INSTRUMENT DEMONSTRATION SUMMARY

Topic: MALDI LTQ XL – small molecule imaging
Time: Wednesday 3:00 pm

Background
Most traditional MALDI matrices are small molecules that are present in great excess compared to the analytes of interest. The matrix molecules are readily ionized, easily form complexes with salts (such as sodium and potassium ions), and form many fragment ions upon laser irradiation. As a result, multiple signals are generated in the low molecular weight region of the spectrum (<1000 Da). Unambiguous identification of a low molecular weight analyte is therefore challenging based solely on the molecular weight of the ion. Tandem MS may be performed on a low molecular weight ion of interest in order to improve both selectivity and sensitivity. This workshop will highlight the use of MS/MS strategies for imaging low molecular weight analytes from tissue.

Highlights
- Optimizing fragmentation conditions
- Evaluating specificity via blanks and controls
- Setting up the MS/MS experiment
- Single scan event
- Multiple scan events
- Setting up imaging parameters (optimizing spatial resolution and analysis time)
- Discussing space charging in a linear ion trap with regards to MALDI-generated ions
- Discussing why automatic gain control is discouraged in imaging experiments
- Normalization strategies
- Strategies to improve spatial resolution

Summary
Promethazine is a first-generation phenothiazine antihistamine with an exact mass of 284.13 g/mol (shown below). In this workshop, the distribution of promethazine in mouse brain will be determined. Brains were obtained from mice either untreated or dosed with promethazine (via tail vein injection, 5x, 20 mg/kg per injection). After sacrifice, brains were flash frozen and stored at -80°C prior to use. Thin sections (10 µm) were obtained on a cryostat, and matrix was applied via manual spray-coating. [Brains were generously donated by W. Pham, Vanderbilt University]