Tandem Analysis: LC^n-MS^n

Bells & Whistles, For What?

Simplicity
Sensitivity
Selectivity
Simplicity

- LC-MS has a practical peptide maximum
  - Too many proteins/peptides swamps the analyzer
  - At best, mixtures of 400-500 peptides are workable

- Fractionating samples increases *Simplicity*
  - Leads to higher overall peptide analysis limit
  - Generates better certainty in protein ID

- Pre-Trypsin: sub-cellular, SEC, affinity

- Post-Trypsin: CX/AX, RP-LC, affinity (ICAT)
  - LC/LC-MS^n

Sensitivity

- Tandem LC allows better peptide separation
  - Reveals lower abundance peptides
  - Better protein ID *sensitivity*

- Affinity purification enriches sample
  - Low abundance proteins become concentrated
  - More *sensitive* and *selective* protein ID

- Tandem MS (or MS^n) provides more data
  - Peptide fragmentation data generates sequence info
  - Peptide sequence gives better protein ID with fewer usable peptide peaks
Collision Induced Decay

- How do we get more info out of MS?
- 1-D Mass Spectrometry
  - Protein Mass
  - Tryptic Peptide Masses: Peptide Mass Fingerprint
- Collision Induced Decay: CID
  - Introduction of large, inert, neutral atoms (Ar, Xe, etc)
  - Peptide-Atom collisions induce peptide fragmentation
  - Peptides typically fragment along peptide bond
  - Forms series of ‘b’ & ‘y’ ions
- 2nd-D Mass Spectrometry: ‘b’ & ‘y’ ion mass

‘b’ & ‘y’ Ion Review

- Same as those seen in MALDI PSD
- ‘b’ ions retain amino terminus
- ‘y’ ions retain carboxy terminus
‘b’ & ‘y’ Peptide Sequence

K T N P V F E P I L V D T T G S

MS^n: Triple Quadrupole

- Q1: Peptide mass filter
- Q2: RF only (Everything transmitted)
  - Acts as collision cell for CID
  - Introduce inert collision gas (He, Ar, Xe)
- Q3: Scan CID fragment masses
**MS^n**: Quadrupole-TOF

- Same concept as Triple Quad
- Remove Q3, add TOF
- Higher Sensitivity
  - Simultaneous analysis of fragments
  - Requires less sample – not steady stream
  - TOF reflectron inherently more sensitive
- No upper m/z limit
- Greater mass accuracy

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**MS^n**: Quadrupole-Ion Trap

- Ion traps can store and accumulate ions
- CID is carried out *inside* ion trap
- Fragments are selectively ejected
  - Scan all resonance ejection frequencies
  - Generate full fragment mass spectrum
- Advantages
  - Attomole sensitivity can be obtained
  - Multiple rounds of fragmentation can be applied
- Disadvantages
  - PTM scanning (precursor/neutral loss) are not practical with Q-Traps
Accurate Quantitation in MS

- Relative abundance not perfect:
  - Variations in ionization, sample preparation
  - Low signal, high complexity decrease accuracy

- Isotope Enrichment
  - Metabolic labeling with stable isotopes
  - Isolate protein, Mix, trypsinize
  - Mass spectra will have mass doublets
  - Relative intensity of doublets = relative abundance

- ICAT / iTRAQ Tagging

Isotope Coded Affinity Tag

- Uses protein reactive group to tag samples
- Include an affinity tag for sample enrichment
  - Usually biotin group
  - Some biotin tags are chemically removable
- Contain different isotopes (\(^1\)H/\(^2\)H or \(^{12}\)C/\(^{13}\)C)
- Labeled peptides from different samples differ by defined mass amounts
- Results in peak ‘splitting’
  - Relative abundance in sample = Relative signal in MS
  - Normalizes for variations in ionization, sample prep, etc.
**ICAT – D/H, Non-Cleavable**

ICAT Reagents:
- Heavy reagent: d8-ICAT (X = deuterium)
- Light reagent: d0-ICAT (X = hydrogen)

**ICAT – 12C/13C, Cleavable**

Affinity Tag (Biotin)  Acid Cleavage Site  Isotope Coded Tag
- Heavy: 9 x 13C (236 amu)
- Light: 9 x 12C (227 amu)

Protein Reactive Group (Iodoacetamide)
iTRAQ

- Similar in theory to ICAT tags
- Uses a set of different isobaric tags
  - Reporter mass tag
  - Mass balance
- Tag is liberated upon CID
- Reporter masses detected in a noise/signal free region of mass spectra (~120 \( m/z \))
- Peptide (b/y ion) peaks are not split as in ICAT
iTRAQ Mass Spectrum

Full scan mass spectrum obtained via HPLC tandem mass spectrometric analysis on QSTAR XL

MS/MS of m/z 483.9

control
Treatment 1
Treatment 2
Treatment 3