Ultracool NMR technology

Cryogenically cooled probes allow significant inroads into drug discovery and characterization.

BY KIMBERLY L. COLSON

Recent progress in NMR spectroscopy has delivered important capabilities to drug discovery researchers. One of the most notable is the development of cryogenically cooled NMR probes, which have delivered the single largest increase in sensitivity in NMR spectroscopy in the last few decades. These sensitivity enhancements enable pharmaceutical researchers to observe samples that were considered too small just a few years ago and to increase sample throughput up to 16-fold.

Cryogenic mechanics

Cryogenically cooled probe technology takes advantage of the inverse relationship of the signal-to-noise ratio (S/N) to the temperature of the radio frequency (RF) electronics. By reducing the temperature of the NMR coil assembly and the preamplifier, researchers can achieve up to a fourfold S/N enhancement. The coil assembly and preamplifier are cooled using cold helium gas in either an open- or a closed-loop cooling system. In the open-loop system, cold helium gas is produced from boil-off of liquid helium. Open-loop systems are less common in commercial products because of the need for frequent liquid-helium replenishing, which typically limits experiment times to a few days and is therefore not suitable for many NMR applications.

Commercial vendors commonly use the closed-loop cooling system (Figure 1), where helium gas is compressed in one chamber and then allowed to expand in a second chamber, relying on the ideal gas law ($PV = nRT$, where P is pressure, V is volume, n is the number of moles of gas, R is the gas constant, and T is temperature) to produce cold helium gas. Vacuum-insulated parts in the cooling system allow the coil assembly to reach very low temperatures (approaching 25 K), which substantially decreases noise. Thermal isolation in the probe is critical to allow nearly room-temperature samples to be measured a few millimeters from the cold coil assembly.

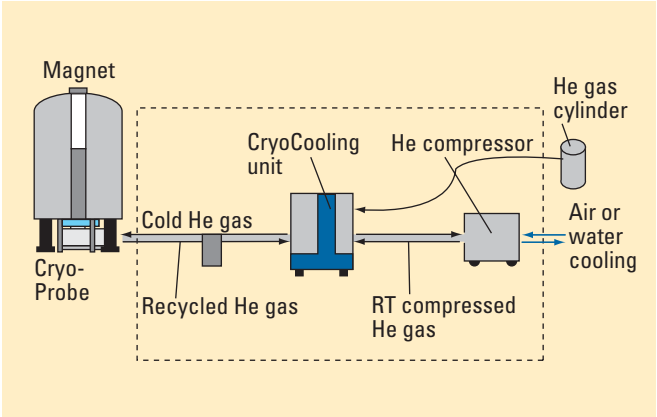

Figure 1. Keeping your cool. The Bruker BioSpin CryoProbe Accessory is a closed-loop cooling system. The helium cylinder is used for system purges during probe changes and provides makeup gas. Helium compressors generate substantial heat and are therefore cooled with air or water. (Image courtesy of the author.)

High-throughput screening

An ideal application of cryogenically cooled probe technology is the screening of large numbers of potentially bioactive molecules against protein targets, where the probes can dramatically improve sample throughput. A leader in the use of NMR for screening is Abbott Laboratories (*http://abbott.com*), where Steve Fesik, vice president of cancer research, and Philip Hajduk, group leader for NMR-based screening, developed the method of "SAR (structure–activity relationship) by NMR" and other NMR-based screening strategies for drug discovery and design (*1*).

"Our laboratory utilizes a fragment-based approach to build and optimize new ligands for protein targets," explains Hajduk. "This fragment-based approach allows the scientist to determine the function, shape, and size characteristics of fragments that can improve the potency and pharmacokinetic properties of lead compounds." Their laboratory was the first in the pharmaceutical industry to use Bruker BioSpin's CryoProbe technology (*www.bruker-biospin.com*) and discovered several lead compounds using the SAR by NMR method. The increased sensitivity of the CryoProbe enabled the researchers to examine proteins that could not be evaluated using conventional NMR probes because of limited protein solubility.

