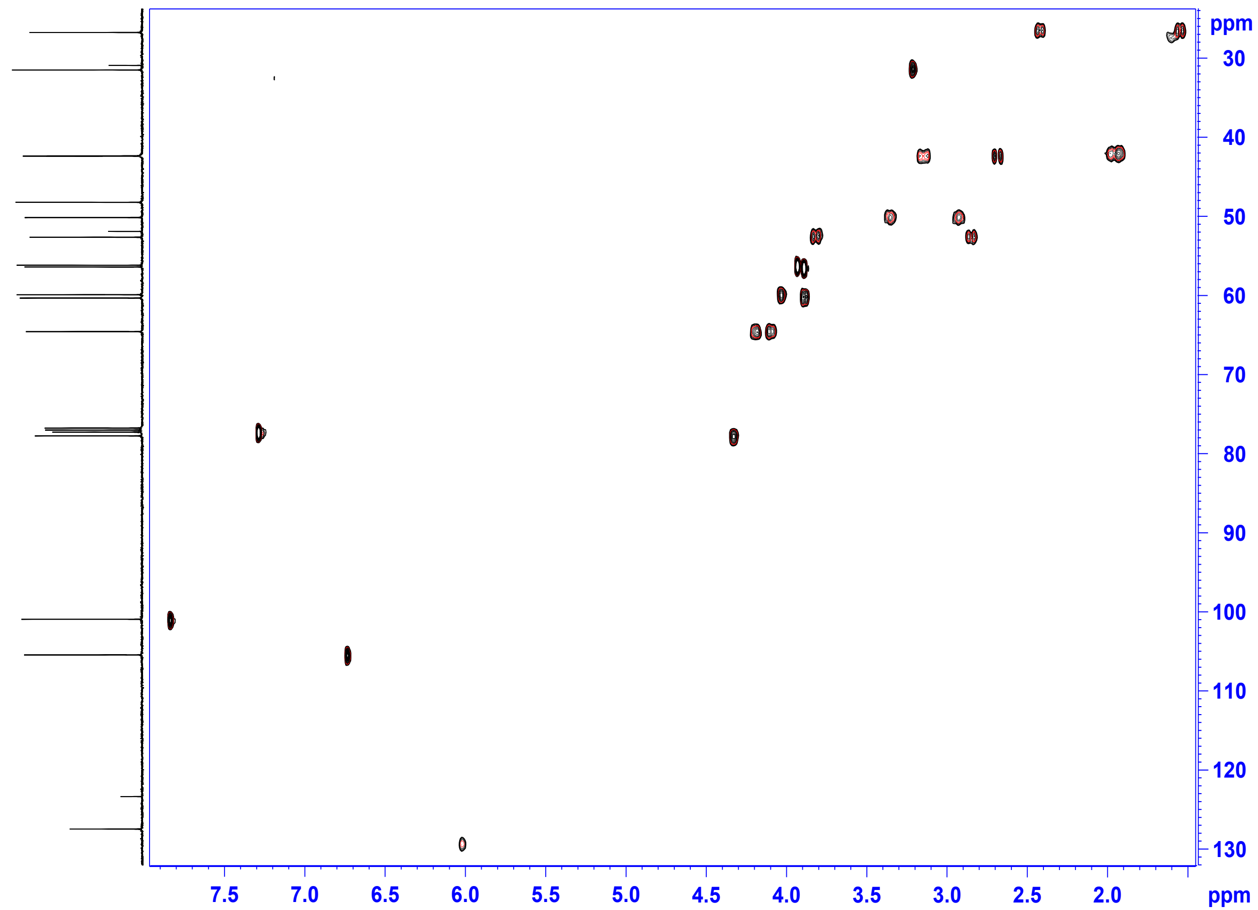
5mm TCI CryoProbe™ $^1H\{^{13}C, ^{15}N\}$

^1H, ^{13}C
HSQC:

200µg
brucine

NS=1

3 min 50 sec



Slide from Bruker.

