Outline: CryoProbes

- CryoProbes
  - CryoProbe Prodigy Overview
  - Helium CryoProbe Overview
  - Cooling Unit CU/2 & CU/3
  - New Options and Models
  - 3mm TCI 800 MHz – 1 GHz
  - 5mm TXO vs. TCI
  - 5mm BBO 800 MHz
CryoProbe™ Prodigy
Economical and Affordable

- Price significantly less than a conventional He-cooled closed loop CryoProbe
- Impressive performance boost with **minimum operating** and **maintenance costs**
- **Long service intervals** of two years
- Probe package comprises only control unit and a liquid N2 vessel
- **Easy siting** with no additional infrastructure
<table>
<thead>
<tr>
<th>CryoProbe Prodigy</th>
<th>400 MHz</th>
<th>500 MHz</th>
<th>600 MHz</th>
<th>700 MHz</th>
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<td>Prodigy BBO</td>
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<td>Prodigy TCI</td>
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17.5mg Quinine (100mM) in DMSO in a standard 5mm tube.

Expt. Time: 16 h
15mM Ajmalicin in DMSO-d$_6$

2D 1H-$^{13}$C HMBC

ns = 2;

TD = 4k x 256

12 min
CryoProbe Prodigy TCI 500 MHz
$^{1}H-^{13}C$ NOESY-HSQC

- Lb-FABP 1.3 mM
- $N_s = 8$
- $TD = 2k \times 512$
- 120 ms mixing
- 90 min

CryoProbe™ Prodigy

Summary

• Sensitivity enhancement
  ➢ **2...3** for **all nuclei** (equivalent to >300 MHz higher field)
  ➢ More than **5 x faster** compared to equivalent RT probe
  ➢ Increased **sample throughput** or **reduction in sample amount**
    for labs in academia and industry

• Exciting features for
  ➢ Routine & research applications
  ➢ Small molecule structural work
  ➢ Bio-NMR: excellent solvent suppression, power handling
16 years CryoProbe success story

>1600 CryoProbes delivered to customers

... the evolution is going on!
CryoProbe product portfolio is growing
CryoCooling Unit Generations & Support

• **Cooling Unit CU/2:**
  - End of production 2005
  - **End of Service (EOS) January 2018,** support on best effort at reasonable endeavour basis

- Z49290 – 2m
- Z70377 – 3m
- Z70917 – 4m
- Z70929 – 5m
- Z74368 – 6m
- Z71863 - ICE
CryoCooling Unit Generations & Support

• **Cooling Unit CU/3:**
  • End of production 2006
  • **End of Service (EOS) January 2018,** support on best effort at reasonable endeavour basis

• Z74852 – 2m
• Z74853 – 3m
• Z74854 – 4m
• Z74855 – 5m
• Z74856 – 6m
• Z74857 - ICE
### CryoProbe Portfolio (Spectroscopy) Helium cooled

<table>
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<th>CryoProbes</th>
<th>400</th>
<th>500</th>
<th>600</th>
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5mm TXO CryoProbes

- Temperature range: -40° C...+80° C (optional 0° C...135° C)
- All nuclei benefit from cold preamplifier:
  - $^{13}\text{C}$, $^{15}\text{N}$, $^{1}\text{H}$, $^{2}\text{H}$
- Z-Gradient

- Two versions:
  - $^{13}\text{C}$ optimized (default)
  - $^{15}\text{N}$ optimized (optional: ~30% gain for $^{15}\text{N}$, at the expense of $^{13}\text{C}$ SiNo)
5mm TCI CryoProbes

- Temperature range: $-40^\circ\text{C}...+80^\circ\text{C}$ (optional $0^\circ\text{C}...135^\circ\text{C}$)
- Cold preamplifier: $^1\text{H}$, $^{13}\text{C}$, $^2\text{H}$,
  - New: optional $^{15}\text{N}$ cold preamplifier
- Optional: $^1\text{H}$ tuneable to $^{19}\text{F}$
- Z-Gradient
  - Optional XYZ-Gradient
5mm QCI HPCN CryoProbes

- Temperature range: -40° C...+80° C (optional 0° C...135° C)
- Cold preamplifier: $^1$H, $^{13}$C, $^2$H,
  - New: optional $^{31}$P or $^{15}$N cold preamplifier
- Optional: $^1$H tuneable to $^{19}$F
- Z-Gradient
10mm DUL CryoProbes

