### Outline
- Liquid Chromatography
- Electrospray
- MS Acquisition
- Peptide Fragmentation
- PTM Detection
- Protein ESI-MS

### Terminology
- ESI
- Liquid chromatography
- Acquisition
- DDA (data dependent acquisition)
- MS1 vs MS2
- Precursor ion
- MS/MS
- Product Ions
- b-ion, y-ion
A. Obtain protein-complex
B. Separate proteins on 1D gel
C. Excise protein bands > trypsin digestion
D. Liquid Chromatography-ESI-Mass Spectrometry
Example Sample Analysis Workflow

1. Liquid Chromatography (LC): Peptide Separation
2. Electrospray Ionization (ESI)
3. Mass Spectrometry (MS)
Liquid Chromatography: Separation Technique

Mixtures are separated or partially separated before MS analysis with Chromatographic Technologies
Liquid Chromatography Basic Overview

“... modern Liquid Chromatography (LC), uses a liquid mobile phase to transport the sample components through a column packed with a solid material - the stationary phase.” Reference: http://www.earl2learn.com

http://www.chemistry.adelaide.edu.au/external/soc-rel/content/lc-col.htm

Mikhail Tswett (1872 – 1919)

From Tswett’s notebook (1910) on the early chromatographic experiments: plant pigments were passed through calcium carbonate using petroleum ether
Liquid Chromatography, Peptide Elution Profile 1D LC

- Peaks represent analyte elution profiles
- Increased Retention Time = increased peptide hydrophobicity on a C18 column
• **Column 1** Elution Profile
• Use fraction collector – collect peptides in separate tubes

Liquid Chromatography, Peptide Elution Profiles
2D LC

**Column 2**: LC-MS Peptide Elution Profiles
Example Sample Analysis Workflow

1. Liquid Chromatography (LC): Peptide Separation
2. Electrospray Ionization (ESI)
3. Mass Spectrometry (MS)
Electrospray Ionization (ESI)
Produce Analyte Ions

GOAL: eliminate solvent, get analyte into the gas phase; apply high voltage to a liquid to create aerosol

Electrospray Ionization

Ref: IJAC Vol 2012 ID 282574
Example Sample Analysis Workflow

1. Liquid Chromatography (LC): Peptide Separation
2. Electrospray Ionization (ESI)
3. Mass Spectrometry (MS)
EXAMPLE Workflow: MIXTURE OF UNKNOWN PROTEINS

Typical Numbers of Proteins Identified:

• 1D LC-MS: up to 300
• 2D LCMS: up to 5000

(sample and dynamic range dependent)
EXAMPLE:
MIXTURE OF UNKNOWN PROTEINS

Data-Dependent Acquisition Scan Mode (DDA)
on the Mass Spectrometer
1 Dimensional LC-ESI-MS Elution Profile

RT: 0.67 - 73.58

Retention time 39 minutes → 39.08

Next slide: see Full Scan/Survey
Scan at 39 minutes

Retention time 39 minutes

1 Dimensional LC-ESI-MS Elution Profile
MS1 Data Acquisition

- MS1 spectrum (below)
- Peaks below represent unfragmented peptide m/z values

Mass Spectrum at 39.22 minutes shows co-eluting peptides
Data Dependent Acquisition:
- Top 6 (most abundant) Peaks Identified
- Next 6 Scan Events are MS/MS

Sequential MS/MS scan events are triggered for the 6 most abundant peaks
MS2 Data Acquisition (1st peak triggered in DDA)
MS/MS spectrum
Precursor 851.95 m/z
Tandem Mass Spectrometry (MS/MS)

- Select Precursor Peptide
- Isolate Precursor ions in Collision Cell
- Fragment precursor ions with Collision Induced Dissociation (CID)
- Measure $m/z$ of Product ions
Collision Induced Dissociation (CID or HCD)

**Q1**
Quadrupole Mass Filter

\[ \text{+LLLYSSQICK}^+ \]

**q2**
Collision Cell

\[ \text{+LLLY} \text{SSQICK}^+ \]
\[ \text{+LLL} \text{YS} \]
\[ \text{N}_2 \]

