Biophysics 101: Genomics & Computational Biology

Section 9: Mass Spectrometry and Proteomics

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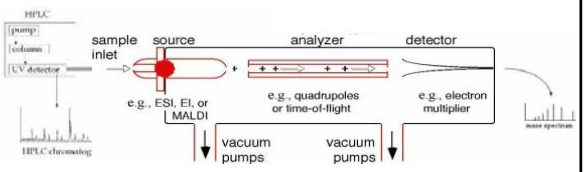
Nov. 18th 2003

Mass Spectrometry



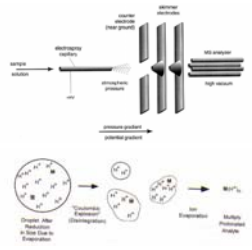
- Macromolecular MS only became feasible with past ~15 yrs with advent of new milder ionization methods:
  - Electrospray ionization (ESI)
  - Matrix-assisted laser desorption/ionization (MALDI)
- Molecular weight precision ~± 0.01%

Instrumentation

- Purpose – to measure the mass of molecules
- Three Components
  - Ion Source – Convert molecules to ion's in the gas phase – Nebulize and charge
  - Mass Analyzer – Separate or filter the mixture of ions by their mass to charge ratio (m/z)
  - Detector – Detect ions and abundances and convert to electrical signal to produce a digital response

Instrumentation

- Ion-Source
  - API (Atmospheric Pressure Ionization) or Electrospray (ESI)
  - High pressure gradient, heat and electric field aids ionization and nebulization
  - Charges in solvent collapse onto analyte
  - One of the first methods used to ionize large bio-molecules

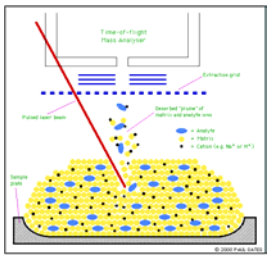


Instrumentation

- Ion Source
  - MALDI (Matrix Assisted Laser Desorption Ionization)
  - Analyte, matrix and cation (H+) are co-crystalized
  - Matrix has absorbance at the wave length of the laser
  - Laser excites matrix, energy is transferred to analyte as matrix evaporates leaving bare analyte molecules
    - (At some point the cation is transferred from solution to matrix and then to the analyte)
  - Charged analyte ions are accelerated through a voltage gradient to the mass analyzer

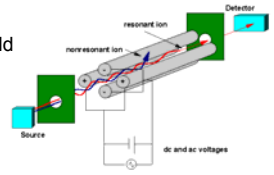
Instrumentation

- Ion Source
  - MALDI (Matrix Assisted Laser Desorption Ionization)



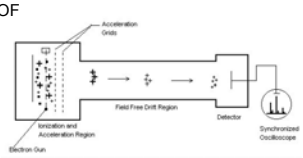
Instrumentation

- Mass Analyzers
  - quadrupole mass spectrometer (Most popular method)
    - DC and alternating RF voltages applied to four parallel rods creates electric field that focuses ions of a particular m/z towards the detector
  - Relatively easy to build
  - Limited Mass Range
  - Need multiple analyzers for ms/ms



Instrumentation

- Mass Analyzers
  - Time of Flight (TOF)
    - Ions are accelerated through a voltage gradient into a field free tube towards a detector
    - Velocities resulting from kinetic energy generated at gradient determines "time of flight"
    - Lighter ions – Lower TOF
  - Unlimited mass range
  - Fast scan times
  - High quality spectra

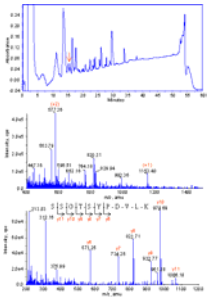


Instrumentation

Instrument	Application	Comments
ESI-Triple QP	LCMS, tandem MS	Limited Mass Range, relatively easy to make
ESI-QP Ion-Trap	LCMS, tandem MS	Limited mass range, trap improves sensitivity and automated analysis
MALDI-TOF	Analysis of purified samples	Low sample consumption, tolerant of buffer and salt contaminants (Ex. 2D Gel samples)
QP-TOF	LCMS, Tandem MS	High mass accuracy, fast scan times.
Fourier (transform ion cyclotron resonance)	LCMS, Tandem MS, Top-Down MS (Fragment large biomolecules)	Very High mass accuracy, ability to fragment very large molecules

