Pep3D

Day 2

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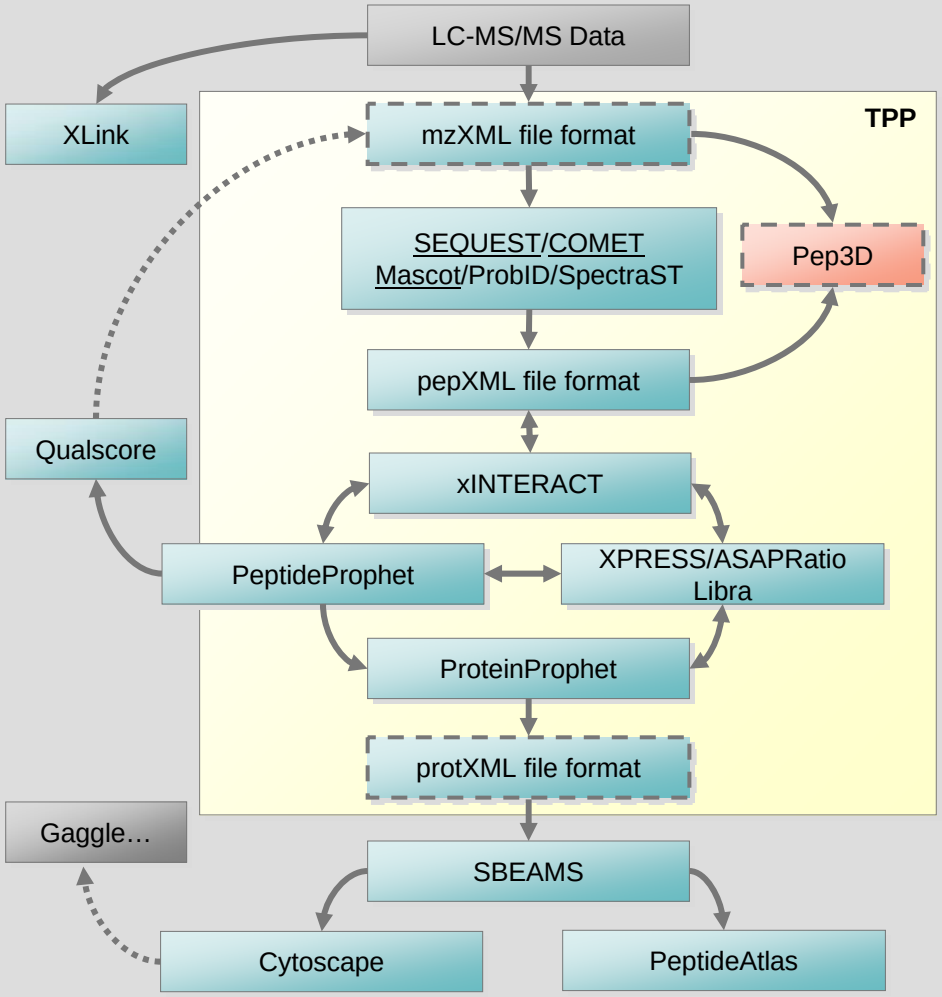
INSTITUTE FOR Systems Biology

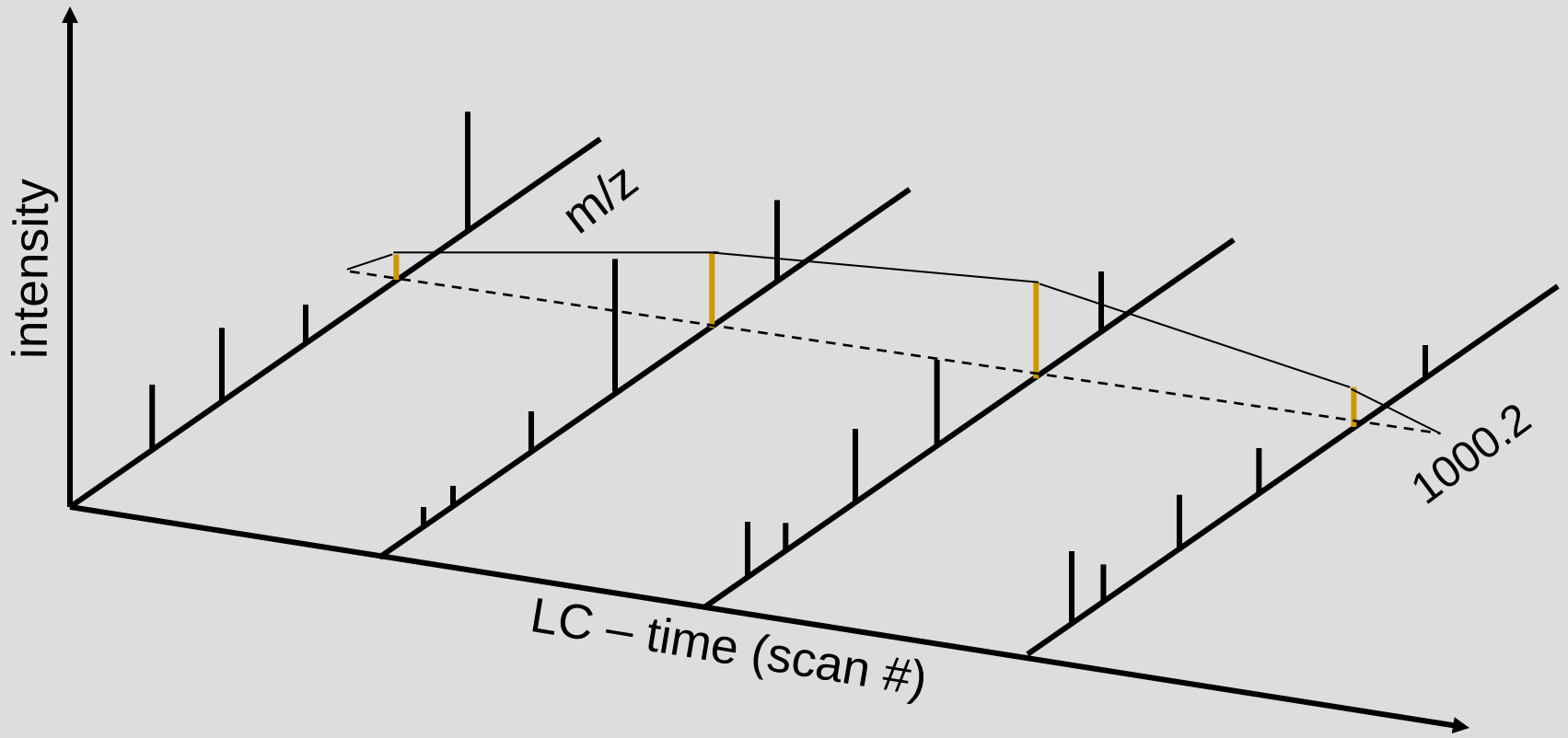
Pep3D


LC-MS/MS Data
XLink
TPP
mzXML file format
SEQUEST/COMET
Mascot/ProbID/SpectraST
Pep3D
pepXML file format
Qualscore
xINTERACT
PeptideProphet
XPRESS/ASAPRatio
Libra
ProteinProphet
protXML file format
Gaggle…
SBEAMS
Cytoscape
PeptideAtlas

