A

LC/GC Mass Spectrometry

Peptides (proteomics)

Metabolites
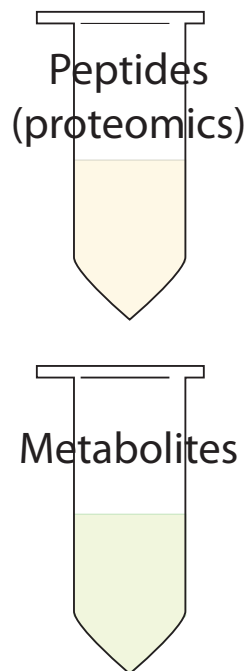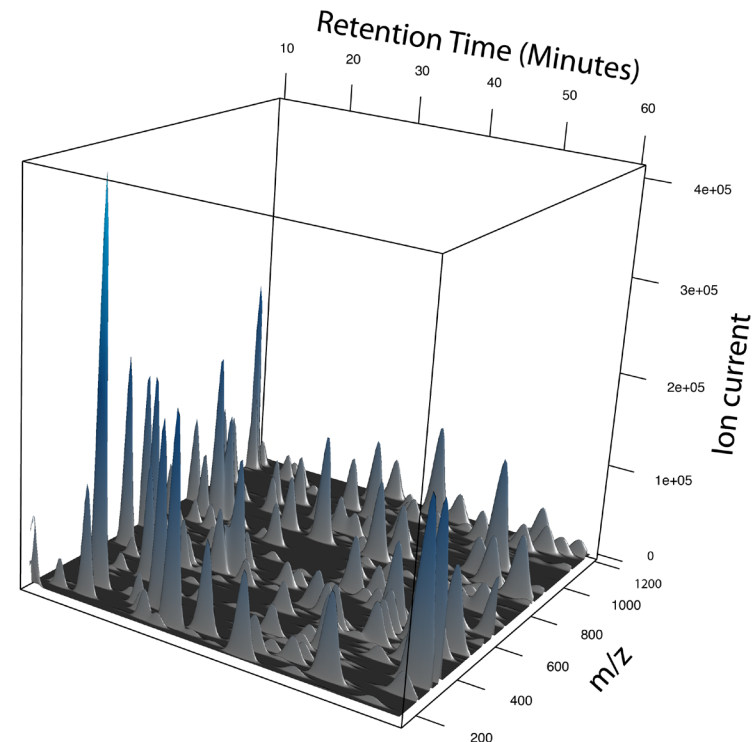
- Distinct ions possess a characteristic m/z and retention time.

- At each time, ions, with possibly overlapping m/z will be detected, and the ion count of ions with each m/z is recorded.

- Secondary MS (MS2) may be used to aid in ion identification or quantification.

Machine Output

Retention Time (Minutes)

Ion current

m/z

Ion Identification

MS2
- m/z of daughter ions and parent ions are matched to possible peptides/metabolites.

Standards
- Pure standard with measured m/z and RT matched to current ions.

- Allows absolute quantification.
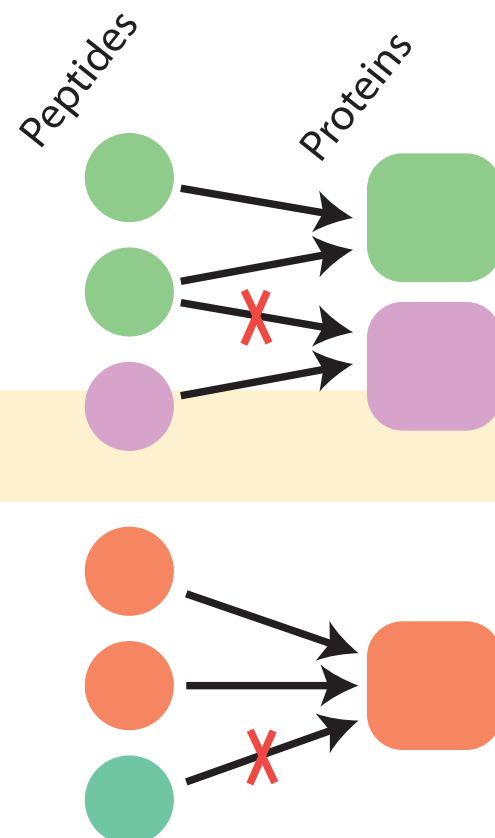
Peptide/metabolite-level analysis

- Based on the experimental design, estimate the main effects for each specie.

