NMR is inherently insensitive, and conventional NMR hardware generally requires several milligrams of protein sample. However, there has been substantial recent progress in development of highly sensitive microcoil NMR probes for biological NMR. This technology exploits the concept that miniaturization of the receiver coil leads to a concomitant increase in mass sensitivity (S/N per mg of solute), since S/N for a given amount of sample is approximately inversely proportional to the diameter of the coil. As a result, microcoil probes are highly suited for mass sensitive applications, where the mass of the solute is limited, yet the solute is reasonably soluble. Microcoil NMR probes offer several other practical advantages over conventional 5-mm probes, including enhanced solvent suppression, improved salt tolerance, ease of shimming, and improved RF homogeneity. In the first published example of its kind, the NESG determined the structure of a small (68-residue, 8.6 kDa) β-barrel protein using only 72 μg of protein with a Bruker 1-mm TXI MicroProbe (7 μL sample volume) (1). The data were acquired in approximately twice the spectrometer time but on 1/20th the mass of protein compared to a conventional 5-mm NMR sample (1600 μg). The backbone rmsd between mean coordinates of the ensembles of conventional and microprobe structures is 0.7 Å, demonstrating that high-quality data can be obtained with < 100 μg protein samples (1).

The NESG consortium has recently begun to use the even more sensitive Bruker 1.7-mm MicroCryoProbe™ (30 μL sample volume), which marries the microcoil concept with the 3 to 4-fold increase in S/N afforded by cryogenically cooled coils in the probe. Using this probe at 600 MHz, we have determined complete resonance assignments and 3D structures for four proteins ranging from 6 - 15 kDa in size, at 0.5 - 1 mM protein concentration. A representative example, comparing structures obtained using 300 μL and 30 μL samples at the same concentration is shown in Fig. 1. Accordingly, we can now routinely determine high-quality 3D structures of small (< 15 kDa) proteins by NMR using 70 – 300 μg samples. This opens many new applications for NMR in PSI3:Biology projects: The unique value of the MicroCryoProbe will be in the investigation of proteins that can only obtained in minute amounts, e.g. using cell free and other eukaryotic expression systems (2).

**Fig. 1** Ribbon representation of NMR structures of NESG target VpR247, MW 11 kDa, solved using a B800 (5-mm cryoprobe; 300 μL volume, cyan; PDB id 2KIF) and the B600 (1.7-mm MicroCryoProbe; 30 μL volume, green; PDB id 2KIM). The same protein sample at 0.9 mM was used for both studies.