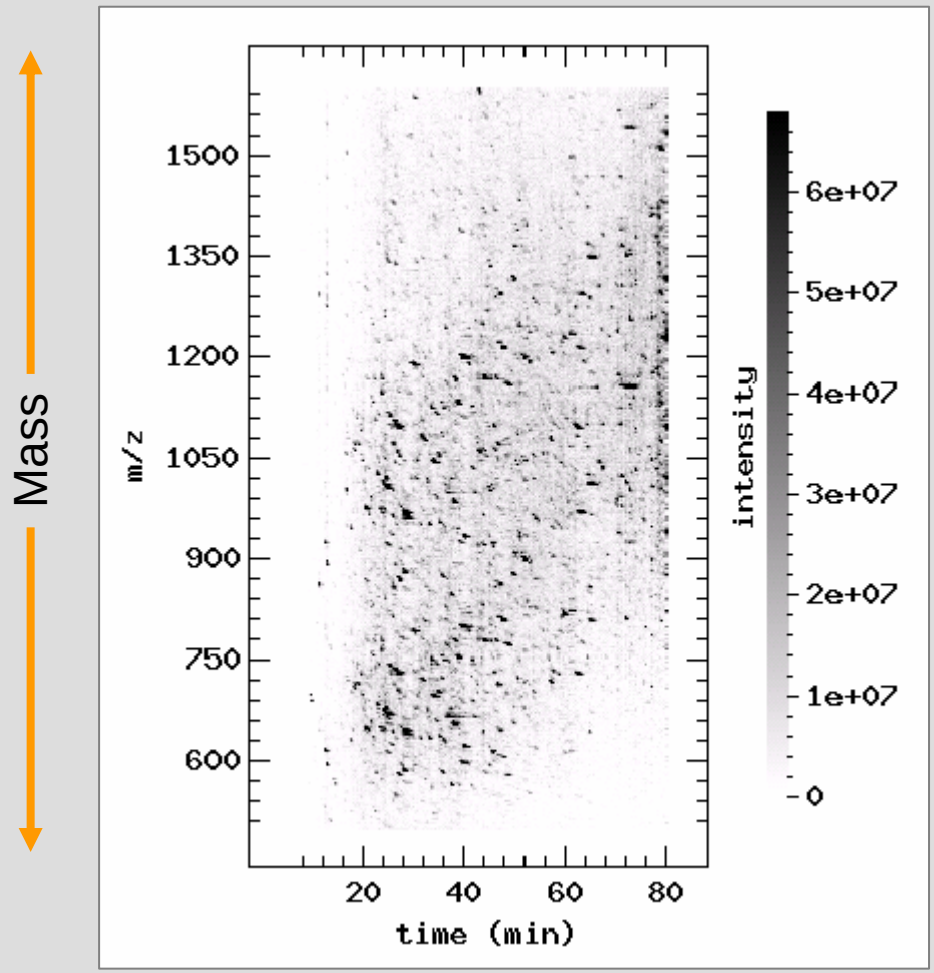
Why visualize LC MS data?

- A single MS spectrum provides only a snapshot in time.
- MS/MS spectra lead to peptide identifications, but typically only the most abundant signals are examined.
- How do we know what else is occurring?
- For a more holistic view we can look at the entire LC MS space.
- The most intuitive method is visualization.

