New Capabilities with X-Detection

Cooled ^{13}C and ^{15}N Preamplifiers and TXO CryoProbes

High-field high–resolution NMR in structural biology has been very successful using inverse detection schemes. The most popular CryoProbe models for macromolecular work are therefore triple resonance inverse (TCI) or quadruple resonance inverse (QCI) probes. Active (cryogenically cooled) preamplifier electronics for ^{13}C have been standard for a number of years, for almost all CryoProbes.

Over the past decade, this increased sensitivity has supported the development of direct ^{13}C detection.[1] Direct ^{13}C detection is now an established, powerful, complementary technique, especially for the study of intrinsically disordered proteins (IDP) and proteins with paramagnetic centers.[2] For very large globular proteins, ^{1}H and ^{13}C approach their natural limits, while the sharp ^{15}N lines of a few Hz line width still offer long magnetic coherence lifetimes and high resolution.

Recently, a collaboration between groups in Harvard and Tokyo[3] has demonstrated great potential for using ^{15}N direct detection in combination with TROSY. Additionally, the need for perdeuteration is reduced when using the ^{15}N detected approach, thus facilitating protein expression and detection of amide protons involved in hydrogen bonds.

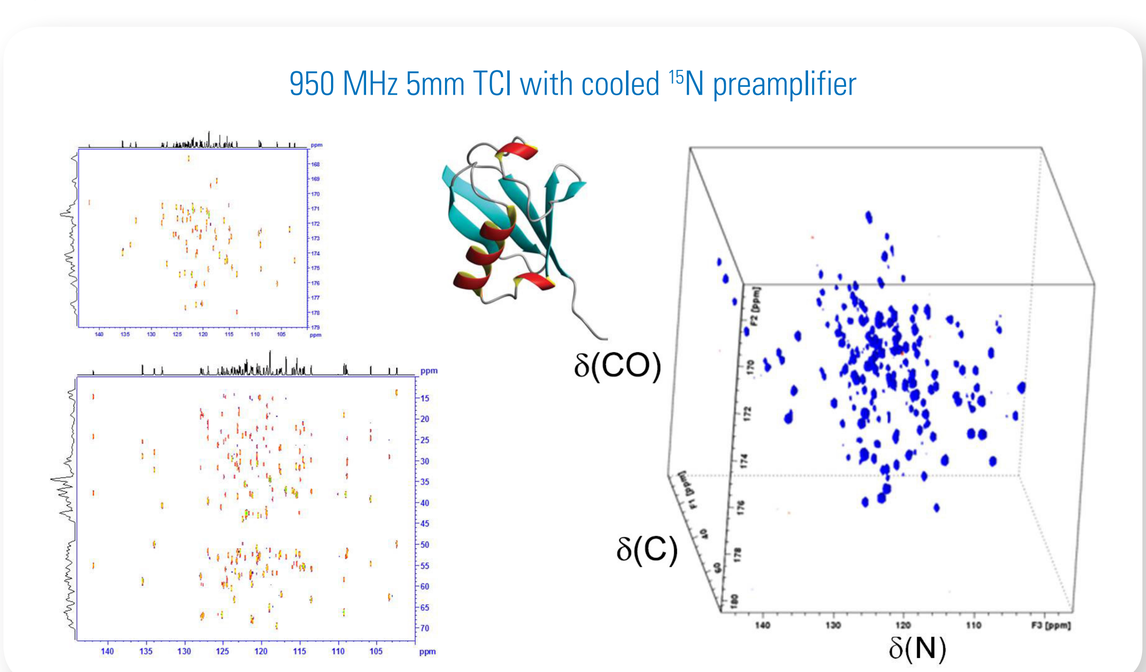

Fig. 2 1.8mM $^{13}C/^{15}N$ labeled Ubiquitin, ^{15}N detected refocused INEPT at 950 MHz: Top: 298 K, bottom 278 K. Reduced HN-solvent exchange shows larger benefit for many resonances in ^{15}N detection as compared to signal broadening due to slower tumbling rates. Typical linewidth 3-8 Hz. Adiabatic ^{13}C (Chirp, 48 kHz sweep) CPD decoupling. NS = 256, experimental time 7 min 47 sec

950 MHz 5mm TCI with cooled ^{15}N preamplifier

Fig. 3 1.8mM $^{13}C/^{15}N$ labeled Ubiquitin. Left: ^{15}N detected 2D C-N and CO-N projections of 3D (H)CCCON. NS = 16, TD = 2k x 64 x 96; total experimental time 37 hours.

