

MS Scheme for Complex Protein Samples (i.e. 3-5 or more); fractionations by SCX or 2D PAGE are carried out by the customer, only the C18 RP runs are carried out by the core.

- 1) Strait LCMS analysis on C18 RP column with 1 hr gradient.
- 2) ID-PAGE followed by 1D LCMS runs on 7-14 fractions making up the entire lane. [in this case dark bands should be separated from lighter areas that contain lesser amounts of protein.]
- 3) MuDPIT; *multidimensional protein identification technologies*
[strong cationic separation of peptides or proteins followed by analysis of the final fractions on a C18 RP column with 1hr gradient.]
- 4) Gas-phase fractionation (i.e. same sample is injected a number of times and run with a focused mass range that increases with each injection allowing for a more in depth analysis of each complex fraction.)

MS Scheme for Purified (semi-purified proteins, i.e. IP/ID-PAGE or 2D-PAGE)

IP – 1) 70% goes for 1D-PAGE in-gel digestion (MALDI-Tof/Tof)

2) optionally, 30% goes for in-solution digestion (LC/MS)

2D PAGE (in-gel digestion yields ~20 μ L of solution)

1) MALDI-Tof/Tof (top 10 ions are fractionated in MS/MS)

[MASCOT score i.e. > 100 – done, <100 got on to #2]

2) Concentrate samples with focus chip, or zip tip

[MASCOT score i.e. > 100 – done, <100 got on to #3]

3) A 10 μ L injection is separated and run by LC/MS