**Detector**

Measure \( m/z \) values of product ions

\[ \text{N}_2 \text{ collision gas} \]

Collision Induced Dissociation
1) Electric potential applied to collision cell
2) Ions accelerated to high kinetic energy
3) Ions collide with neutral gas molecule
4) Kinetic energy is converted to internal energy
5) Chemical bond breakage occurs

Tandem Mass Spectrometry
1\textsuperscript{st} measurement (MS1) = Intact Peptide \( m/z \)
2\textsuperscript{nd} measurement (MS2) = Peptide fragment ion \( m/z \) values

**PRECURSOR ion** 612.8 \( m/z \)
(LLLSSQICK) filtered in Q1
Predictable Fragment Ions Types from Peptide Dissociation

- Peptide Backbone has 3 Bond Types (peptide bond is the weakest of the 3 bonds)
- Bond Breakage Yields Complimentary Ion Types, e.g., b- and y-type
Peptide Fragment Ions (or product ions)

Peptide MQIFVKTTLTK
- 604.8572 \( m/z \) precursor ion, monoisotopic
- 1208.7077 Intact Peptide Mass, monoisotopic

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Peptide Tandem MS can provide Unambiguous Evidence for Location of Amino Acid Modifications

Rule: Experimental data must contain fragment ions that provide site localization evidence
Tandem MS (or MS/MS) for Identification of Amino Acid Post-translational Modification (PTM) Site

MQIFVKT LTK \( MW_{\text{mono}} = 1208.7077 \)

MQIFVKTpLTK \( MW_{\text{mono}} = 1288.6740 \) (phosphorylation +80 Da = HPO₃)

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Mass shifts will occur only in fragments containing the phos-Thr, therefore location of MODIFICATION can be pinpointed.
Predictable Mass Shifts for Phosphorylated Fragments
MQIFVKT<sup>p</sup>LTK

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Mass shifts will occur only in fragments containing the phos-Thr, therefore location of MODIFICATION can be pinpointed.
Predictable Mass Shifts for Phosphorylated Fragments

\[ \text{MQIFVK}^\text{TpLTK} \; (+80 = \text{HPO}_3); \quad \text{MW}_{\text{mono}} = 1288.67 \]

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Mass shifts will occur only in fragments containing the phos-Thr (bold), therefore location of MODIFICATION can be pinpointed.
EXAMPLE: MS2 Spectrum and Confirmed Peptide Match

Protein ID: Tyrosine-protein kinase JAK3 (human)
Intact Protein Electrospray Mass Spectrometry
Intact Protein Electrospray - Mass Spectrum

Mathematically deconvoluted Data \( \rightarrow \) Protein MW

PROTEIN CHARGE SERIES

12359 +/- 2
Intact Protein ESI-MS
Cytochrome C, 12361 Da

\[
\frac{m}{z} = \frac{12361 + 12}{12} = 1031
\]

Charge = +8

\[
\frac{m}{z} = \frac{12361 + 8}{8} = 1546
\]

Relative Abundance

\[
1545.7
\]
Intact Protein Electrospray-MS
Infusion of Solubilized Relatively Pure Protein

- Relatively pure sample
- 20 – 50 µM protein concentration
- Detergent and salt free solution
- Typical solvent: 50:50, acetonitrile:water, 0.1% formic acid
- Difficult (but possible) to achieve high quality data
- Non-covalent interactions are retained with ESI (not MALDI), usually with neutral/basic buffer system and a lot of trial and error
Glycosylated Intact Protein ESI Mass Spec

Intermediate 1 Protein
Intermediate 1: Deconvoluted MS Data
Using Bayesian Reconstruct Tool; Theoretical mass = 50140.01 Da; error=4 ppm

Mass, amu

Intensity, cps

50140  .23

Sialic acid

Δ 291

Fucose

Δ 656

Δ 146

NAcNeu
Gal
GlcNAC

Δ 656

Δ 146

49849  50229  50497  50797

500e4  505e4  510e4

+K

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