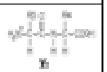
Tandem Mass Spectrometry
(ms/ms)

- Hit peptide with high energy gas and fragment
- Trade off between identification and quantization
  - Mass Spec will not be collecting quantitative data when it is fragmenting peptide



Tandem Mass Spectrometry
(ms/ms)

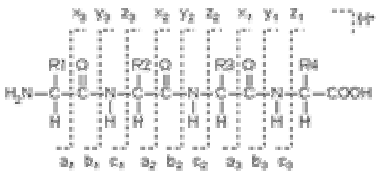
Peptide Fragmentation

x, **y**, z	Fragment between aa residues with charge retained on C-Terminal	
a, **b**, c	Fragment between aa residues with charge retained on N-Terminal	
Neutral Loss, NH_3 and H_20 loss	R, K, Q, N can lose NH_3 and S, T, E, D can lose H_2O	
Internal Fragments	Double fragmentation of the peptide bond – Common at Prolines	
Immonium Ions	A single aa chain fragment	

Tandem Mass Spectrometry
(ms/ms)

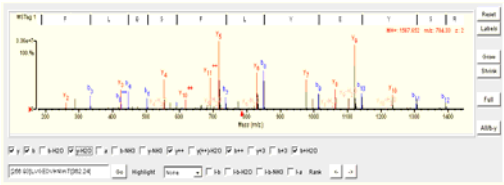
Peptide Fragmentation Nomenclature

- Integers indicate amino acid closest to charged terminus
- b-NH_3, y-NH_3 contains one residue with ammonia neutral loss
- b++ a double charge b ion
- Fragments generated dependent on peptide sequence



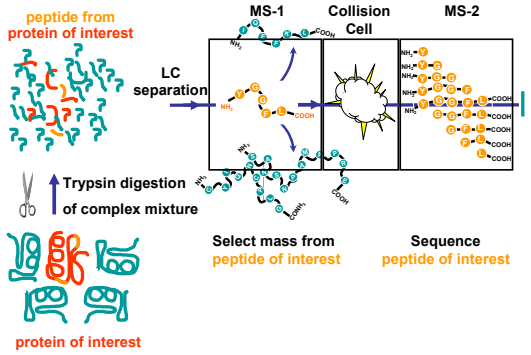
Tandem Mass Spectrometry
(ms/ms)

Spectral Interpretation

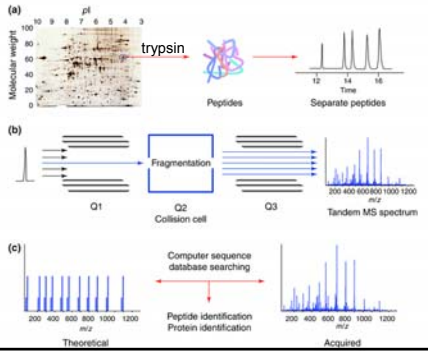


- Manual interpretation
- de novo sequencing-*Graph Algorithms, dynamic programming,*
- *Sequence spectrum correlation – Match experimental spectral pattern to pattern predicted from known sequence databases*

Peptide Sequencing by LC/MS/MS

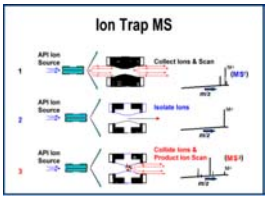


Standard proteome analysis by 2DE and MS

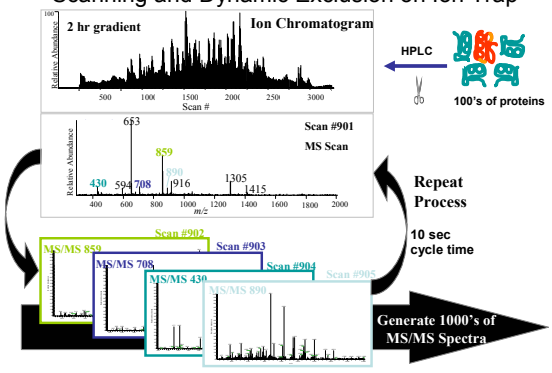


LC-MS Technology

- Data Dependent MS/MS
  - Quadrupole Ion Trap
  - Fill the trap, scan for most intense, refill trap with desired mass, fragment
  - Allows for dynamic fractionation of peptides
  - Higher Throughput
  - Complex Mixtures
    - Complexes
    - Body Fluids
    - Media