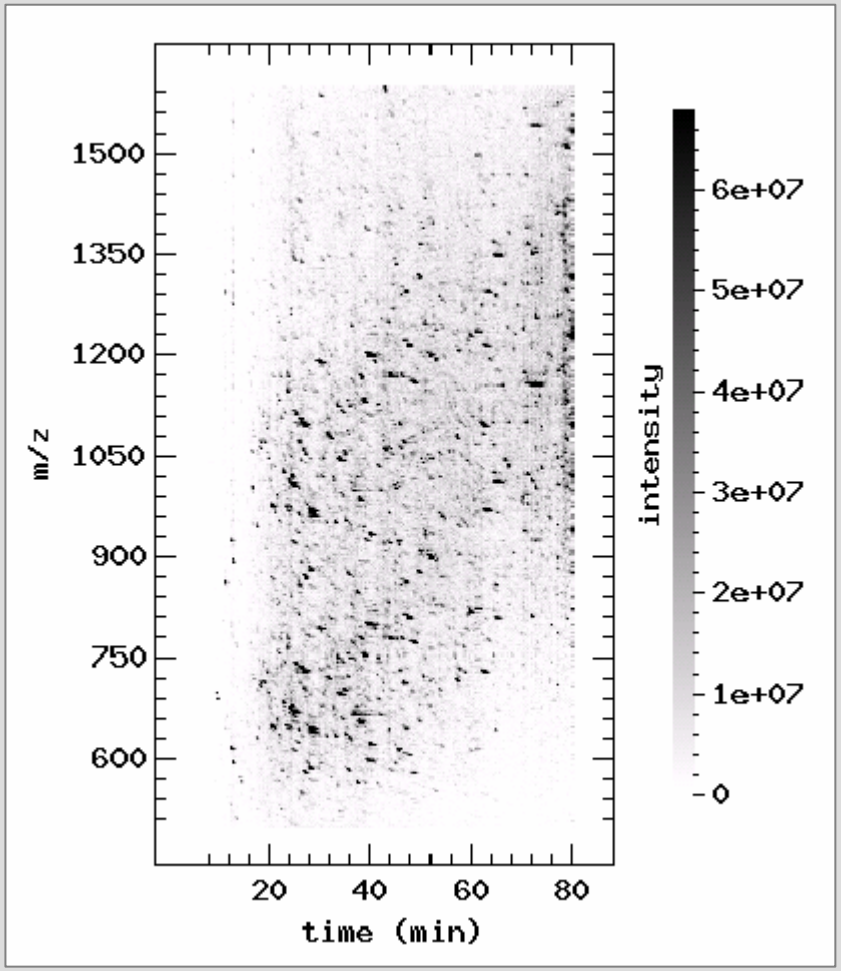
LC-MS Mass vs. Intensity vs. Time



That looks like a gel…

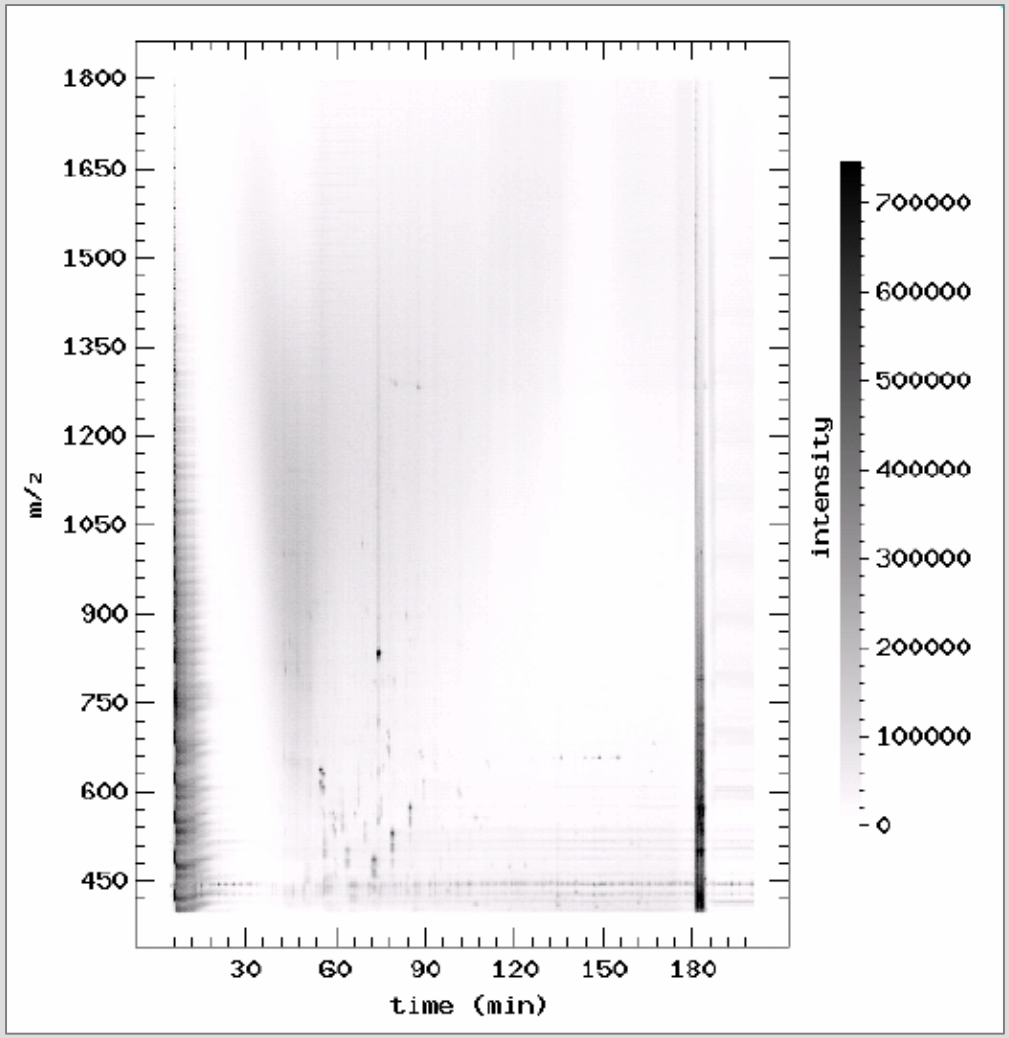


Good Sample / LC Separation



Plenty of well-localized spots without any particular large-scale pattern.

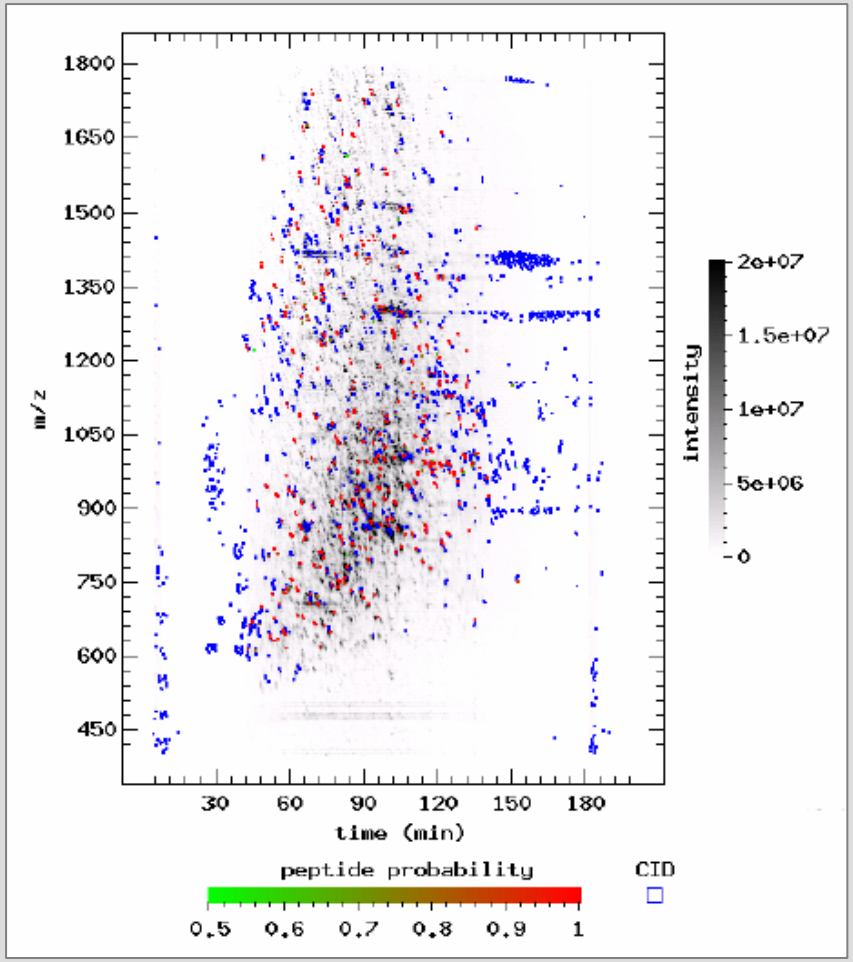
Where's the Sample



- Very few localized spots
- Mainly background noise
- Distinguishable from no-spray

Peptide / CID (0/311 = 0)