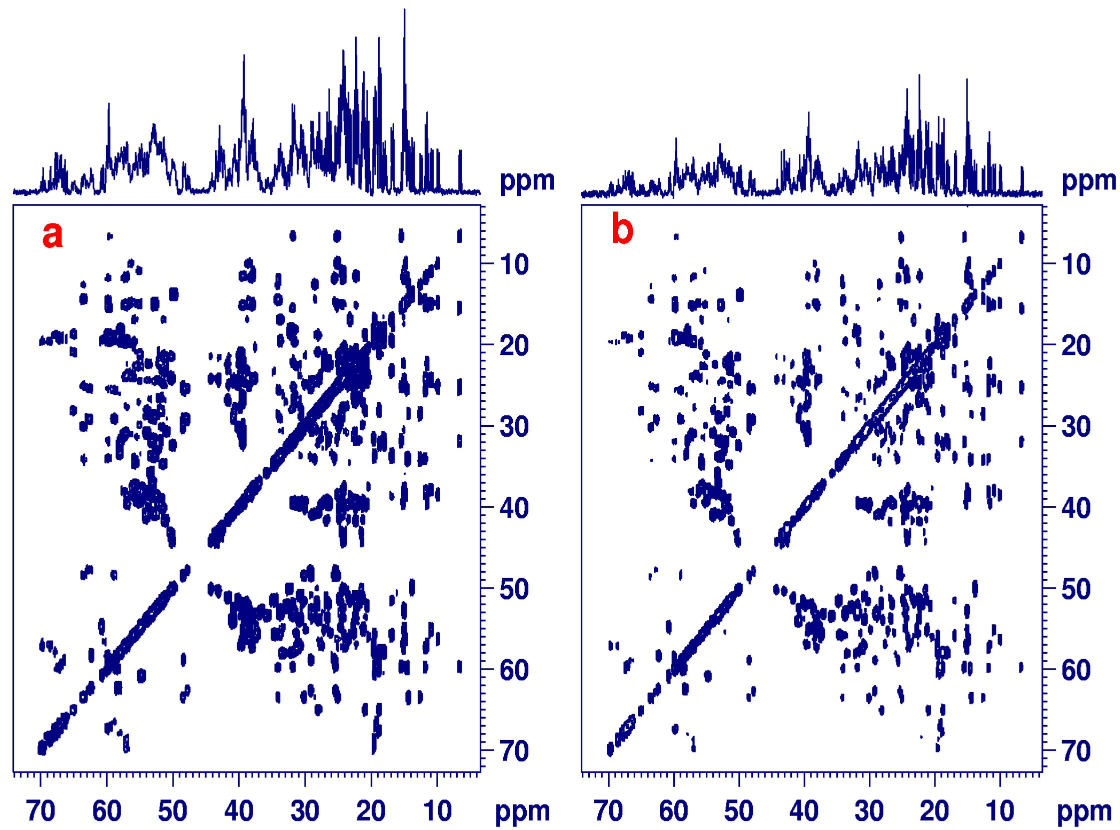
5mm TCI CryoProbe^{TM} ^{1}H{^{13}C, ^{15}N}

^{13}C-detection in biomolecular NMR spectroscopy

^{13}C-^{13}C-TOCSY

1mM ^{15}N,^{13}C-labeled ubiquitin

Recorded with the CryoProbes, as follows:
a) 500MHz DUL
b) 600MHz TCI, ^{15}N decoupled



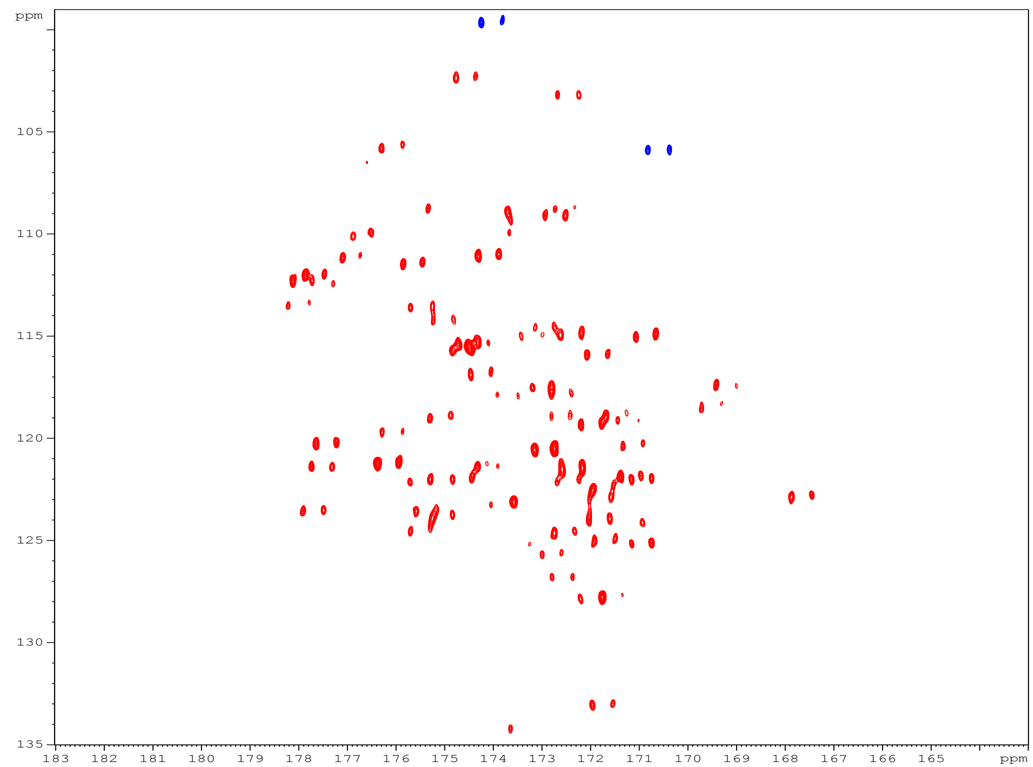
Slide from Bruker.