- The variance of species varies greatly.

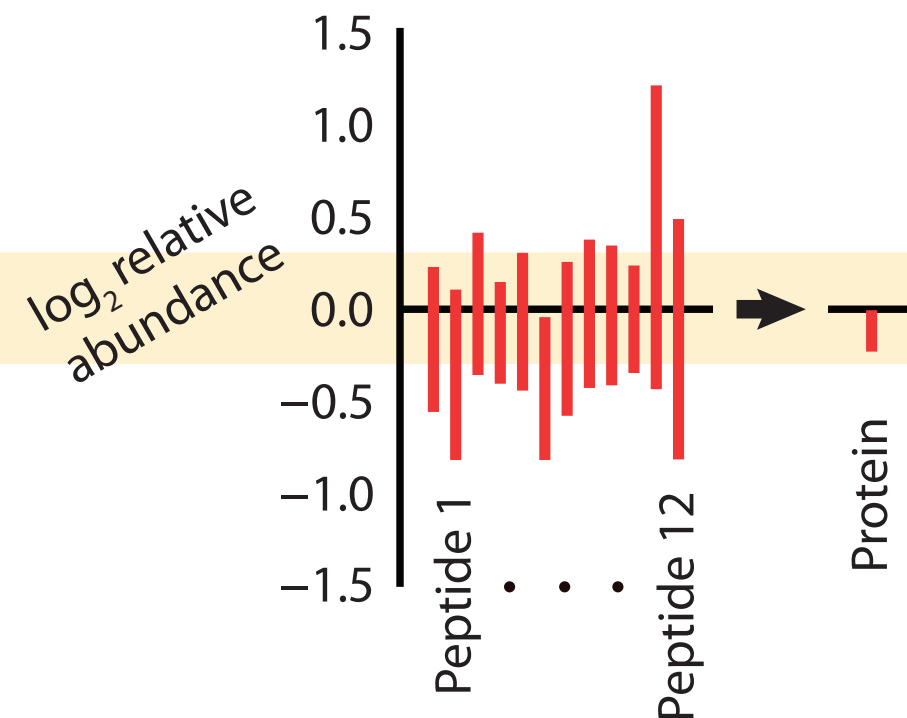- Individual measurements may be more variable than expected due to low signal quality.

B

Peptide-to-protein matching

Possible Proteins

Measured peptides

```
1 0 0 0 0 ...
1 0 0 0 0 ...
0 1 0 0 0 ...
1 1 0 0 0 ...
0 0 1 0 0 ...
⋮ ⋮ ⋮ ⋮ ⋮
```
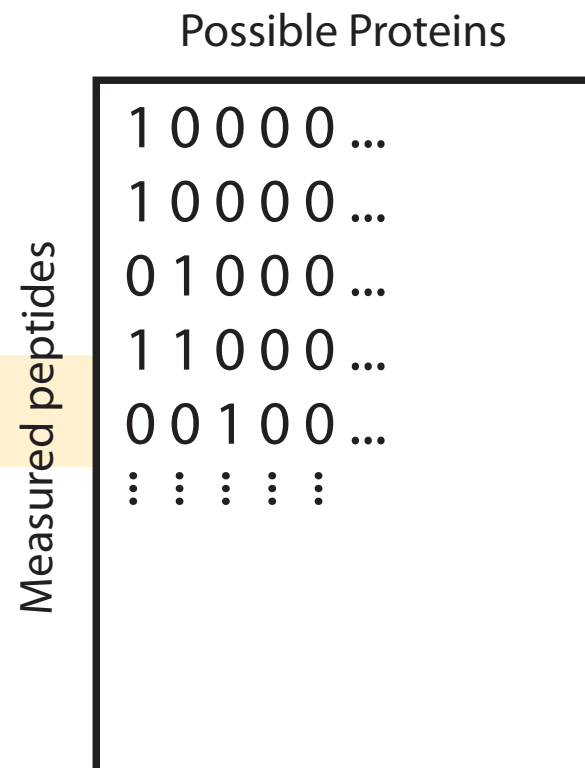
Remove unsupported proteins and divergent peptides

Peptides   Proteins

Aggregate peptides to estimate protein abundance and variance

$\log_2$ relative abundance

1.5
1.0
0.5
0.0
−0.5
−1.0
−1.5

Peptide 1 ... Peptide 12

Protein

Downstream Applications