2-D Gel – Mass Spectrometry

Pick a Spot, Any Spot…

- 2-D gel analysis generates spots of “pure” protein
- Visualize by stain
- Pick by hand
- Robotic spot picking
  - True –Omics technology
  - High-throughput, *en masse*
  - Can be user directed or automated
Who Cares About MW?

- Mass spectrometric analysis on pure, whole protein will generate
  - Accurate molecular mass
  - Isotope distribution

- SDS-PAGE gives MW to 10-30% accuracy

- Does a MW to 1Da and isotope distribution assist in protein identification?

Peptide Mass Fingerprinting

- Specific, limited fragmentation of pure protein
  - Enzymatic – Trypsin, Endopeptidase LysC, AspN
  - Chemical – CyanogenBr (Met), NBS (Trp)

- Clean-up of resultant peptide mixture

- Mass Spec (MALDI-TOF) of peptides

- Spectrum gives unique peptide “fingerprint”

- Bioinformatics takes over
  - Fingerprint databases
  - Theoretical in silico protein digestion – More -Oomics
**In-Gel Proteolytic Digestion**

- Simplest procedure is to digest in-gel
- Trypsin digestion is most common
- Small (~23 kDa) – Easily diffuses into gel
- High specificity – C-term of R, K, except X-Pro
- Resistant to buffer, salts, chaotropes
- Pure protein is readily available
- Generates ~500-2500 kDa fragments

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**Peptide Mass Fingerprint**

Peptide Mass Fingerprint

**Cytolysin III Tryptic Digest**

![Mass Spectrum Graph](image)
Post Source Decay

- MALDI ain’t so “soft” after all…..
- Five-Point Palm Exploding Heart Technique
- After acceleration, ions collide with matrix/gas
- Collisions generate sub-peptide fragments
- Fragments have same v as parent, different KE
- Since TOF uses velocity as indicator of mass…
- Use “ion gate” as mass filter
- Use gridded reflector to spread fragments

PSD Reflection & Detection
Peptide Fragments

- Fragmentation most often at peptide bond
- Generates series of “b” and “y” ions
- Other minor fragments can occur
- Post-Transl. Mod are usually lost – Not ID’d
Alternative Fragmentation

Typical Peptide

\[ \text{NH}_2 - C - C - N - C - N - C - N - C - C - CO_2H \]

Peptide Fragmentation

\[ \begin{align*}
\text{N-terminal} & : R_1 \quad R_2 \quad R_3 \quad R_4 \\
\text{C-terminal} & : R_4 \quad R_3 \quad R_2 \quad R_1 \\
\end{align*} \]

\[ a_1 \quad b_1 \quad c_1 \quad a_2 \quad b_2 \quad c_2 \quad a_3 \quad b_3 \quad c_3 \]


PSD Peptide Sequencing

\[ \text{K} \quad \text{T} \quad \text{N} \quad \text{P} \quad \text{V} \quad \text{F} \quad \text{E} \quad \text{P} \quad \text{I} \quad \text{L} \quad \text{V} \quad \text{D} \quad \text{T} \quad \text{T} \quad \text{G} \quad \text{S} \]
Molecular Scanning

- New approach, not quite perfected yet
- Whole 2-D gel analysis
- Tryptic digest of entire 2-D gel
  - Parallel In-Gel Digest (PIGD)
  - One-Step Digestion-Transfer (OSDT)
  - Double Parallel Digestion (DPD)
- Transfer to PVDF membrane
- Direct analysis of membrane by MALDI-TOF
  - Membrane adhered to plate with vacuum grease
  - Matrix deposited on membrane
  - Largest membrane analyzed 4 cm²

Molecular Scanning

- Proteomics equivalent to microarray
- In order to catch up:
  - More complete digestion
  - More complete transfer to PVDF
  - Better visualization of peptides on PVDF
  - Better resolution – In gel & In MS
  - Larger MALDI platforms
  - More automation of each step
- When optimized, has potential to increase high-throughput protein identification 10X