5mm TCI CryoProbe™ $^{1}H\{^{13}C, ^{15}N\}$

^{13}C-detection in biomolecular NMR spectroscopy

^{13}C,^{15}N-HSQC:
^{15}N-decoupling during acquisition

1mM ^{15}N,^{13}C-labeled ubiquitin



Slide from Bruker.

Strategies for Backbone Assignment of Large Proteins

#1 ***TROSY-HNCA, TROSY-HNCO and MQ-HACACO (^{13}C-obs.)***

Pervushin & Eletsky, J. Biomol. NMR, 25 (2003) 147-152

#2 ***TROSY-HNCO, TROSY-HN(CA)HA, MQ-HACACO (^{13}C-obs.)***

Hu, Eletsky & Pervushin J. Biomol. NMR, 26 (2003) 69-77

- **Set of experiments with both, ^{1}H and ^{13}C-detection**
- **Best sensitivity therefore required for both, ^{1}H and ^{13}C**
- **TCI CryoProbe offers an elegant solution**

This slide is modified from Bruker's slides.

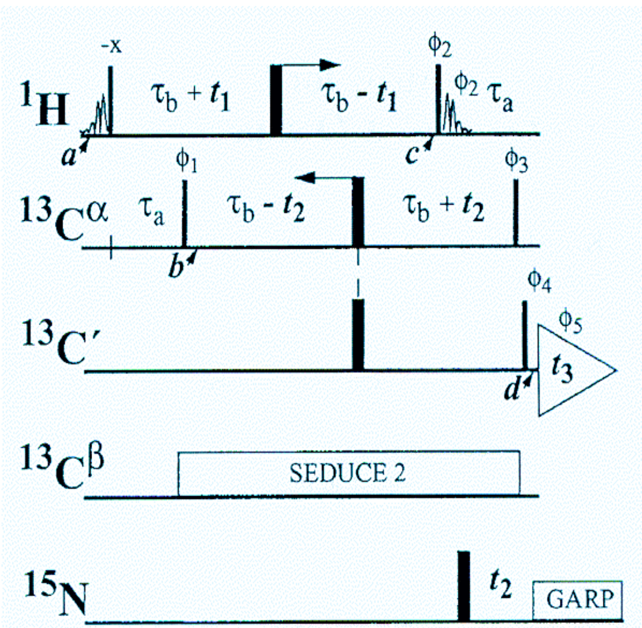
New Strategies for Backbone Assignment of Large Proteins

