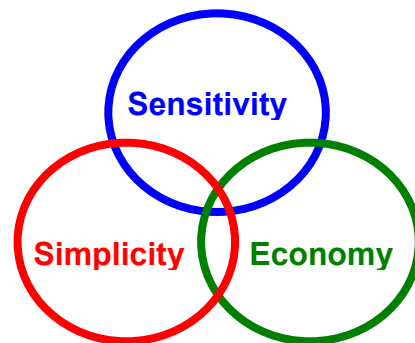
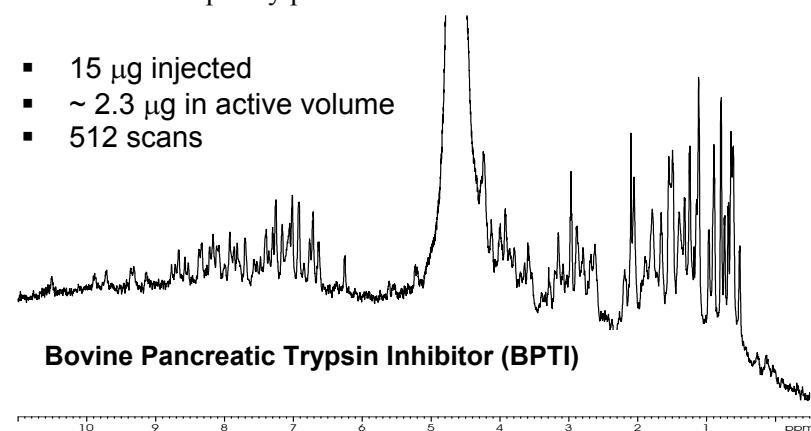


Structural Proteomics using NMR Spectroscopy?



Structural elucidation and backbone assignment can now be accomplished with NMR using only a fraction of the mass of protein previously required, significantly reducing the challenges associated with expression.

Using the recently released Protasis/MRM TXI HCN z-Gradient CapNMR™ probe, researchers at The Scripps Research Institute (San Diego, CA) and at Sequoia Sciences (San Diego, CA) have acquired a full compliment of data for complete structural assignment of a protein from microgram amounts. To demonstrate the detection sensitivity of MicroFlow NMR, these researchers initially evaluated a BPTI sample of 0.24mM concentration on an existing ICG probe. A 10 μ L injection of sample results in only 2.3 μ g of protein residing in the 1.5 μ L active volume of the capillary probe.

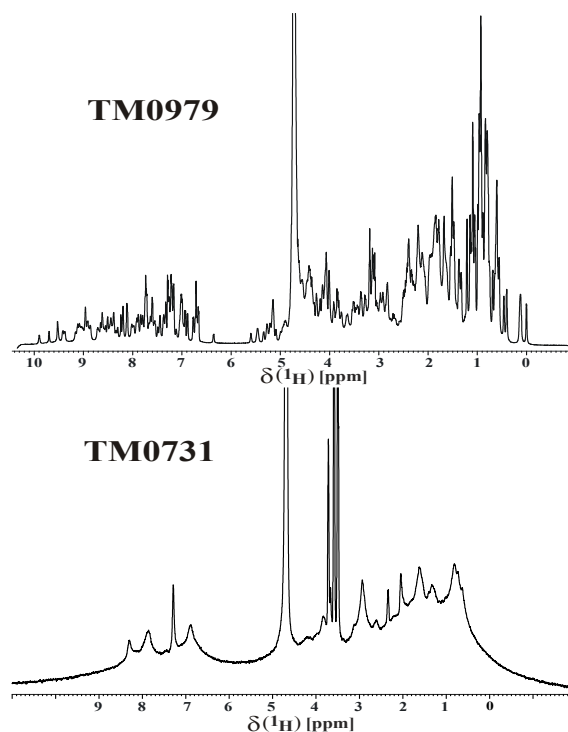


Data courtesy of Wolfgang Peti, Ph.D.
The Scripps Research Institute, San Diego, CA, USA, and
Mark O'Neil-Johnson, Ph.D.
Sequoia Sciences Inc., San Diego, CA, USA.

