Cryogenically cooled probes for Bio-NMR:
Probe geometry & sample losses

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Outline

• NMR & Sensitivity: optimizing probes for different applications
• Probe geometry and Mass Sensitivity
• Helium Cooled CryoProbes
  • Optimizing sensitivity for different samples & different applications
  • Salt & ionic strength
  • 3mm TCI vs. 5mm TCI CryoProbe
  • Observe (TXO) vs. Inverse (TCI) CryoProbe
• X-nucleus detection and sample losses
What is critical to NMR?

• Sensitivity
• Sensitivity
• Sensitivity
• ...

• Sensitivity comparison
  – At constant concentration
  – At constant mass
RF design & RF efficiency: $B_1/I_{\text{coil}}$

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

- Optimize RF coil design & RF coil materials
- Reduce RF coil diameter
<table>
<thead>
<tr>
<th>Probehead Diameter [mm]</th>
<th>1</th>
<th>1.7</th>
<th>3</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume [μl]</td>
<td>5</td>
<td>30</td>
<td>184</td>
<td>565</td>
</tr>
<tr>
<td>„Comfort Concentration“ [mM]</td>
<td>30</td>
<td>10</td>
<td>2.5</td>
<td>1</td>
</tr>
<tr>
<td>Sample amount [μMol]</td>
<td>0.150</td>
<td>0.300</td>
<td>0.460</td>
<td>0.565</td>
</tr>
<tr>
<td>Sample mass if MW = 300 Da [mg]</td>
<td>0.45</td>
<td>0.90</td>
<td>1.38</td>
<td>1.70</td>
</tr>
</tbody>
</table>
CryoProbe: Coil Temperature & Resistance

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

S/N versus Temperature (K)
Probe: sample & RF coil 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
  ~85 K (CryoProbe Prodigy)
  ~15-25 K (He-cooled CRP)
Acqueous samples & CryoProbes

- Typically some ionic strength is present
  - Buffer
  - Charges in Sample
  - Salt
CRP:
Signal-to-noise ratio (S/N) and noise sources

Influence of different noise sources:

\[ \frac{S}{N} \sim \sqrt{P_{\text{Coil}} (T_{\text{Coil}} + T_{\text{Preamp}}) + P_{\text{Sample}} (T_{\text{Sample}} + T_{\text{Preamp}})} \]

Electronics noise contribution:  
* probe performance

Sample noise contribution:  
* ionic strength

\( P_{\text{Sample}} \) is close to the physical limit for “pure” magnetic field generation for all Bruker probes since “last century”.

All Bruker Cryoprobes are virtually free of unnecessary E-Field and therefore optimal for ALL sample, including lossy solvents.
CRP:
Signal-to-noise ratio (S/N) and noise sources

- Organic solvent, e.g. CDCl3
- Pure Water, no salt
- low / moderate salt concentrations
- high salt concentrations
Signal-to-noise ratio (S/N) and noise sources

\[ \frac{S}{N} \sim \frac{1}{\sqrt{P_{\text{Coil}} \left( T_{\text{Coil}} + T_{\text{Preamp}} \right)}} \]

Intermediate Regime => Probe and sample dependent

\[ \frac{S}{N} \sim \frac{1}{\sqrt{P_{\text{Sample}}}} \]

Sample limited

Salt Conductivity
Signal-to-noise ratio (S/N) and sample diameter

Comparison 5mm round tube vs. 3mm round tube in 5mm 700 MHz TCI

S/N

Salt concentration NaCl [mM]

5mm (600 μl)
3mm (200 μl)
Signal-to-noise ratio (S/N) and sample diameter

Comparison 5mm round tube vs. 3mm round tube in 5mm 700 MHz TCI

- **Constant Mass**

![Graph showing S/N vs. Salt concentration NaCl [mM] for 5mm and 3mm round tubes]

- **5mm**
- **3mm**

- **S/N**
  - 3.00
  - 2.50
  - 2.00
  - 1.50
  - 1.00

- **Salt concentration NaCl [mM]**
  - 0
  - 200
  - 400
  - 600
  - 800
  - 1000

- **Comparison highlights**
  - **2.6x** increase
  - **2.8x** increase
Pulse length & sample diameter for lossy solvents

If sample noise dominates

- PW shorter with smaller tubes
- $PW \sim \sqrt{k_1 R_c + k_2 R_s}$
- $PW \sim \sqrt{\text{Loss}} \sim r_2$

- For higher (> 150 mMol) salt concentration it is better to use smaller diameter tubes
Signal Density in a non-lossy sample

Sample

Signal density in cross section

Signal density projected on x-direction

Change in Signal [x]