MQ-HACACO with direct ^{13}C-detection

1 mM, 35%-^{2}H,^{13}C,^{15}N-labeled chorismate mutase 44 kDa

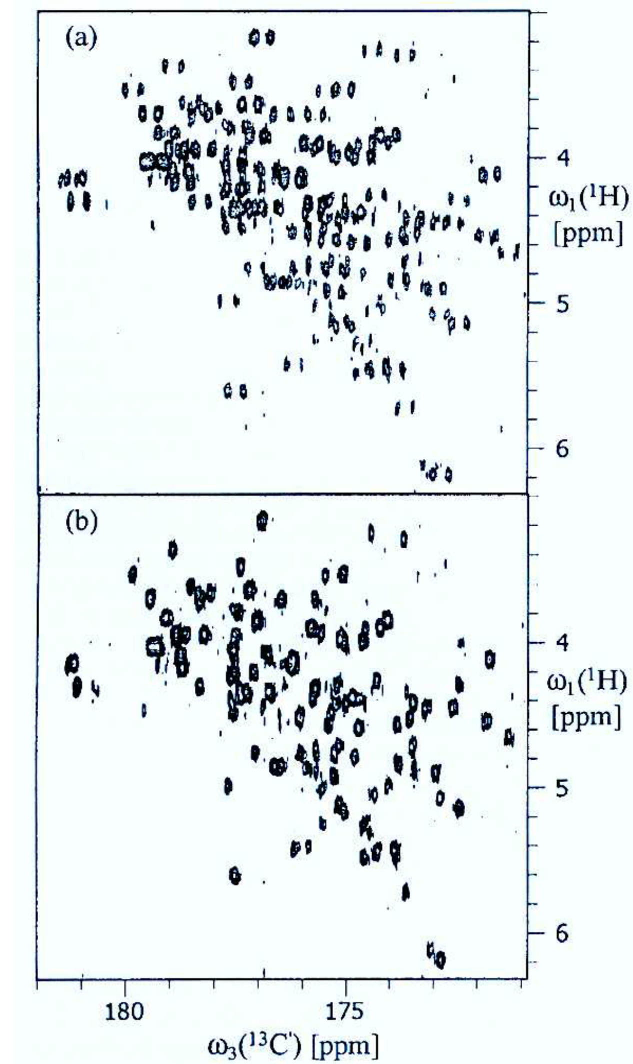
TXI CryoProbe 600MHz

- favourable MQ relaxation properties
- signal detection via the carbonyl-C antiphase doublet

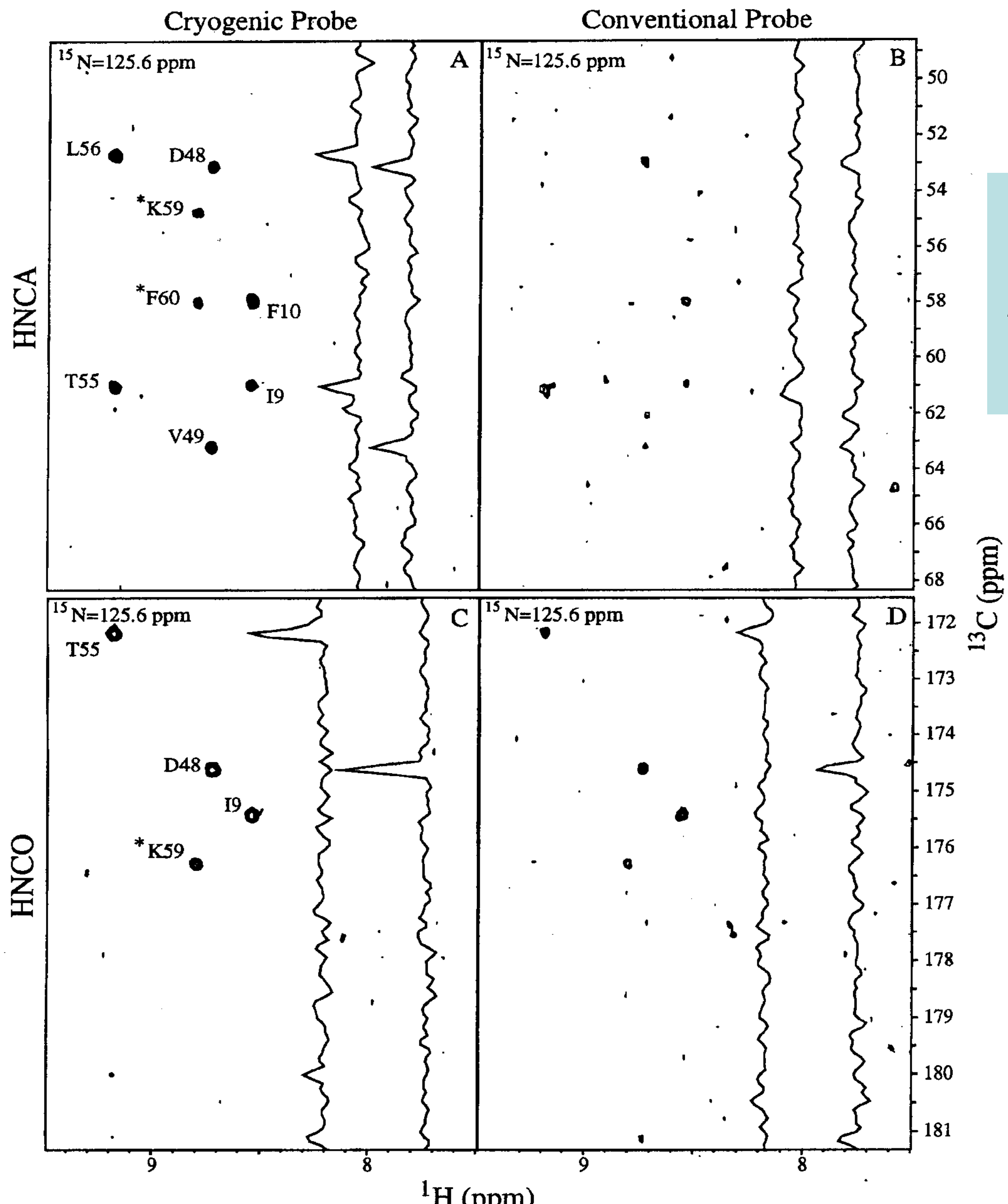


Pervushin & Eletsky, J. Biomol. NMR, 25 (2003) 147-152

First 2D plan of a 3D MQ-HACACO , (22 hr acquisition time). 600 MHz CryoProbe.