Similarly, Jonathan Moore, head of structural biology, and colleagues at Vertex Pharmaceuticals (*www.vrtx.com*) use cryogenically cooled probe technology with Moore's SHAPES method of NMR screening (*2*). According to Moore, "The SHAPES strategy combines nuclear magnetic resonance (NMR) screening of a library of small druglike molecules with a variety of complementary methods, such as virtual screening, high-throughput enzymatic assays, combinatorial chemistry, X-ray crystallography, and molecular modeling, in a directed search for new medicinal chemistry leads." This improves the quality of leads determined by high-throughput screening and increases the hit rate of potential drug targets.

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By studying the ligand rather than the protein using the SHAPES method, Vertex researchers can explore protein targets too large for other methods such as SAR by NMR. By combining SHAPES with the increased sensitivity of CryoProbe technology, Vertex has increased its hit rate 10-fold and reduced data acquisition time 16-fold compared with a conventional NMR probe, substantially increasing productivity.

Metabolite analysis

Cryogenically cooled probe technology can also be used to analyze drug metabolites, which provide important insight into the biological activity and toxicity of potential drug targets. Traditionally, metabolites have been difficult to study because they are typically isolated only in small quantities. With the increased sensitivity available with the new technology, however, studies on metabolites are providing new structural information not available just a few years ago. The new information will assist pharmaceutical companies in developing drugs with improved biological and toxicological profiles.

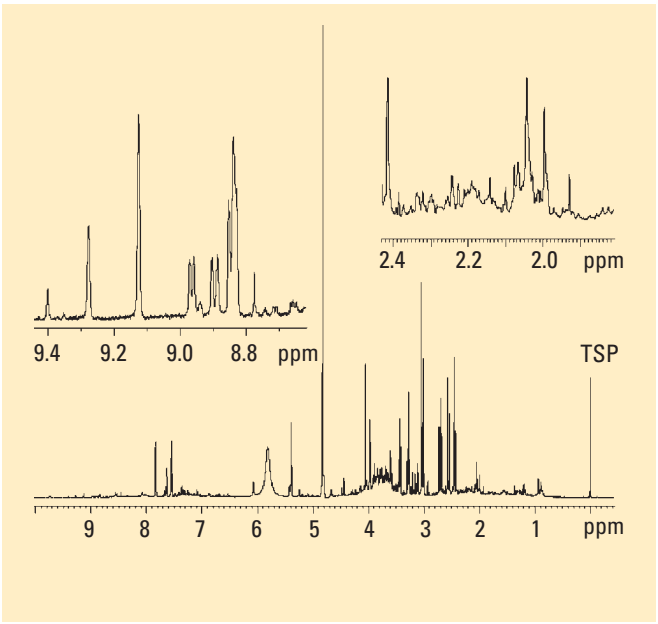

Figure 2. Metabolic profiling. Researchers used a 5-mm 500-MHz Bruker TXI z-gradient CryoProbe to acquire a ^{1}H-NMR spectrum of rat urine collected 24 h after dosing with pharmaceutical agent. The concentration of the metabolites is calculated using the internal standard TSP added at a concentration of 1 mM. (Image courtesy of the author.)

According to Frank Koehn, associate director of natural products and structural chemistry at Wyeth Ayerst (*www.wyeth.com*), "CryoProbe technology has had a significant impact in the way almost all problems are approached. We routinely utilize the technology to elucidate the structures of complex biologically active natural products and drug metabolites that are often available only in microgram quantities. In addition to the obvious advantages of enabling our group to analyze smaller and smaller samples, the CryoProbe has also affected the way we approach other types of problems. For example, with the added sensitivity provided by the CryoProbe, we can now routinely acquire long-range ^{1}H–^{15}N correlation data in minutes rather than hours or days (*3*). This difference in experiment time moves this experiment from something that would only be invoked to answer special problems to a routine experiment that can be used to very easily answer a wide variety of structural problems."

"Another experiment that has also come into more routine use is the ^{1}H-detected ACCORD-ADEQUATE experiment (*4*). This experiment, which yields valuable information on ^{13}C–^{13}C connectivity while taking advantage of detection through the more sensitive ^{1}H nucleus, was in the past only reserved for extremely soluble small-molecular-weight samples [100–300 Da] due to its inherent low sensitivity. However, with the new CryoProbe, this experiment can routinely be performed on 10 mg of moderate-molecular-weight compounds (500–600 Da) in an overnight acquisition. This powerful experiment can now be regularly used to answer some of the most difficult structural problems, especially in ^{1}H-deficient systems."