[Graph showing change in signal density along x-direction]
Sample Noise for a lossy sample

\[
\text{curl } E = -\frac{dB}{dt}
\]

\[
 j = \sigma E
\]

\[
\text{Noise}_{\text{SampleCrossSection}} = \iint_{xy} j^* E = \iint_{xy} \sigma E^2
\]
Signal-to-Noise in Cross-Section

Signal-to-Noise / cross section

~ 250mM NaCl

Noise Power Density in Cross-Section
Remove areas that do not contribute to enhance the sensitivity with Bruker Shaped Tubes

Sample Volume:

- Bruker shaped tube 350 µl
- Compared to standard tube 600 µl
Comparison 5mm round tube vs. 3mm round tube 
& Bruker shaped tube in 5mm 700 MHz TCI

Signal-to-noise ratio (S/N)

S/N

Salt concentration NaCl [mM]

Volumes:
5mm  600 μl
Shape 350 μl
3mm  200 μl
Sample loss [shape/salt]
Inverse Sample loss [salt/temperature]
Which geometry to chose?

- 5 mm round tube / 5 mm shigemi
- 3 mm round tube / 3 mm shigemi
- Shaped tube
- Slotted shigemi
Which geometry to chose?

- $^1$H detected low salt @ constant concentration: 5mm
- $^1$H detected low salt @ constant mass: 3mm
- $^1$H detected medium/high salt @ constant concentration: 3mm / shaped tube (keep $^1$H pulse width < 12us)
- $^{13}$C detected @ any condition: maximize number of spins in active volume
Sample loss [solvent/temperature]
CryoProbe: 5mm vs. 3mm
800 MHz: 3mm TCI

![Graph showing signal to noise ratio](image)

- **5mm CP: 5mm tube**
- **5mm CP: 3mm tube**
- **5mm CP: shaped**
- **3mm CP**

**Signal to Noise [% of 5mm 0mM]**

**Salt conc. [mol/l]**

- **0**
- **0.05**
- **0.1**
- **0.15**
- **0.2**
- **0.25**
Water suppression: 2mM Sucrose

- 1D $^1$H Presat using composite read pulse & crusher gradient
- 15% splitting
\( ^1\text{H} \) Watersuppression: 2mM Lysozyme

- 1D \(^1\text{H}\) Presat using composite read pulse & crusher gradient
- \( \text{ns} = 8 \)
- \( \text{AQ} = 1\text{s} \)
$^1$H-$^{15}$N HSQC: 2mM Lysozyme

- 2D $^1$H-$^{15}$N HSQC with flip-back & SI at natural abundance
- $ns = 16$
- 128 increments
- $AQ = 80$ms
- Expt. Time 38 min
- 7 uM $^{15}$N species !!
3mm dedicated CryoProbe vs. 5mm

- Higher $^{1}H$ (mass) sensitivity: +25..50% with same sample in 5mm TCI
- $^{13}C$ sensitivity: $\sim$1/3 of 5mm TCI (volume ratio)
- Shorter RF pulses at same power level
- Slightly lower salt dependence compared to 5mm tube in 5mm TCI
CryoProbe: X nucleus detection
CP-TXO vs. CP-TCI
Signal-to-noise ratio (S/N) INVERSE / OBSERVE CryoProbes

Comparison 5mm TCI & TXO

**S/N**

- **TCI using 5mm tube**
- **TCI using shaped tube**
- **TCI using 3mm tube**

**Salt concentration NaCl [mM]**

0 - 500
800 MHz CP-TXO

- 2mM Sucrose in H$_2$O:D$_2$O 9:1
- Presat using composite read pulse & crusher gradient
- Resolution: 13%

Data courtesy of Prof. Ichio Shimada, Dr. Koh Takeuchi, AIST, Tokyo
800 MHz CP-TXO

- 1mM T1RNAse in H₂O:D₂O 9:1
- ¹H-¹⁵N HSQC
- NS = 2
- Expt. Time 10 minutes

Data courtesy of Prof. Ichio Shimada, Dr. Koh Takeuchi, AIST, Tokyo
IDPs & UHF-NMR:
IDPs are challenging for NMR

- Due to lack of 3D structure massively reduced dispersion for $^1$H
- IDPs suffer inherently from much more NMR signal overlap

ubiquitin 295.7 K  \( \alpha \) -synuclein 295.7 K
IDPs & UHF-NMR
IPDs create new requirements for NMR methods and technology

- Highest sensitivity for $^1$H, $^{13}$C, $^{15}$N
- Highest signal dispersion
- Highest fields in GHz range
- $^{13}$C direct detection (5mm CP-TXO, 5mm CP-TCI)
- $^{15}$N direct detection
- ‘In-cell’ NMR
- High-dimensionality, fast experiments (4D, 5D, 6D,...) combined with NUS, APSY, parallel NMR
- New tools: multiple receivers, 3mm CP-TCI, triple-gradient CRP’s
800 MHz CP-TXO