Combined HNCA/HNCO for ^{15}N labeled protein with ^{13}C at Natural Abundance



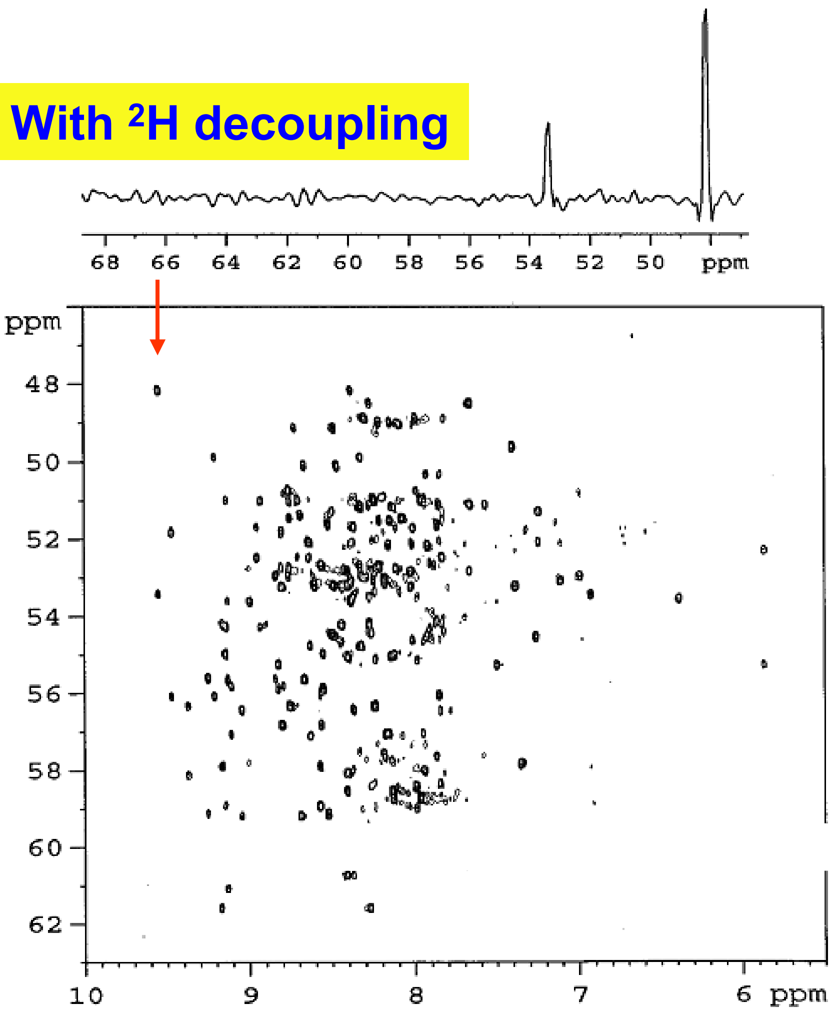
0.5 mM, 62 A.A. Protein
22 hr acquisition time
on a 500 MHz chili probe.