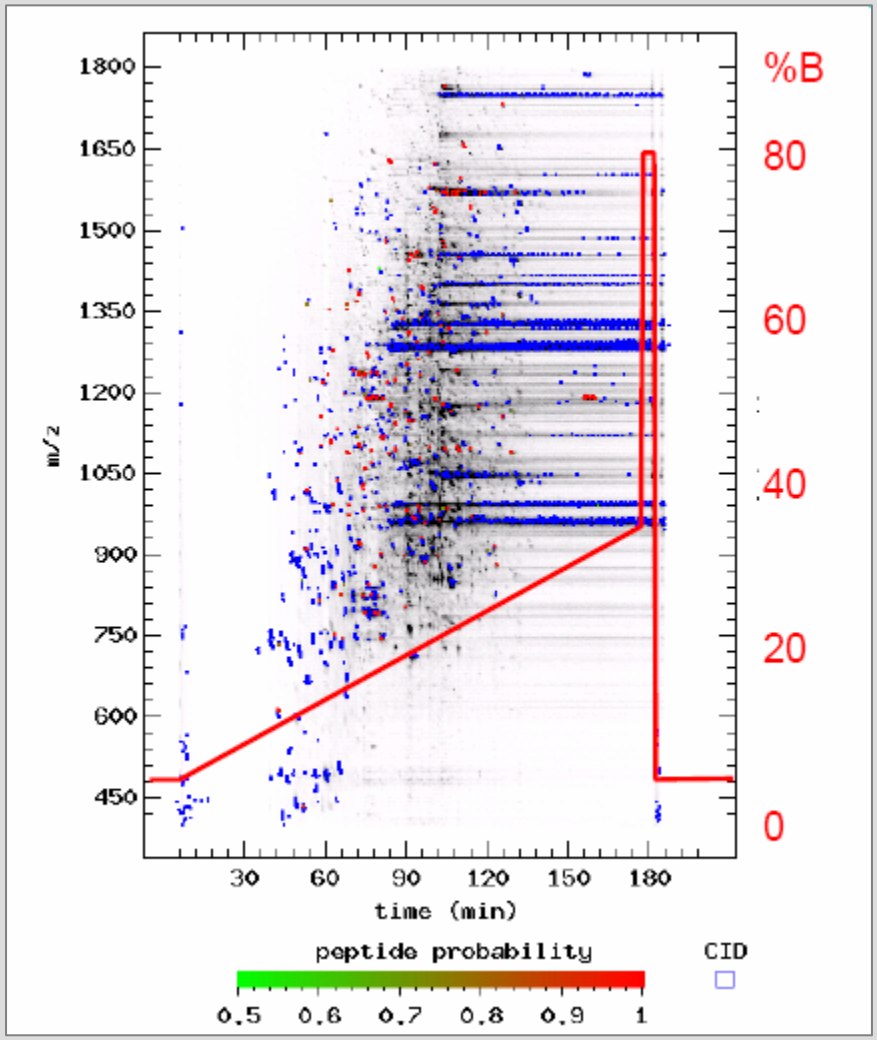
Insufficient Sample Separation



peptide/CID
(527/2150 = 0.25)

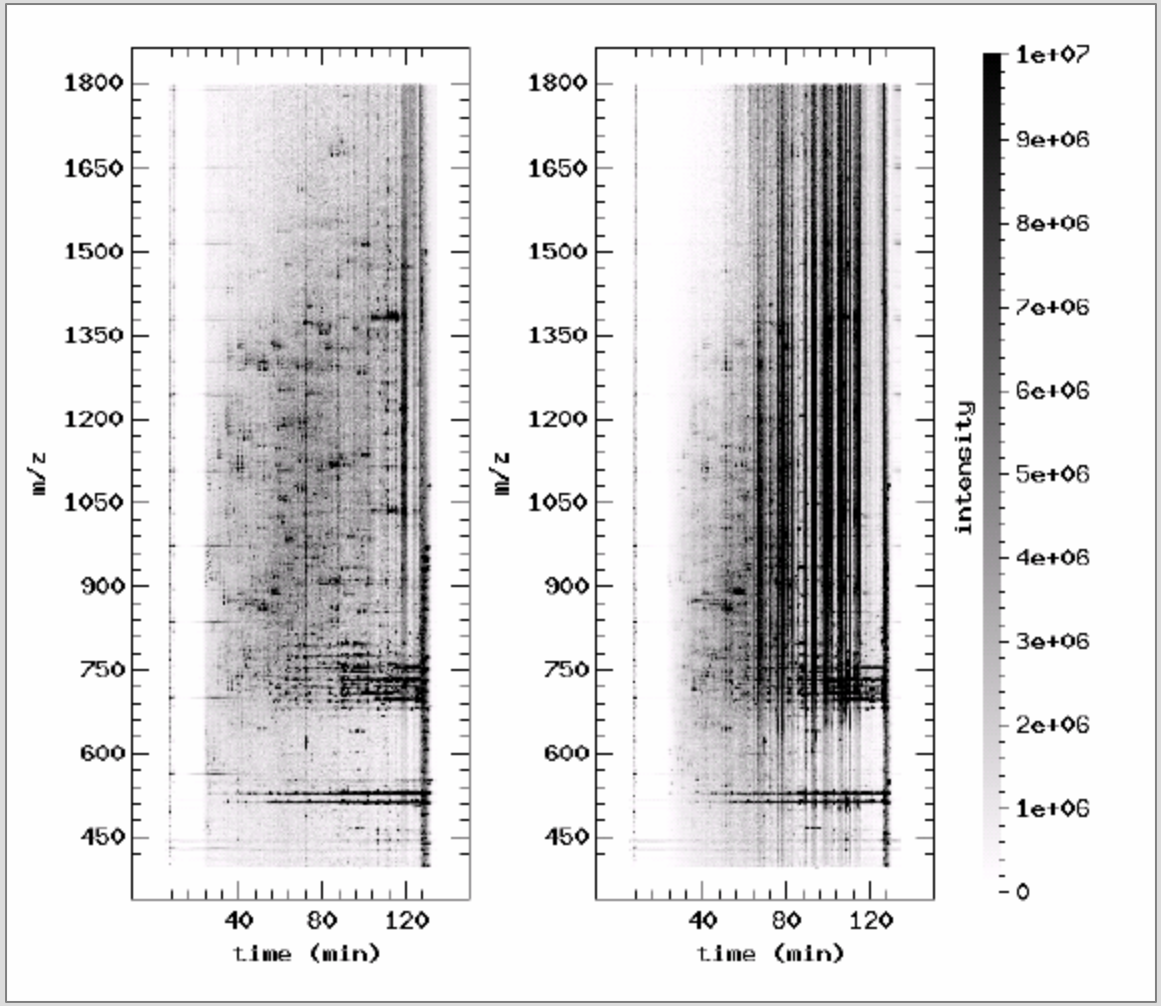
- Peptide ions elute in a narrow zone of the gradient
- Many intense ions not fragmented
- Insufficient fractionation / separation