Dual Receive: 700 MHz 5mm TXO CryoProbe

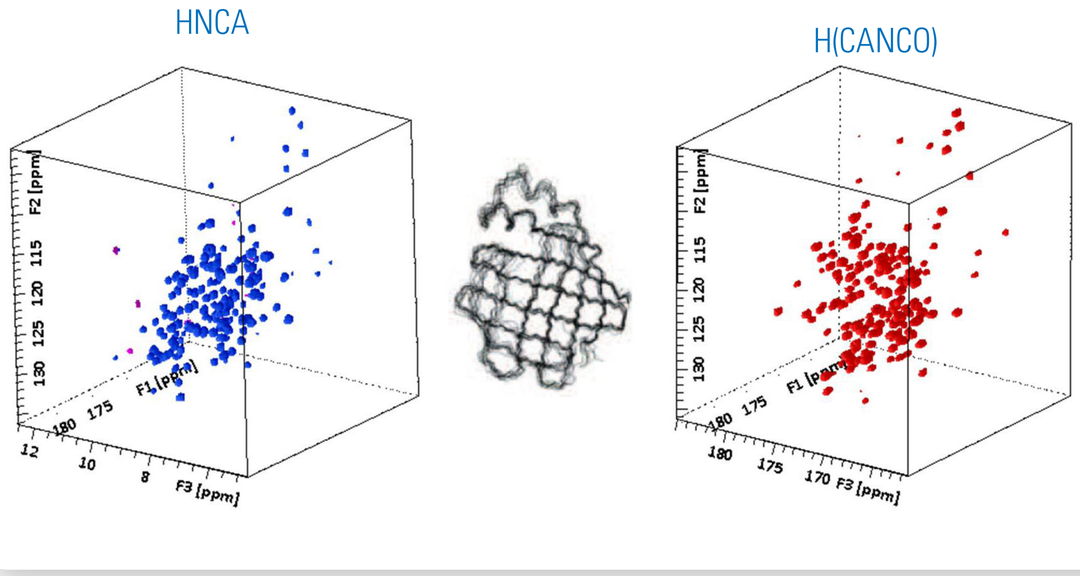

Fig. 1 1.3mM $^{13}C/^{15}N$ labeled Liver Basic Fatty Acid Binding Protein (LB-FABP) dual receiver experiment. Two scans per increment, 12 hours total acquisition time. 1k x 40 x 32 complex points after IPAP processing. Structure ensemble from Vasile et al. J Biomol NMR; 25, 157-160, 2003.

950 MHz 5mm TCI with cooled ^{15}N preamplifier

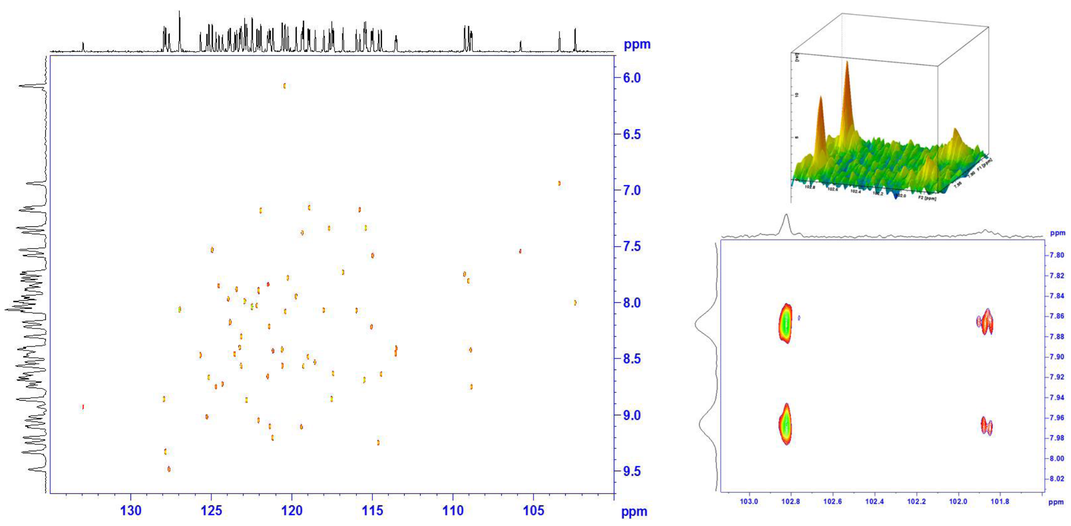

Fig. 4 1.8mM $^{13}C/^{15}N$ labeled Ubiquitin. Left: ^{15}N detected high resolution 2D refocused and **fully decoupled HX-INEPT** (298 K). NS = 4, TD = 4k x 128; total experimental time 11 minutes. Right: zoom in to a single cross-peak when acquiring a **fully coupled HX-INEPT** illustrating the TROSY effect. Data acquired at 278 K.

Direct Observe CryoProbes offer several advantages over indirect probes. The increased X-nucleus sensitivity makes the development and use of ^{13}C-^{1}H dual-receive experiments much more attractive, as the relative sensitivity between the two nuclei is much more balanced and hence the compromises to be taken are significantly reduced when concatenating two experiments. In addition, the effect of ionic strength on ^{1}H sensitivity is reduced, as the filling factor for ^{1}H is lower compared to inverse probes.

References

1. Bermel W, Bertini I, Felli IC, Kuemmerle R, Pierattelli R, 2003, J Am Chem Soc 125:16423-9; Bermel W, Bertini I, Felli IC, Piccioli M, Pierattelli R, 2006, Prog Nucl Magn Res Spec 48:25–45
2. Bertini I, Lee YM, Luchinat C, Piccioli M, Poggi L, 2001, ChemBioChem2:550-558
3. Takeuchi K, Arthanari H, Shimada I, Wagner G. 2015, Nitrogen detected TROSY at high field yields high resolution and sensitivity for protein NMR, J. Biomol. NMR, 63(4):323-31

Summary

- ^{13}C and ^{15}N detection is attractive for IDR & IDP.
- ^{13}C detection is a well established alternative to traditional indirect detection.
- ^{15}N detection shows potential especially for high molecular weight globular macromolecules.
- Active preamplifier electronics for ^{13}C and ^{15}N are available for new TCI's and TXO's.