Kupce et al., *J. Biomol. NMR* , 27: 175-179, 2003

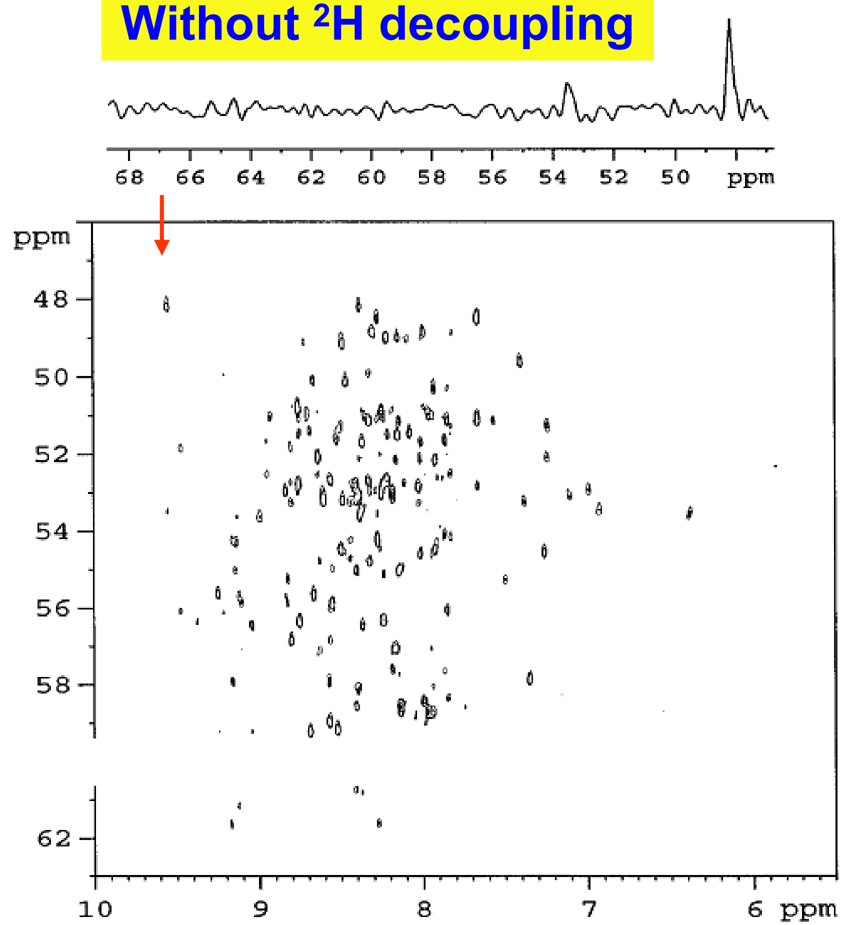
Both the 500 MHz and 600 MHz CryoProbes Have ^2H Decoupling Capacity.

First 15N plan (1H, 13C) of constant time-HNCA experiments for ^2H, ^{13}C, ^{15}N-Didomain, (pH 7.5, 295 K). NS=32. AV600-IBMS.