Non-optimal Gradient



- Empty elution space at the beginning
- Crowded in the middle
- Horizontal streaks at the end

Bad RP Column



Same sample run on 2 different columns

peptide/CID

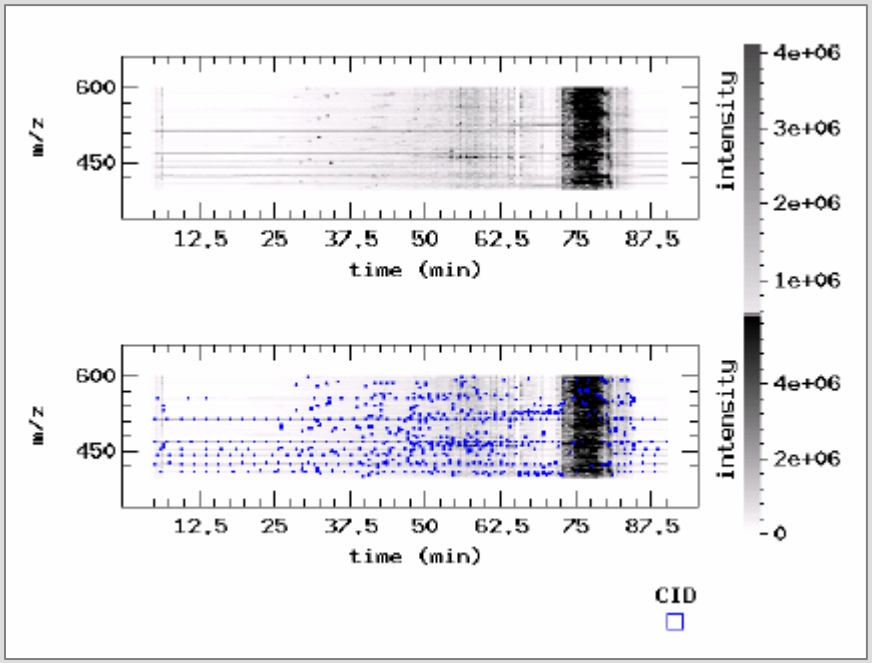
(354/3004 = 0.12)

(224/2760 = 0.08)

37% less IDs

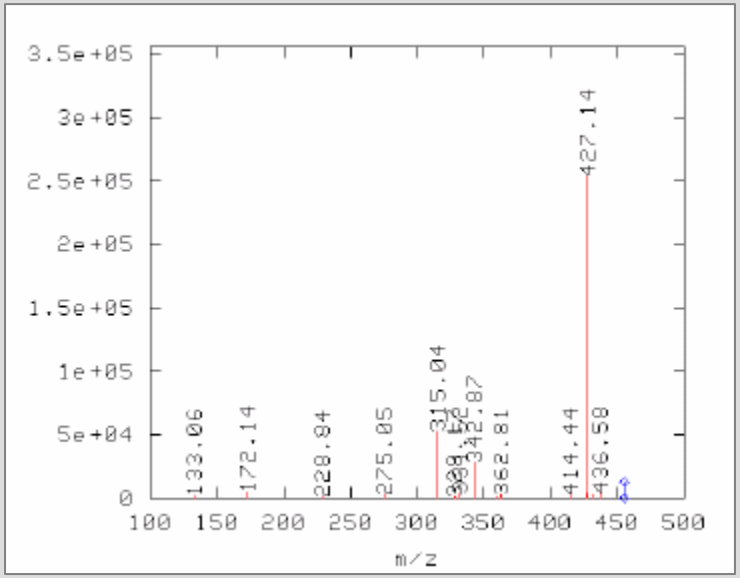
Quantitation also suffers

Chemical Contamination

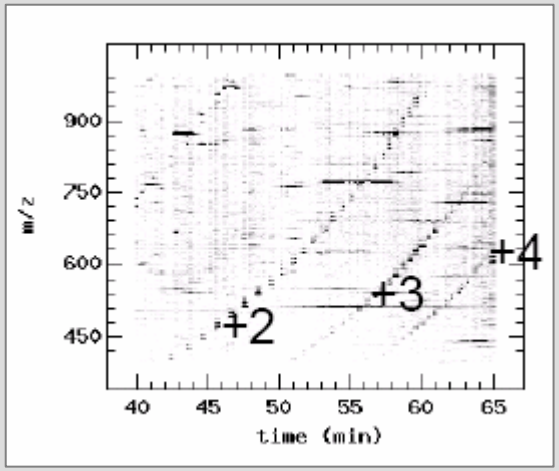


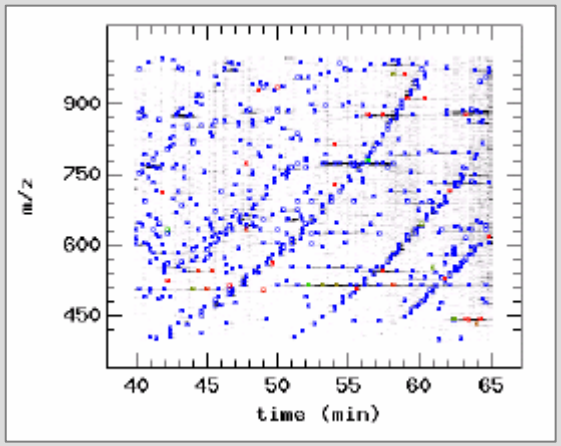
CID (588); no positive ID

- Long horizontal streaks
- Low m/z values
- Singly charged ions
- Wasted CID attempts