Complex Mixture Analysis with Data Dependent Scanning and Dynamic Exclusion on Ion Trap

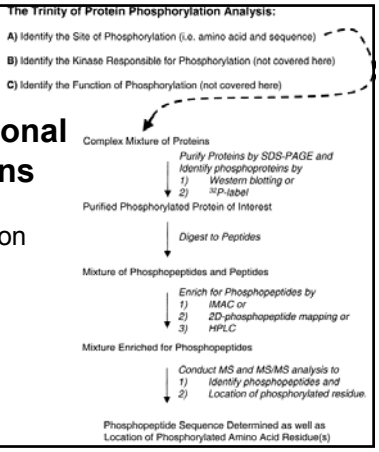


Detection of Post Translational Modifications (PTM's)

- MALTI-TOF Mass Spectrum of tryptic digestion of purified protein (GCSF)
- All of the masses of the detected peptides match except 3227.8 and 3243.8
- These are 16 and 32 amu more then the native N-Terminal peptide MKLMVLQLLLWHSALWTVHEATPLGPAR
- Delta amu of 16 indicates an oxidation. Met is known to be susceptible to oxidation
- If you want to know which amino acid is modified in the 3227.8 amu peptide you need to do ms/ms

Post-translational Modifications

- Phosphorylation
- Ubiquitination
- etc



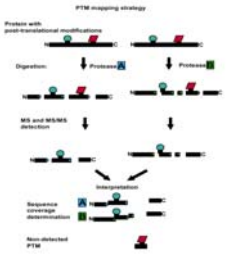
Detection of Post Translational Modifications (PTM's)

Complete characterization is difficult in purified samples and nearly impossible in complex mixtures

Some modifications won't be seen since peptide is not detected or not identified

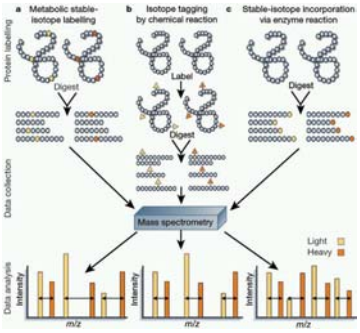
FTMS and top down proteomics is enhancing the ability to analyze PTM's in Complex Mixtures

2D Gel analysis, antibody identification and affinity purification helps

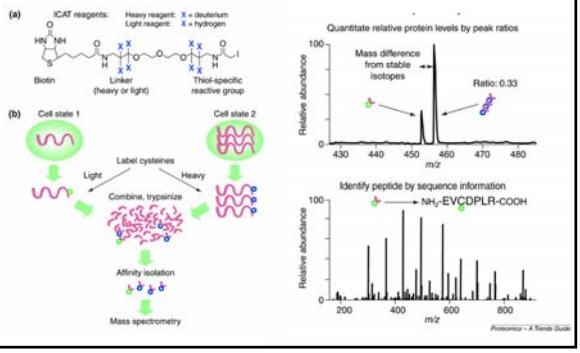


Proteomic analysis of post-translational modifications
Matthias Mann & Ole N. Jensen, Nature Biotechnology 21, 255 - 261 (2003)

Quantization
Stable-isotope protein labeling

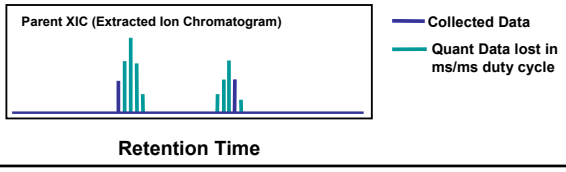


Quantization
Isotope-coded affinity tag (ICAT)



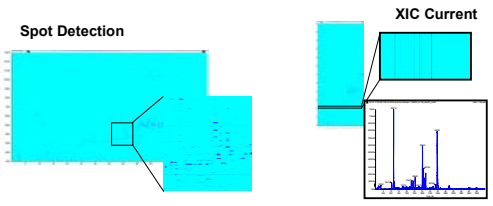
Quantization
ApproxiQuant

- Spectrum Mill feature
- Absolute intensity determined by intensity data collected for each parent ion
- Compare intensity data from identical peptides across samples
- Approximate method since data lost in duty cycle