With ^2H decoupling

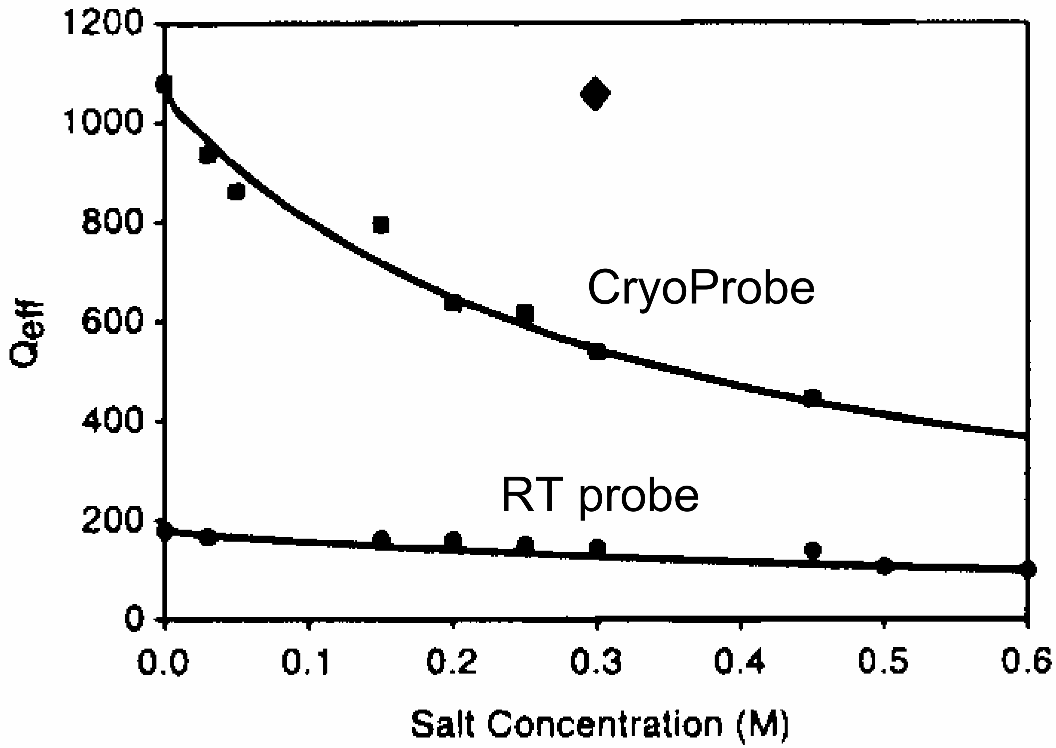


Without ^2H decoupling



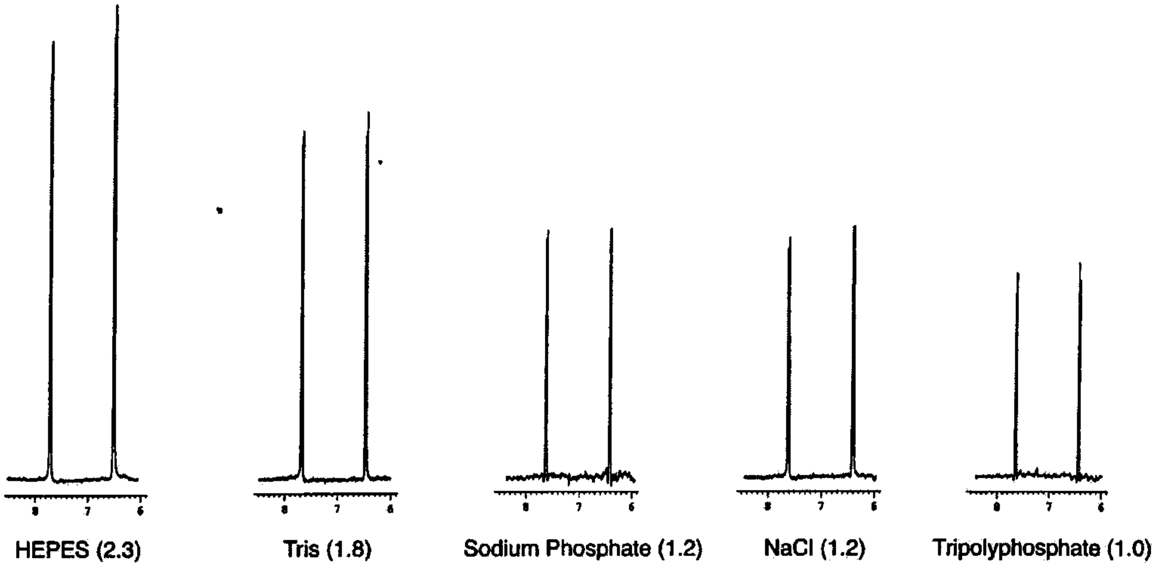
Effects of Salts on the Sensitivity of a CryoProbe

4824 *J. Am. Chem. Soc., Vol. 122, No. 19, 2000*



Flynn et al. *J. Am. Chem. Soc.*, 2000, 122, 4823-4824

Effects of Different Types of Conducting Salts on the Sensitivity of a CryoProbe



S/N shown in parenthesis

The decisive factor in degrading the sensitivity of a cryogenic probe is not just the salt concentration itself but the conductivity which is a function of both the concentration and the mobility of the salts.

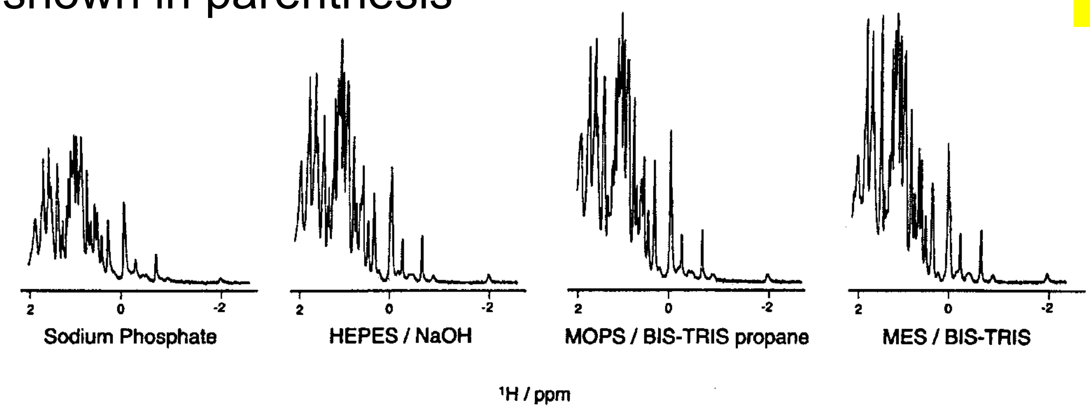


Figure 3. One-dimensional spectra of a 1 mM lysozyme sample measured in 50 mM sodium phosphate, 50 mM HEPES/NaOH, or 50 mM MOPS/BIS−TRIS propane buffer, all pH 7, and in 50 mM MES/BIS−TRIS, pH 6.0. Only the most high-field-shifted regions of the spectra are shown.