0.24mM BPTI
20mM NaP pH 6, 10% D₂O
Bruker Avance 600 MHz Spectrometer
Protasis/MRM CapNMR ICG probe
1.5 μ L active volume
Manual injection, injection volume 10 μ L
512 scans, 5 min. acquisition time

Using NMR Spectroscopy as a High Throughput Screening Tool for Folded Proteins!

To further demonstrate the detection sensitivity of the TXI probe, these researchers initially assessed the ability of the probe to serve as a screening tool. They found that, using only microgram amounts of protein, it is possible to test the folded state of a protein. This tool is extremely vital for testing soluble expressed and purified proteins in the context of structural proteomics initiatives. Only well-behaved and folded proteins are taken forward for structure determination, and therefore the efficiency of the process is dramatically improved if this information can be obtained up-front. As an example, the 1D ¹H NMR spectra of two test proteins TM0979 (well-behaved folded) and TM0731 (poor behavior, not folded) from the hyper thermophile organism *Thermotoga maritima* are shown. Well-behaved proteins can be identified by their chemical shift dispersion in the amide and H α proton region and by their high field shifted methyl groups, because of ring current shifts from interaction with aromatic groups in the protein core.

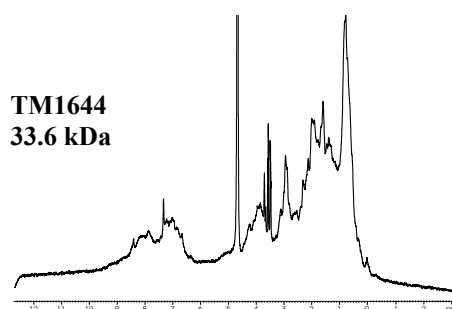
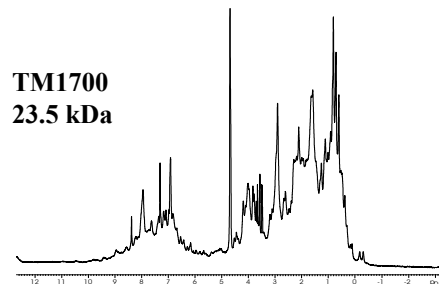
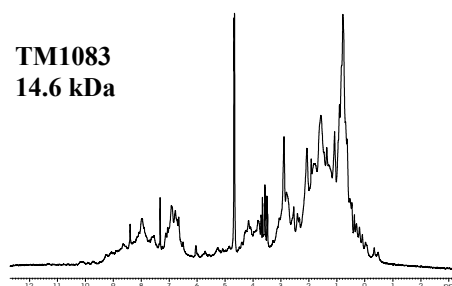
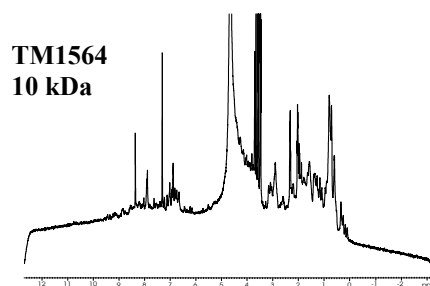


Test proteins TM0979 and TM0731
Thermotoga maritima
20mM NaP buffer, pH 6, 50 mM NaCl, 10% D₂O, 313 K
Bruker Avance 600 MHz Spectrometer
CapNMR TXI probe 1.5 μ L active volume



Can protein screening really be done effectively at microliter size scales?

For purposes of comparison between conventional and microliter size scales, a series of proteins from *Thermotoga maritima* were prepared for screening. Conventional experiments at The Scripps Institute have been carried out in the past using 96 separate proteins from TM. For microliter scale comparisons, 24 of the 96 TM proteins ranging in size from 10kDa to 40kDa were randomly selected. The statistics from the conventional experiments were tallied and compared against statistics from the 24 randomly chosen TM proteins. Examples of the ¹H NMR spectra are provided, and the data from the comparison study tabulated below. These data demonstrate the applicability of capillary-scale NMR as a screening tool for the wider basis set of proteins from *Thermotoga maritima*.