- Temperature range: 0° C...+135° C
- Cold preamplifier: $^1$H, $^{13}$C, $^2$H,
- Z-Gradient (2 G/cmA)
- New: multi-nucleus “MNP” 10mm CryoProbe
  - Up to 5 X nuclei ($^{31}$P-$^{15}$N frequency range)
800 MHz 3mm TCI
3mm 800 MHz TCI $^1$H Watersuppression: 2mM Lysozyme

- 1D $^1$H Presat using composite read pulse & crusher gradient
- ns = 8
- AQ = 1s
3mm TCI 800 MHz $^1$H-$^{15}$N HSQC: 2mM Lysozyme

- 2D $^1$H-$^{15}$N HSQC with flip-back & SI at natural abundance
- ns = 16
- 128 increments
- AQ = 80ms
- Expt. Time 38 min
- 7 uM $^{15}$N species !!
800 MHz 5mm BBO
5mm CP-BBO 800 MHz

- 5mm BBO H&F Z-Gradient CryoProbe
  - $^1\text{H} - ^{19}\text{F};$
  - $^{31}\text{P} - ^{15}\text{N};$
  - $^2\text{H}$

- All nuclei benefit from cryogenically cooled preamplifier

- Temperature range
  - $-40^\circ\text{C} \ldots +80^\circ\text{C}$
  - $0^\circ\text{C} \ldots +135^\circ\text{C}$
5mm CP-BBO 800 MHz

- Polymer in TCE-d$_2$
- 1D $^1$H
- NS = 16
- ZG30
- TE = 400 K

x 4096
• Polymer in TCE-d$_2$
• 1D $^{13}$C{$^1$H}
• Ns = 1024
• ZGPG30
• TE = 400 K
• Expt. Time 47 min
5mm CP-BBO 800 MHz

- 10mg $^{195}\text{Pt}$-complex in CD$_2$Cl$_2$
- 1D $^1\text{H}$
- NS = 1
5mm CP-BBO 800 MHz $^{31}\text{P}$ detection

- 10mg $^{195}\text{Pt}$-complex in CD$_2$Cl$_2$
- 1D $^{31}\text{P}$ {$^1\text{H}$}
- NS = 32

$^{31}\text{P}$-$^{195}\text{Pt}$: 1742 Hz
5mm CP-BBO 800 MHz $^{195}\text{Pt}$

- 10mg $^{195}\text{Pt}$-complex in CD$_2$Cl$_2$
- 1D $^{195}\text{Pt} \{^1\text{H}\}$
- NS = 256
- Expt. Time: 1 min 42 sec
5mm CP-BBO 800 MHz $^{195}\text{Pt}$

- 10mg $^{195}\text{Pt}$-complex in CD$_2$Cl$_2$
- $^{195}\text{Pt} \{^1\text{H}\}$
- NS = 128

- T1 inversion-recovery
- T1 = 21 msec
5mm CP-BBO 800 MHz $^{195}$Pt

- 10mg $^{195}$Pt-complex in CD$_2$Cl$_2$
- $^1$H - $^{195}$Pt HMQC
- TD 1k x 128
- NS = 8
- Cnst2 = 50 Hz
- Expt. Time 18.5 min
1 GHz 5mm TCI
ENC 2016 Announcement: World's first shielded **Aeon 1 GHz** System installed

- Active shielding reduces space requirements by > one order of magnitude
- **Aeon 1 GHz** magnets leverage advanced BEST superconductors
- Active refrigeration eliminates LN2, reduces LHe boil-off essentially to zero
- **New GHz-class magnet, novel Cryoprobes, 111 kHz MAS probes, and new NMR methods expand frontiers in structural biology, membrane protein and intrinsically disordered protein (IDP) research**