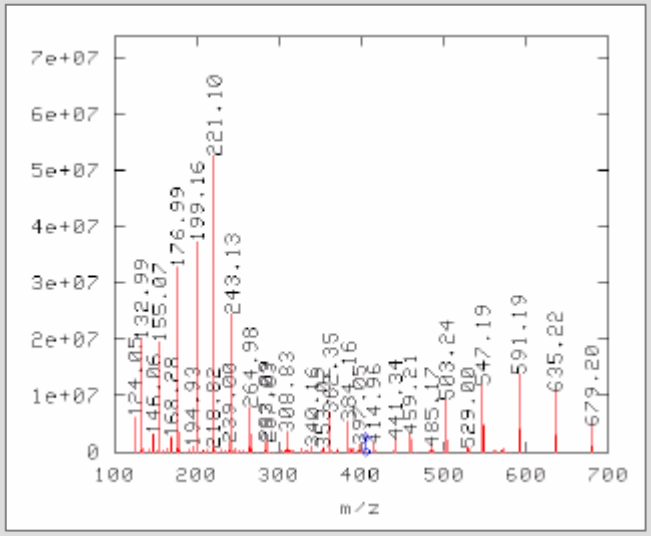


Polymer Contamination





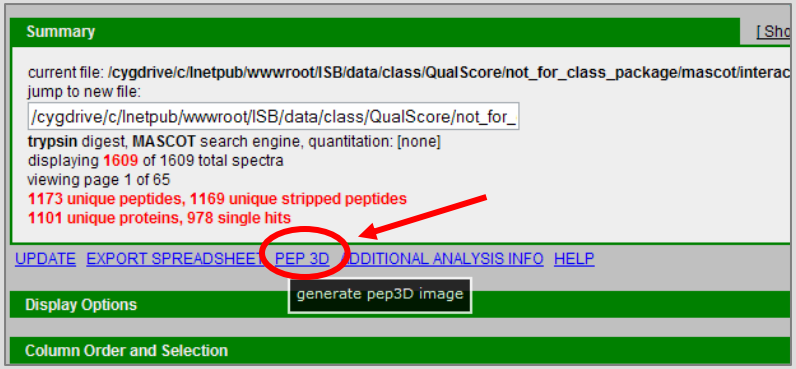
- Localized spots running off diagonally
- Equidistant in m/z
- Almost equidistant in time
- May be ionized in multiple charge states
- Wasted CID attempts



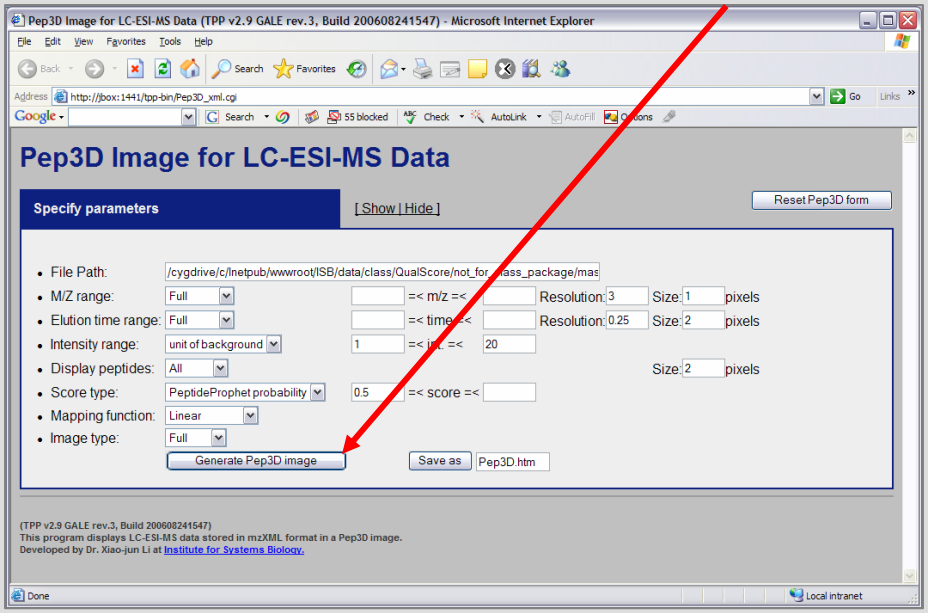
Repeat unit of 44 amu

Pep3D from XInteract

1. Click on PEP 3D link



2. Enter parameters – click Generate Pep3D image



3. Visualize data!

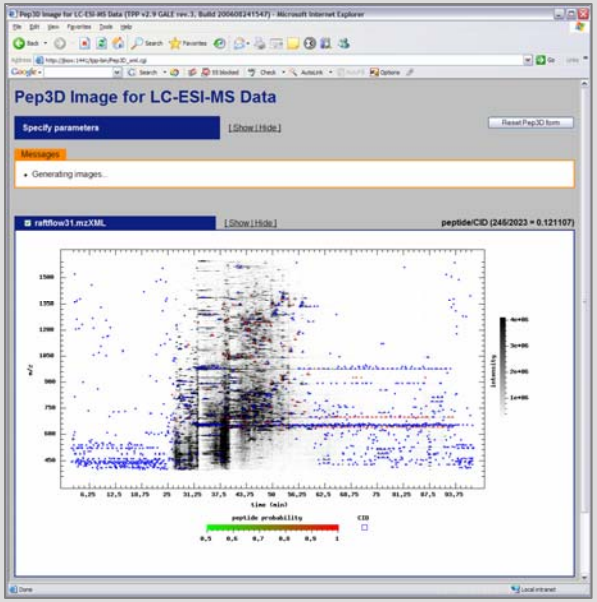
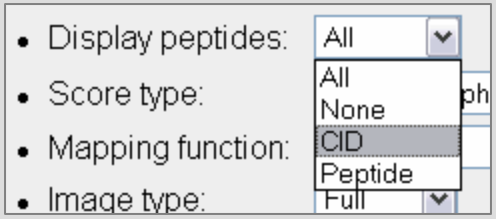


Image types: CID



CID (1198)