All spectra taken at:
303 K, 20 mM NaP pH 6.0, 50 mM NaCl

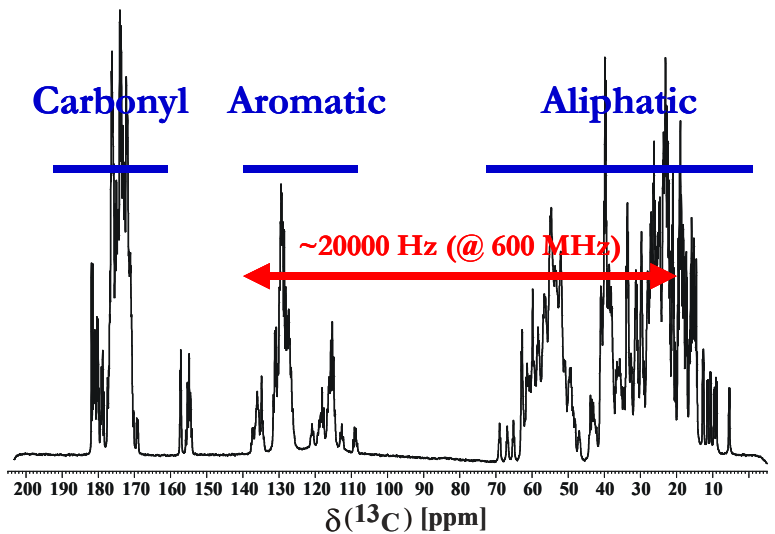
Spectra and TM proteins provided by W. Peti
(Scripps Research Institute), S. Lesley and H. Klock
(Genomics Institute of Novartis Research Foundation)

	Regular	Micro Coil
Total Proteins	96	24
Soluble Proteins	40 (42%)	14 (58%)
Total Folded Proteins	19%	25%
Soluble Folded Proteins	42.5%	42.8%

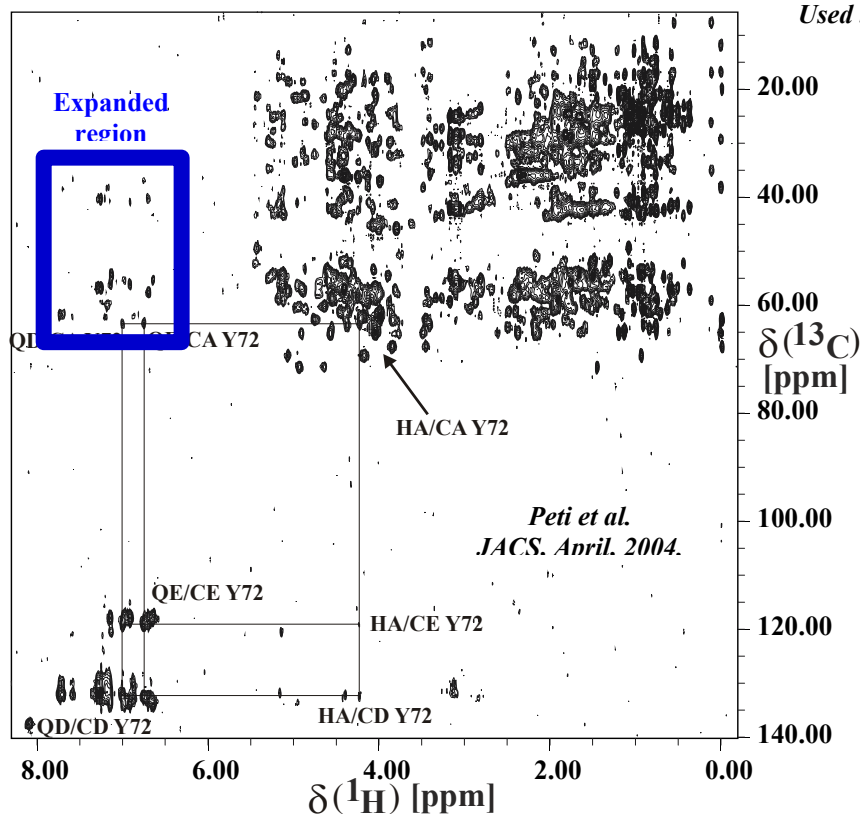
Studying protein expression at the capillary-scale with cost-effective MicroFlow NMR has significant experimental advantages. Up to 100 times less material can be used, often saving days of preparation time. Sample cost savings in studies that use expensive doubly-labeled reagents can easily add up to many thousands of dollars per study. For natural product studies, sample may be limited to microgram amounts by collection efficiencies, in any case. A smooth flowpath ensures that these valuable samples can be easily recollected or directed to mass spectrometry. Microcoils are intrinsically faster and allow researchers to both use less material and less spectral acquisition time, thus dramatically reducing overall experimental timeframes for complicated 2D and 3D proteomic studies. The capability to achieve target protein spectra with as little as a few tens of micrograms of sample makes this a must-have tool in any modern research facility.

