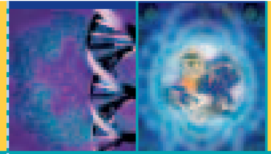

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# NMR at 900 MHz: TROSY NMR

**With increasing interest in the structure of proteins, NMR spectrometers are rapidly becoming key enabling tools for academic and pharmaceutical research. Their use in biomolecular analysis has often been limited, however, as scientists increasingly require technology that can analyse larger and more complex proteins. In order to meet these needs, Oxford Instruments Superconductivity continues to develop and improve its superconducting magnets that form the heart of NMR, culminating recently in the development of the world's first commercial 900 MHz NMR system. In line with this progress, scientists are developing innovative methods of exploiting the advantages that these powerful systems offer.**

In order to obtain high-quality NMR data, several essential features rely on the field strength of the magnet. Resolution is proportional to the magnetic field strength ($B_0$), with spectra becoming increasingly dispersed as the field strength increases. Sensitivity is also improved by approximately the 3/2 power of the field strength. This results in the signal to noise ratio improving by almost 20% in a 900 MHz instrument when compared a to an 800 MHz system, or 84% compared to a 600 MHz.

## Analysing larger molecules

As larger and larger molecules are analysed, the interpretation of results is hampered not only by more complex spectra with more overlap, but also by broader resonance lines as the nuclei of interest relax more quickly. Interference between different relaxation mechanisms results in different relaxation rates for the two components of an N-H doublet, producing an effect known as differential line broadening. This effect is increased in larger molecules.

## Transverse Relaxation Optimised Spectroscopy

First described by Professor Kurt Wüthrich in 1997, the importance of Transverse Relaxation Optimised Spectroscopy (TROSY)[1], together with a body of equally fundamental NMR research, was recognised in 2002 by the Nobel Prize committee. Professor Wüthrich was awarded the Nobel Prize for Chemistry in recognition of his development of NMR spectroscopy for determining the three-dimensional structure of biological macromolecules in solution.

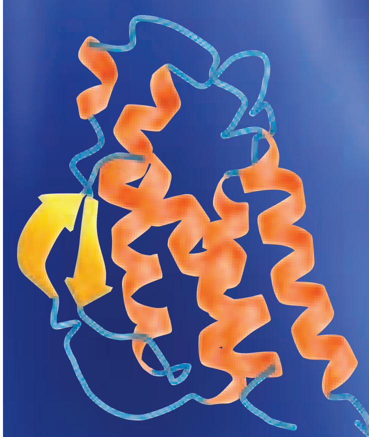
TROSY selects only the narrow spectral component of N-H multiplets, thus dramatically increasing the resolution and sensitivity of large protein spectra recorded at high magnetic field strength[1]. As with the essential NMR features described above, TROSY is dependent on the field strength of the magnet. It provides some benefits at lower magnetic field strengths, but the effects are seen most between 900 MHz and 1 GHz and it is estimated that they will be maximised at around 1.1 GHz.

## Analysing molecules in their natural state

As researchers exploit the ability of high-field NMR to analyse larger and larger biomolecules, it should be remembered that there are other important factors to be considered apart from molecule size. The conditions used should ensure a molecule's structural and functional integrity and ideally should mimic the molecule's natural environment.

The structural determination of integral membrane proteins is essential to the understanding of a wide range of biological functions. Despite this, the database of 3D membrane protein structures is still small, reflecting the difficulties that their analysis causes. Many NMR techniques for membrane proteins rely on detergent micelles to keep the proteins in solution. Although this helps to ensure the integrity of the proteins, the large size of the resulting

*Dr. Ēriks Kupče of Varian R&D explains the significance of superconducting magnet technology to TROSY NMR*



*TROSY NMR can analyse larger and more complex proteins*

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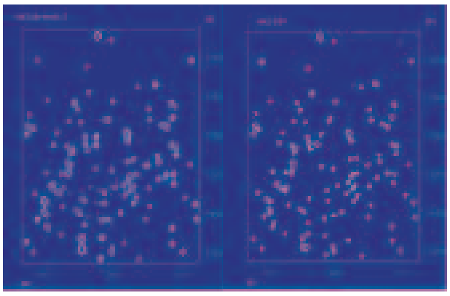

*Figure 1: Resolution: protein NMR at 600 MHz and 900 MHz*
*Data courtesy of Dr. L. Mueller (Bristol-Myers Squibb)*

structures has actually limited the use of NMR in these studies. This is due to the fact that these large structures tumble slowly in solution, leading to large linewidths and making results difficult to analyse.

To demonstrate the use of TROSY in overcoming these difficulties, Fernández *et al*[2] analysed the outer membrane protein X (OmpX) from E.coli, reconstituted in micelles of DHPC (dihexanoyl phospatidylcholine) of approximately 60 kDa. As well as using TROSY, the choice of detergent proved a critical factor for obtaining high-quality spectra of OmpX. Concentrated solutions of DHPC have relatively low viscosity, which supports obtaining spectra with narrow linewidths.

A 3D [$^{15}$N, $^{1}$H]-TROSY-HNCA experiment demonstrated a 10-fold gain in sensitivity compared to the conventional 3D HNCA spectrum. The results allowed the complete polypeptide backbone of the OmpX molecule to be determined. In addition, the data was used to identify regular secondary structure elements and some of the factors influencing the protein's tertiary structure. Although additional work would be required to further refine the protein's structure, this data already demonstrates the potential of TROSY-NMR for studying the structure and function of membrane proteins.

## TROSY: data at 900MHz

The TROSY data in the OmpX experiments was obtained at 750 MHz. As previously mentioned, however, the results of the TROSY effect are seen more at higher field strengths. Figure 1 demonstrates the difference between TROSY data at 600 MHz and 900 MHz.

TROSY also aids in the analysis of much larger proteins. The difference between TROSY and HSQC spectra for the 78 kDa aldolase molecule is demonstrated in Figure 2.

## Conclusions

The development of 900 MHz NMR superconducting magnets offers scientists new opportunities in terms of the size and classes of molecules studied and the detail of results obtained. Although the data obtained at 900 MHz is impressive, researchers are already working to build on this, using for example, cryogenic probes to significantly improve data collection times. Techniques such as TROSY and the newly-discovered G-matrix Fourier Transform NMR (GFT-NMR)[3] will continue this trend, helping researchers to obtain the maximum benefits from NMR at 900 MHz and beyond.

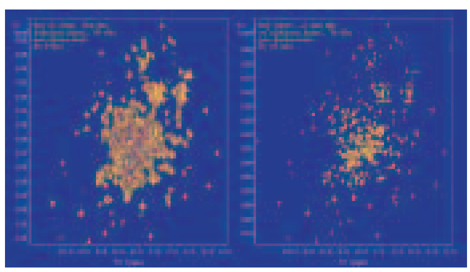

*Figure 2: Difference between TROSY and HSQC spectra for the 78 K Da aldolase molecule*
*Data courtesy of Prof. S. Hommans (University of Leeds)*


References:

1. Pervushin, K, Riek, R, Wider, G and Wüthrich, K. Proc. Natl. Acad. Sci. USA **94**, 12366 (1997).

2. Fernández, C, Adeishvill, K and Wüthrich, K. Proc. Natl. Acad. Sci. **98**, 2358-2363 (2001).

3. Kim, S and Szyperski, T. J. Am. Chem. Soc. **125(5)**: 1385-93 (2003).



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