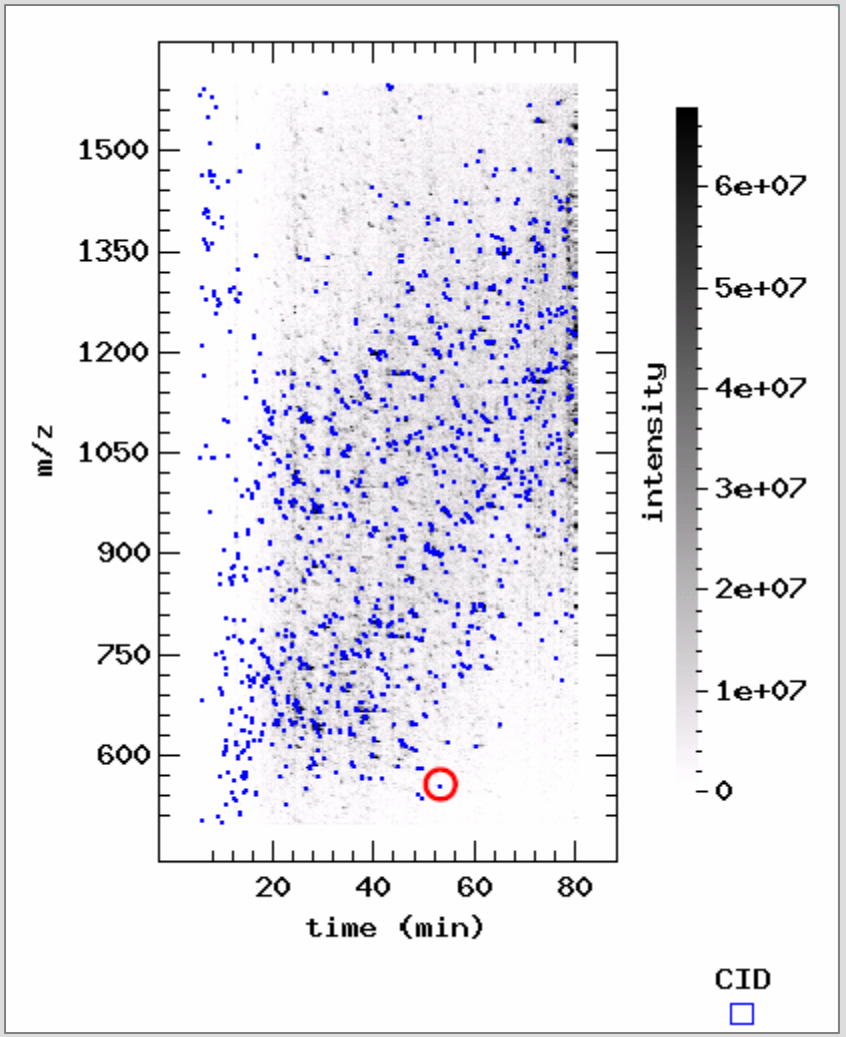
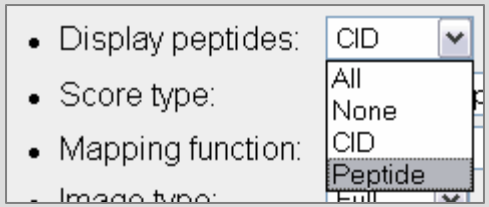


Image types: Peptide



Peptide (221)

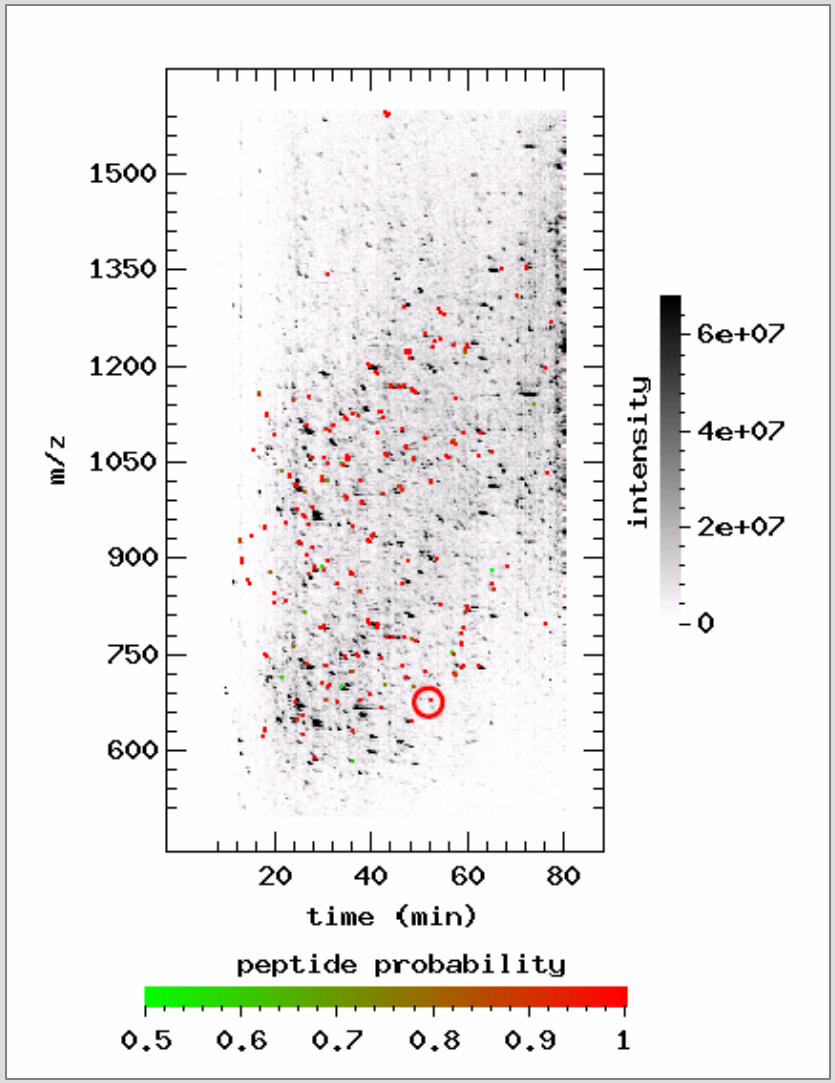
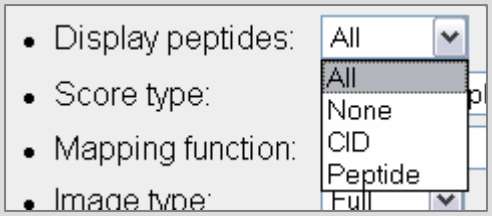
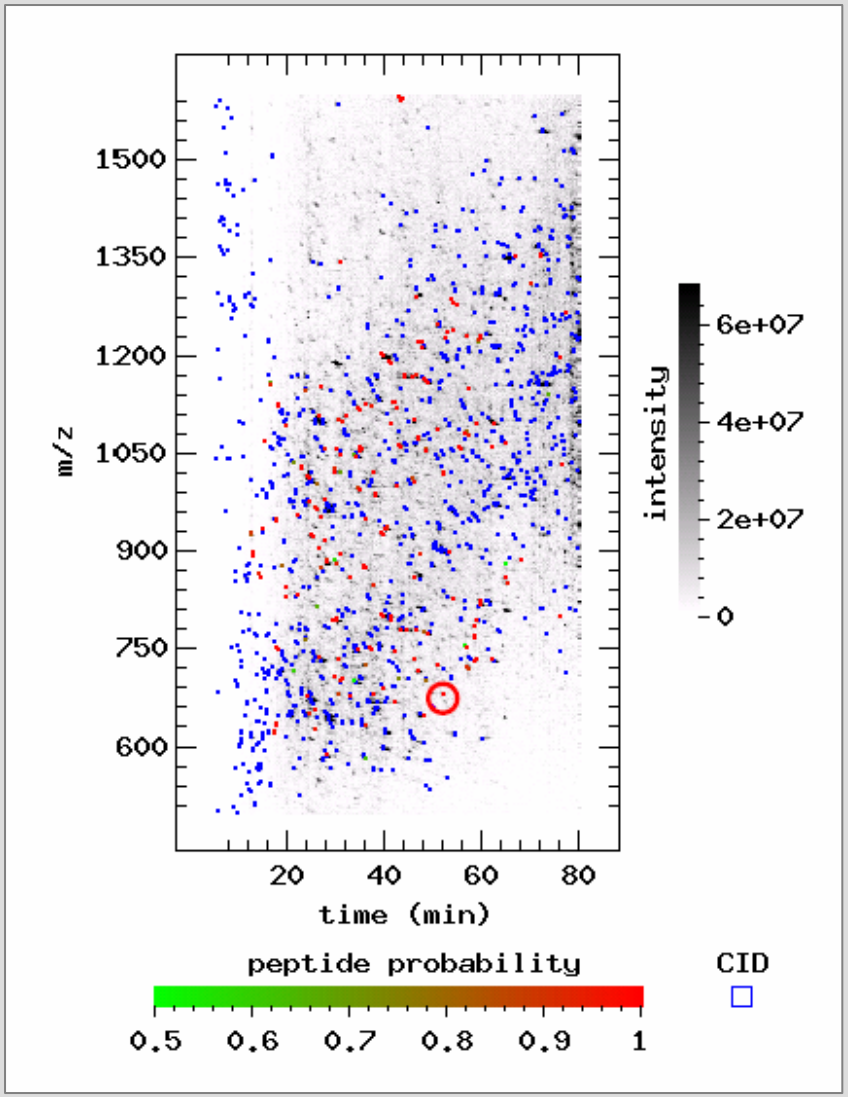