**Aeon 1 GHz** at Research Center for Bio-Macromolecules at University of Bayreuth
2mM Sucrose in H$_2$O:D$_2$O 9:1

Resolution: 10%
Hump: 9.7 / 16.7 Hz

Composite pulse & crusher gradient presat
1 GHz 5mm TCI: CC-TOCSY

C/N-labeled ubiquitin

$^{13}$C detected CC-TOCSY

NS = 16

TD = 4k x 128

Mixing = 20ms @ 13.5 kHz

Expt. Time 40 minutes
1 GHz relaxation-optimized 3D

C/N-labeled ubiquitin

3D BEST-TROSY-HNCOCACB

NS = 2
TD = 1k x 64 x 256

D1 = 200ms

NUS: 12.5 %
CS processing

Expt. Time: 28 minutes

(traditional sampling: 3.75 hours)
CryoProbe: X nucleus detection
CP-TXO vs. CP-TCI
X-nucleus detection: $^{13}\text{C} \ & \ ^{15}\text{N}$

- Relative Sensitivity provided sample is uniformly labeled:
  - $\gamma_{\text{H}} = 26,7522208 \ [10^7 \text{ rad s}^{-1} \text{ T}^{-1}]$
  - $\gamma_{\text{C}} = 6,728286 \ [10^7 \text{ rad s}^{-1} \text{ T}^{-1}]$
  - $\gamma_{\text{N}} = -2,7126189 \ [10^7 \text{ rad s}^{-1} \text{ T}^{-1}]$

- Signal-to-noise is proportional to $\gamma^{3/2}$
  - $\text{S/N}_{\text{carbon}} = 0,13 \ \text{S/N}_{\text{protons}}$
  - $\text{S/N}_{\text{nitrogen}} = 0,18 \ \text{S/N}_{\text{carbon}}$
  - $\text{S/N}_{\text{nitrogen}} = 0,03 \ \text{S/N}_{\text{protons}}$
Probe design principles

Proton on inner Coil: “INVERSE”

X-Nucleus on inner coil: “OBSERVE”

$^1\text{H}$ (Proton)  

TCI, QCI

X-nuclei C, N

DCH, TXO
Probe design principles

Proton on inner Coil: “INVERSE”

$^1\text{H}$ (Proton)

X-Nucleus on inner coil: “OBSERVE”

X-nuclei C, N

X-nuclei C, N, D

TCI, QCI

DCH, TXO

$^1\text{H}$ (Proton)
Signal-to-noise ratio (S/N) INVERSE / OBSERVE CryoProbes

**Comparison 5mm TCI & TXO**

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

**Salt concentration NaCl [mM]**

0 to 500 on the x-axis, 0.00 to 1.00 on the y-axis.
800 MHz CP-TXO

- 2mM Sucrose in H₂O:D₂O 9:1
- Presat using composite read pulse & crusher gradient
- Resolution: 13%

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  \hspace{2cm} \alpha\text{-synuclein} 295.7 K
IDPs & (UHF-)NMR

IPDs create new requirements for NMR methods and technology

- Highest sensitivity for $^1\text{H}$, $^{13}\text{C}$, $^{15}\text{N}$
- Highest signal dispersion
- Highest fields in GHz range
- $^{13}\text{C}$ direct detection (5mm CP-TXO, 5mm CP-TCI)
- $^{15}\text{N}$ 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 H$_2$O:D$_2$O 9:1
- $^{13}$C 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
Direct or indirect?

$^1$H or X detection?
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
$^{15}$N detection: NCO
950 MHz TCI (active $^{15}$N)

C/N-labeled ubiquitin

$^{15}$N detected NCO

NS = 48

TD = $2k \times 144$

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

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

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

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

For the CON/NCO comparison of ubiquitin at 950 MHz and 298 K one obtains \(~2.5:1\)
Fig. 2 Frequency dependence from 100–1800 MHz of the full resonance linewidth 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.** $^1\text{H}^N$ linewidth. 
- **b.** $^{15}\text{N}$ linewidth. 

The calculation used $\Delta \sigma(^{15}\text{N}) = 155$ p.p.m. and $\Delta \sigma(^1\text{H}^N) = 15$ 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 $15^\circ$ for $^{15}\text{N}$ and $10^\circ$ for $^1\text{H}^N$; $d_{\text{N-H}} = 0.104$ nm; effects of long-range dipole-dipole couplings with spins outside of the $^{15}\text{N}-^1\text{H}$ moiety were not considered.
$^{15}$N detected coupled INEPT - TROSY

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

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

NS = 64
TD = 4k x 256

Expt. Time 7 hours
$^{15}$N detected coupled INEPT - TROSY

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

$^{15}$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
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Acknowledgements

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