

Integrating MicroFlow NMR into Fragment-based Drug Discovery

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At the CPFM (Chemical Proteomics Facility at Marquette) we are pursuing fragment-based drug discovery using protein NMR. The facility has two spectrometers:

- 1) Varian 600 MHz NMR spectrometer with cryogenic probe ($^1\text{H}/^2\text{H}/\{^{13}\text{C}\}/\{^{15}\text{N}\}$ detection, z-gradient, variable temp. control, 5mm tube sample format) that employs manual loading of sample tubes, and
- 2) Varian 400 MHz NMR spectrometer equipped with a Protasis CapNMR MicroFlow probe (TXI triple resonance, $^1\text{H}/^2\text{H}/\{^{13}\text{C}\}/\{^{15}\text{N}\}$ detection, z-gradient, variable temp. control, and 10 μL NMR flow cell) that employs automated sample introduction using a LEAP Technologies (CTC Analytics) liquid handler robot and Protasis One-Minute NMR (OMNMR) software.

The CPFM, and associated drug discovery infrastructure, was designed based on lessons gained from previous affiliation with and cofounding of the first NMR-centric rational drug design company (Triad Therapeutics, Inc.) in the late 1990s in southern California, USA.

Currently, we use Microflow NMR for a number of applications, which include:

- (a) Initial quality control on ^{15}N -labeled proteins (to be used in chemical shift perturbation assays),
- (b) Quality-control on chemical compound collections (especially 1D ^1H and 2D COSY), and
- (c) Fragment-based screening using STD (saturation transfer difference) NMR.

The latter is of greatest interest, and applications will be described using an infectious disease drug target (a 120 kDa tetrameric protein). Specifically, we have developed an assay strategy using lower affinity reporter ligands to identify fragments to be used in a fragment assembly drug discovery effort. Complementary use of this fragment assembly strategy with thiol tethering will also be discussed.