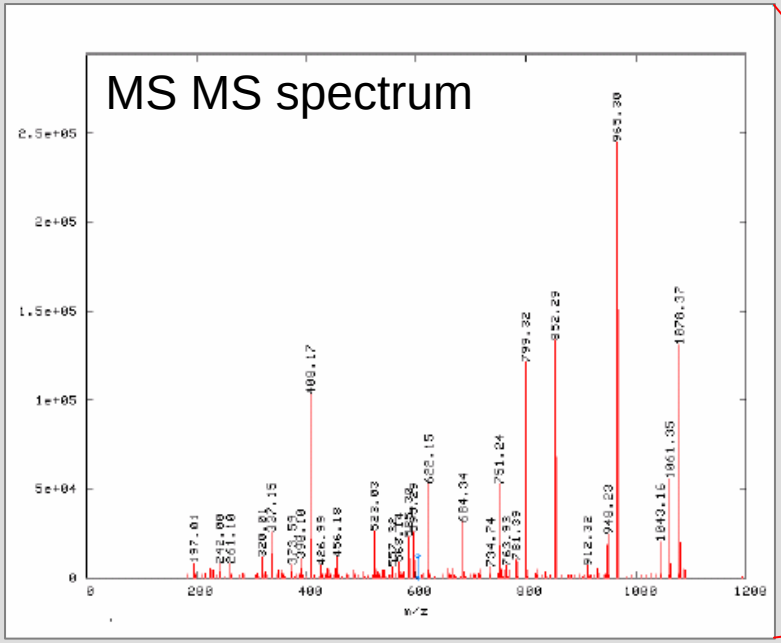
Image types: All

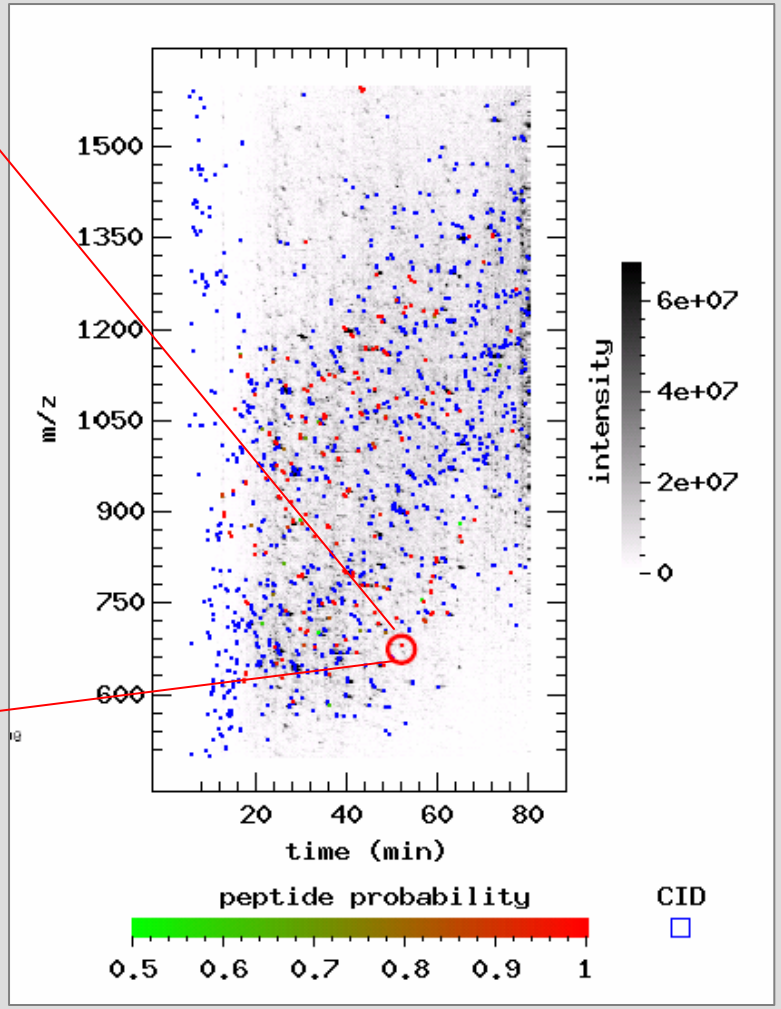


Peptide / CID
(221 / 1996 = 0.18)



Display CID Spectrum





Pep3D Summary

- Pep3D can be used to:
  - Evaluate and optimize sample quality and LC-ESI-MS system performance.
  - Check reproducibility and consistency of different sample analysis
  - Discover biomarkers?

"A tool to visualize and evaluate data obtained by liquid chromatography/electrospray ionization/mass spectrometry", X-J Li et al., *Analytical Chemistry*, 76(13), 2004.