Reference: Kelly et al., *J. Am. Chem. Soc.* (2002), v124, 12013.

Standard Working Parameter Sets for the CryoProbe

Standard working parameter sets for most routine experiments have been generated using ^{13}C, ^{15}N-LBD. Users can quickly set up their experiment with minimal parameter modification.

- **1D Proton**
- **2D Homonuclear Experiments**
- **2D Heteronuclear Correlation Experiments**
- **3D Heteronuclear Editing Experiments**
- **3D Triple Resonance Backbone Experiments**
- **3D Sidechain Resonance Experiments**

All standard parameter files for the CryoProbe start with the words "CRP"
General syntax: CRP_nD_ExperimentType_PulseSequence_ExtraInfo.

For Example:

- **CRP_1D_ZGPGWG**
- **CRP_2D_15N_HSQC_hsqcf3gpph19**
- **CRP_3D_HNCA_hncagpwg3d**

CryoProbe Usage Notes

Instructions NMR-Applications

Typical Pulses for the 5 mm CryoProbe™
500MHz and 600MHz

- Follow the pulse and power limitation (next two pages) ! and our Facility's instructions.
- No simultaneous hard pulses on ^{15}N and ^{13}C, if so, a 3 dB extra attenuation is needed.

  (most Bruker XWINNMR 3.5 use shaped ^{13}C pulses). So, no foreign pulse sequences unless approved by us.
- Due to probe design, water suppression is more difficult.

Pulse and Power Recommendations for CryoProbe™

Note: All values require a minimum repetition period *d1+aq* of 1sec [2)]
<u>Important:</u> observe the separate notes on the following pages!

	5mm 500MHz TXI CryoProbe™	5mm 600MHz TXI CryoProbe™
^{1}H		
hard pulse [3)] (max. length 360°)	8.0 µsec	8.0 µsec
hard pulse for lossy samples	Power level corresponding to 8.0 µsec pulse for non-lossy sample	Power level corresponding to 8.0 µsec pulse for non-lossy sample
trim pulse p28 [4)]	2 msec @ 10 µsec	2 msec @ 10 µsec
TOCSY spin lock [5)]	200 msec @ 25 µsec 400 msec @ 35 µsec	200 msec @ 25 µsec 400 msec @ 35 µsec
ROESY spin lock	Up to CW for power level corresponding to a 100 µsec pulse	Up to CW for power level corresponding to a 100 µsec pulse
WALTZ16 decoupling during 13C-detection	Up to CW for power level corresponding to a 100 µsec pulse	Up to CW for power level corresponding to a 100 µsec pulse
DIPSI2-decoupling in triple resonance	400 msec @ 50 µsec	400 msec @ 50 µsec
^{13}C		
hard pulse [6)] (max. length 360°)	15.0 µsec	15.0 µsec
trim pulse [5)]	2 msec @ 25 µsec	2 msec @ 25 µsec
CC spin lock [5)]	20 msec @ 25 µsec	20 msec @ 25 µsec
GARP decoupling [7)]	140 msec @ 65 µsec	140 msec @ 55 µsec
selective pulses [8)]	Q5: 320 µsec Q3: 256 µsec CHIRP: 2 ms @ 25 µsec	Q5: 320 µsec Q3: 256 µsec CHIRP: 2 ms @ 25 µsec
^{15}N		
hard pulse [6)] (max. length 360°)	40.0 µsec	40.0 µsec
WALTZ16 decoupling [7)]	140 msec @ 200 µsec	140 msec @ 170 µsec

^{15}N		
hard pulse [6] (max. length 360°)	40.0 μsec	40.0 μsec
WALTZ16 decoupling [7]	140 msec @ 200 μsec	140 msec @ 170 μsec
CPMG T2 [9]	250 msec @ 80 μsec (see warning [9])	250 msec @ 80 μsec (see warning [9])
^{2}H		
hard pulse (max. length 360°)	150 usec	150 usec
WALTZ16 decoupling	100 msec @ 250 μsec	100 msec @ 250 μsec
Z-Gradient		
Absolute max. current [10]	10 A	10 A
Max.overall length [11]	10 ms @ 10 A	10 ms @ 10 A

Table 1

Pulse and Power Recommendations for CryoProbe™

Warning !!

Since 15N T2 measurements use CPMG (trains of hard 15N pulse with very short interval delays), excessive heating can occur and may damage the Cyroprobes. No such type of experiments shall be performed on our CryoProbe systems !!