Northeast Structural Genomics Consortium
HTP NMR Screening Using Micro Cryo Probe

As part of our efforts to develop improved methods for NMR protein sample preparation and structure determination, the NESG has implemented a HTP NMR screening pipeline (Fig. 1) for construct and buffer optimization featuring a 1.7-mm MicroCryo NMR Probe (1). The process is feasible because the newest generation micro NMR probes require only small amounts of protein, typically 30 - 200 μg in 8-35 μL volume. The SPiNE database integrates NESG protein sample production data, Bruker IconNMR software controlling a Bruker B-ACS 60 robotic sample changer, and a Bruker 600 MHz TCI 1.7- mm MicroCryoProbe™. These integrated software tools reduce the need for operator intervention during setup and data acquisition, and simplify the process of archiving the resulting large volumes of 1D and 2D NMR screening data. The Bruker TCI 1.7 MicroCryoProbe™ has a mass sensitivity (S/N per microgram of protein) that is an order of magnitude higher than a conventional 5-mm probe; using 30 mL samples containing ~100 mg of protein sample each, 60 1D spectra are collected in a 24 hr period. Samples providing useful 1D NMR data are then assessed using 2D ^{1}H-^{15}N-HSQC (2 – 6 hrs per sample). The results are scored with respect to spectral quality (1,2) and used to identify samples to be promoted to full structure analysis (with ^{15}N,^{13}C and possibly ^{2}H enrichment). The HTP robotic system is also used to compare buffer conditions or multiple constructs of the same protein in order to prepare an optimized sample.

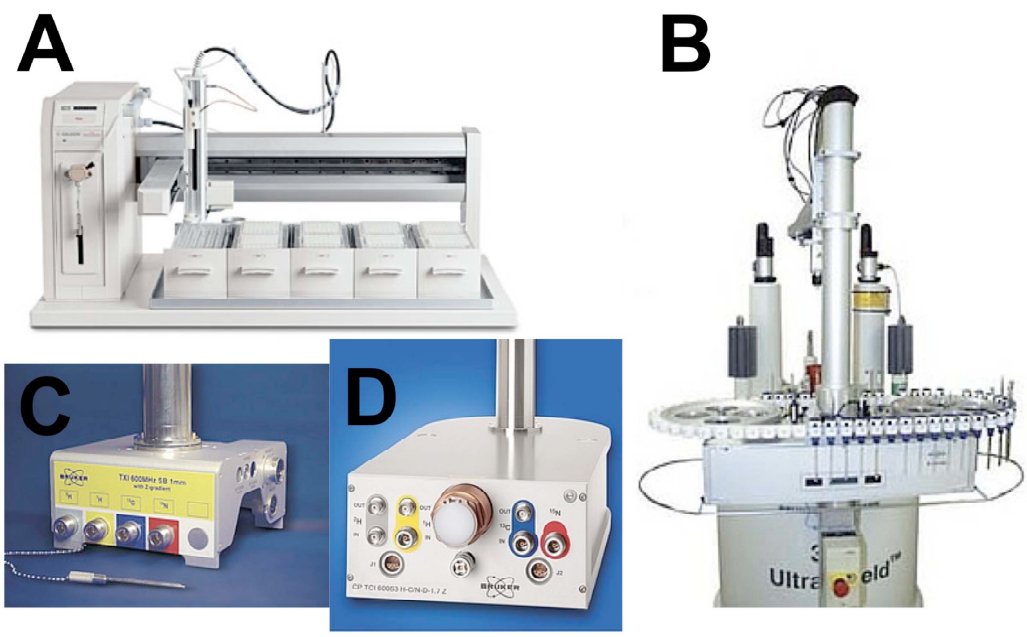

Fig. 1. Labor intensive steps have been moved to robotic handlers: A) Gilson 96-well filling station transfer samples from Eppendorf tubes to microtubes; B) Bruker B-ACS 60, a sixty position sample changer for automated run using Bruker IconNMR; C) 1-mm Bruker TXI 1 Microprobe; D) 1.7-mm Bruker TCI 1.7 MicroCryoprobe used for data collection on a Bruker Avance 600 MHz spectrometer.

1. Rossi, P.; Swapna, G.V.T.; Huang, Y.J.; Aramini, J.M.; Anklin, C.; Conover, K.; Xiao, R.; Acton, T.B.; Ertekin, A.; Everett, J.K.; Montelione, G.T. J. Biomol. NMR 2010, 46: 11 - 22. A microscale protein NMR sample screening pipeline.

2. Snyder, D.A.; Chen, Y.; Denissova, N.G.; Acton, T.; Aramini, J.M.; Ciano, M.; Karlin, R.; Liu, J.; Manor, P.; Paranji, R.; Rossi, P.; Swapna, G.V.T.; Xiao, R.; Rost, B.; Hunt, J.; Montelione, G.T. J. Amer. Chem. Soc. 2005, 127:16505-16511. Comparisons of NMR spectral quality and success in crystallization demonstrate that NMR and X-ray crystallography are complementary methods for small protein structure Determination.