Quantization
MS-Only

- Avoid any ms/ms analysis. create ms scans only-Perform ms/ms after quantization and data analysis
- Component Detection
  - generating XIC's for all mass ranges or do spot detection
- Need to align components since elution times on LC may vary



Quantization

Method	Comments
Stable-isotope protein labeling	Need to synthesize proteins in presence of isotope, experimental design issues of two-color system
ICAT	Enriches (and limited) for peptides with particular amino acids (Ex. Cys), experimental design issues of two-color system
AproxiQuant	Very approximate absolute quantization, easy to implement and use
LC-MS Only	Very good absolute quantization, computationally challenging (Component detection, alignment, noise), identification requires additional steps

Computational Methods for Identification of proteins

- de novo sequencing of ms/ms data
  - *Graph Algorithms, dynamic programming*
  - *Rarely give exact sequence information*
  - *Computationally expensive*
  - *Often used to interpret good quality spectra that can't be interpreted by other methods (Last Chance)*
- *Comparison of experimental data to sequence database*
  - *Peptide Mass Fingerprint, Protein Identification*
  - Quality of match highly dependent on mass accuracy of instrument

Computational Methods for Identification of proteins

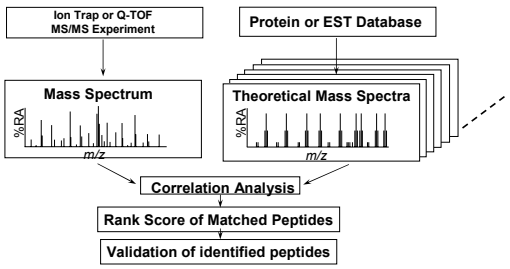
- *Peptide Mass Fingerprint*
  - *Compare spectra of peptides from experimental digestion of purified protein to theoretical digestion of sequence database*
    - *The better the experimental digest corresponds to the theoretical digest the higher the score*
      - *Mass Accuracy, incomplete digestion, contaminations, PTM's all affect the result*
- *PeptIdent* – includes pI (SwissProt)
- Mascot (MatrixScience)
- MS Fit SpectrumMill (Agilent)

Computational Methods for Identification of proteins

- *MS/MS Ion or MS-Tag Search*
  - *Compare spectra of experimentally fragmented peptide (ms/ms) to theoretical fragmentation of predicted sequence database peptides*
    - *Uses parent mass and fragment ion-masses*
  - *Does not require a purified protein*
    - *Not always easy to predict how a peptide will fragment*
      - *Incomplete fragmentation along peptide bonds, neutral losses, differing intensities*
- MS Tag SpectrumMill (Agilent)
- Sequest
- Mascot (MatrixScience)

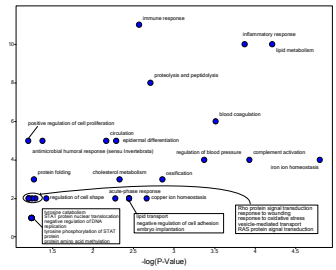
Computational Methods for Identification of proteins

- *MS/MS Ion or MS-Tag Search*



Mixture Functional Classification

- Pie Charts often show subjective representation of complex mixture
- Use statistical categorization method



Sample – Synovial fluid of arthritis patients

Category Set (n) – GO Process

Scoring Method – Fishers Exact

Universe (N)– All Human LocusLink

Resources

- Base Peak (http://www.spectroscopynow.com)
- http://www-methods.ch.cam.ac.uk/meth/ms/theory/maldi.html
- http://www-methods.ch.cam.ac.uk/meth/ms/theory/quadrupole.html
- http://elchem.kaist.ac.kr/vt/chem-ed/ms/ionizatn.htm
- http://www.spectroscopynow.com/Spy/basehtml/SpyH/1,1181,4-1-2-0-0-news_detail-4729775854-842,00.html
- http://www.matrixscience.com/help/fragmentation_help.html
- http://www.nature.com/cgi-taf/DynaPage.taf?file=/nbt/journal/v21/n3/full/nbt0303-255.html
- http://www.spectroscopynow.com/Spy/pdfs/1519_a.pdf (TOF)
- http://david.niaid.nih.gov/david/ease.htm (Categorization)