- 1mM T1RNAse in H$_2$O:D$_2$O 9:1
- $^{15}$N data: refocused INEPT
- NS = 128
- Expt. Time 3 minutes

Data courtesy of Prof. Ichio Shimada, Dr. Koh Takeuchi, AIST, Tokyo
800 MHz CP-TXO

- 1mM T1RNAse in H₂O:D₂O 9:1
- ¹³C detected COCA CTIA
- NS = 1
- Expt. Time 10 minutes

Data courtesy of Prof. Ichio Shimada, Dr. Koh Takeuchi, AIST, Tokyo
800 MHz CP-TXO

- 1mM T1RNAse in H$_2$O:D$_2$O 9:1
- $^{13}$C detected (H)COCA IARE
- NS = 1
- D1 = 100ms!
- Expt. Time 71 seconds!

Data courtesy of Prof. Ichio Shimada, Dr. Koh Takeuchi, AIST, Tokyo
800 MHz CP-TXO

- 1mM T1RNAse in H₂O:D₂O 9:1
- 3D BEST-HNCOCA CB
- TD 2k x 32 x 128
- NS = 2
- D1 = 100ms
- Expt. Time 22.5 min

Data courtesy of Prof. Ichio Shimada, Dr. Koh Takeuchi, AIST, Tokyo
Sample loss [frequency]

Solvent dependence $\sim \nu^4$
Salt dependence $\sim \nu^2$
$^{13}$C detection: CON
950 MHz TCI

C/N-labeled ubiquitin
$^{13}$C detected CON using IPAP virtual homodecoupling

NS = 2
TD = 1k x 800
Expt. Time 32 minutes

298 K
$^{15}$N detection: NCO
950 MHz TCI (active $^{15}$N)

C/N-labeled ubiquitin

$^{15}$N detected NCO

NS = 48

TD = 2k x 144

Expt. Time 150 minutes
$^{13}\text{C CON vs. } ^{15}\text{N detection NCO}$

For the CON/NCO comparison of ubiquitin at 950 MHz and 298 K one obtains $\sim2.5:1$ for $^{15}\text{N detected NCO}$, 150 min.

$^{13}\text{C detected CON}$, $30\text{ min}$

$^{15}\text{N detected NCO}$, $150\text{ min}$

$^{13}\text{C vs. } ^{15}\text{N detection: neglecting relaxation one expects theoretically 5:1}$

For the CON/NCO comparison of ubiquitin at 950 MHz and 298 K one obtains $\sim2.5:1$.
$^{15}\text{N}$ detected HX-INEPT

950 MHz TCI
C/N-labeled ubiquitin, 298 K

$^{15}\text{N}$ detected HX-INEPT

NS = 4

TD = 4k x 128

Expt. Time 11 minutes
**TROSY: $^1$H & $^{15}$N Linewidth**

**The second decade — into the third millennium**

Kurt Wüthrich

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**Fig. 2** Frequency dependence from 100–1800 MHz of the full resonance line width at half height for amide groups in TROSY experiments calculated for three correlation times of $\tau_c = 20$, 60 and 320 ns, which represent spherical proteins with molecular weights of 50,000, 150,000 and 800,000 $M_r$. **a**, $^1$H$^N$ linewidth. **b**, $^{15}$N linewidth. (The calculation used $\Delta\sigma^{(15N)} = 155$ p.p.m. and $\Delta\sigma^{(1H)} = 15$ p.p.m.; axial symmetry was assumed for both tensors; the angle between the principal tensor axis and the N–H bond was assumed to be 15° for $^{15}$N and 10° for $^1$H$^N$; $d_{\text{H–N}} = 0.104$ nm; effects of long-range dipole–dipole couplings with spins outside of the $^{15}$N–$^1$H moiety were not considered.)
$^{15}\text{N}$ detected coupled INEPT - TROSY

TCI 950 MHz
C/N-labeled ubiquitin, 298 K

$^{15}\text{N}$ detected HX-INEPT, fully coupled: illustrating the TROSY effect

NS = 32
TD = 4k x 256

Expt. Time 3.5 hours
$^{15}\text{N}$ detected coupled INEPT - TROSY

TCI 950 MHz
C/N-labeled ubiquitin, 278 K

$^{15}\text{N}$ detected HX-INEPT, fully coupled: illustrating the TROSY effect

NS = 32
TD = 4k x 256

Expt. Time 3.5 hours
$^{15}$N detected coupled INEPT - TROSY

950 MHz TCI
C/N-labeled ubiquitin

$^{15}$N detected HX-INEPT, fully coupled: illustrating the TROSY effect as a function of temperature

298 K

278 K
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