Key to the biological activity of many pharmaceutical agents is the relative stereochemistry of the ^{1}H atoms or the substituents in the molecule. NMR spectroscopy can be used to determine the relative stereochemistry through the analysis of ^{1}H–^{1}H or ^{1}H–^{13}C coupling constants (*5*). An extremely useful experiment for determining the coupling constants within the molecule is the phase-sensitive heteronuclear multiple-bond correlation (HMBC) experiment (*6*). This experiment, however, is not widely applied to pharmaceutical drugs because of its inherently low sensitivity. Koehn reports that the high sensitivity of cold probes has revolutionized the use of this experiment so that it can be widely applied to pharmaceutical agents. Using CryoProbes, Koehn's laboratory routinely applies this method to samples available in quantities as low as 100 µg to gain complete relative stereochemical information.

Koehn continues: "High-resolution CryoProbes are a valuable adjunct to LC-NMR. When working on mass-limited multiple-component mixtures from drug metabolism, natural products, or combinatorial synthesis, conventional LC-NMR-MS is used to obtain individual one-dimensional spectra of each component of the mixture. Once the subspectra are identified, we use the CryoProbes to measure 2-D experiments on the remaining mixture. This enables the structure determination of mixture components (often minor) without full chromatographic separation of the entire mixture."

Likewise, researchers at Wyeth Ayerst use cryogenically cooled probes for pharmaceutical profiling, performing diffusion-based NMR experiments that measure physical and biochemical properties of drug candidates at low concentration. These experiments "are extensively used to study drug–protein interactions where either the drug or protein target is limited in mass or solubility," according to Koehn.

Structural studies

Another prominent pharmaceutical company has been using a cryogenically cooled probe for three primary applications: NMR-based screening using saturation transfer difference (STD) spectroscopy; protein construct screening; and confirming hits

from high-throughput biological screening or STD NMR-based screening (*7*). Using the cryogenically cooled probe, researchers can screen individual samples in as little as 10 min, study proteins that are difficult to crystallize, and establish the binding sites of small molecules on proteins in a couple of hours rather than overnight. The increased S/N of the spectra is especially important in the analysis of complex spectra such as those obtained from urine, which is composed of many important metabolites (Figure 2). Therefore, to this company, the cryogenically cooled probe provides rapid turnaround time, which results in faster decision-making and lets researchers study low-abundance molecules that were previously too difficult to analyze.

Although most pharmaceutical researchers use cryogenically cooled probe technology exclusively for the detection of ^{1}H nuclei, Srinivasan Rajan, a senior fellow at Novartis (*www.novartis.com*), finds that using it for ^{13}C detection is also invaluable. With a ^{13}C-optimized CryoProbe, Rajan can rapidly acquire data on typical synthetic samples in drug development to verify the chemical structure. Such information is used to determine whether inconsistencies in biological assays are the result of an unstable compound or an ambiguity in the structure. Complete ^{13}C and ^{1}H chemical shift information allows proper interpretation of the NMR data and provides accurate chemical structure information for the project team to assess the correlation of the structure with biological activity.

The bottom line

Cryogenically cooled probe technology has been widely and rapidly adopted by the pharmaceutical industry as an essential tool. Of the CryoProbes delivered to date by Bruker BioSpin, 54% are used in the pharmaceutical industry. Although still in its infancy, this product has had a major impact in the past three years and will continue to play a role in revolutionary new methods of drug discovery research for many years to come.

References

(1) Shuler, S. B.; et al. *Science* **1996,** *274,* 1531–1534.
(2) Fejzo, J.; et al. *Chem. Biol.* **1999,** *6,* 755–769.
(3) Sheng, S.; van Halbeek, H. *J. Magn. Reson.* **1998,** *130,* 296–299.
(4) Williamson, R. T.; et al. *Magn. Reson. Chem.* **2001,** *39,* 544–548.
(5) Marquez, B. L.; et al. *Magn. Reson. Chem.* **2001,** *39,* 499–530.
(6) Seki, H.; et al. *Tetrahedron* **2000,** *56,* 2935–2939.
(7) Mayer, M.; Meyer, B. *Angew. Chem., Int. Ed.* **1999,** *35,* 1784–1788.

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