The Power of Solenoid Coil Architecture: A New Way for Aromatic Side Chain Assignment in Proteins

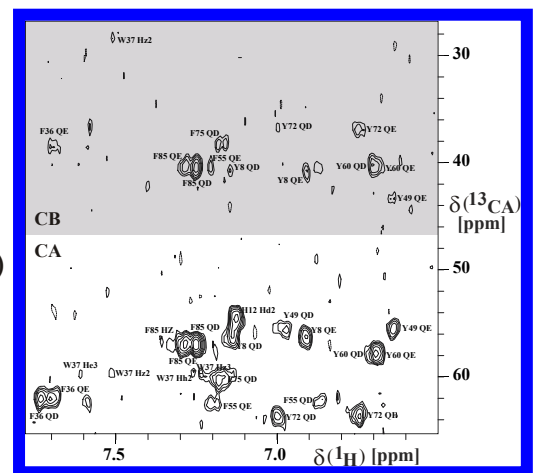
Taking advantage of the excellent radio-frequency capabilities of this probe, which are a result of the solenoid coil design, we have demonstrated for the first time, TOCSY transfer between the aliphatic-aromatic region in a HCCH-TOCSY spectrum. The most important step in the assignment process of the aromatic residues is to connect the C β /H β chemical shifts of tyrosine, phenylalanine, histidine and tryptophan with the H δ protons in the aromatic ring. Different *heteronuclear through bond correlation spectra* have been described in the literature, but most lack sensitivity and ease of handling. Therefore, unlabeled protein samples, where the amide protons are completely exchanged with D₂O (which reduces the chemical shift overlap in the aromatic chemical shift region), are widely used to achieve the assignment of the aromatic side chains.



The biggest obstacle for an effective, quick and reliable assignment has always been the large carbon chemical shift differences between aliphatic and aromatic residues (average ~ 13200 Hz at 14.1 T) creating a requirement for high RF-power demand for a full TOCSY transfer across aliphatic and aromatic residues. Using a Protasis/MRM TXI HCN z-Gradient CapNMR probe, it was possible to achieve an aliphatic-aromatic transfer as shown in the aliphatic-aromatic HCCH TOCSY spectrum below.



Used by permission, Figure 3, Peti et al. *JACS* 126:18, pp. 5873-5878, April, 2004.



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E-mail: info@protasis.com

Aliphatic-aromatic HCCH TOCSY spectrum from the same TM0979 sample used for sequential backbone assignment. For the TOCSY, a FLOPSY-16 mixing sequence in a z-filter with a 20 kHz field was used. The easy assignment of Tyrosine 72 in TM0979 is indicated.