Proteomics: Large-Scale Identification of Proteins

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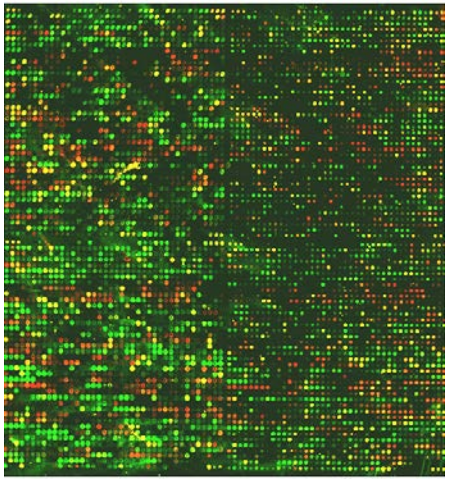
This Lecture

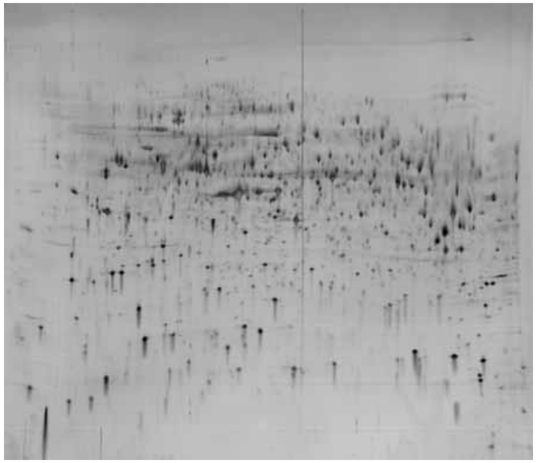
- Proteomics
- Separation
- Identification: Mass Spectrometry

Proteomics

- Genomics =
  Determining the genome of a species
- Transcriptomics =
  Determining the mRNA of a cell / tissue / state
- Proteomics =
  Determining the proteins in a cell / tissue / state
- Proteomics and transcriptomics have mostly identical goals
  - Understanding the processes happening in a cell
  - Differentiate between states, tissues, developmental state, ...
  - Biomarker: Finding protein/mRNA/... (forms, concentrations) that are characteristic for a certain phenotype (e.g., a disease)
- Metabolomics, epigenomics, bibliomics, ...

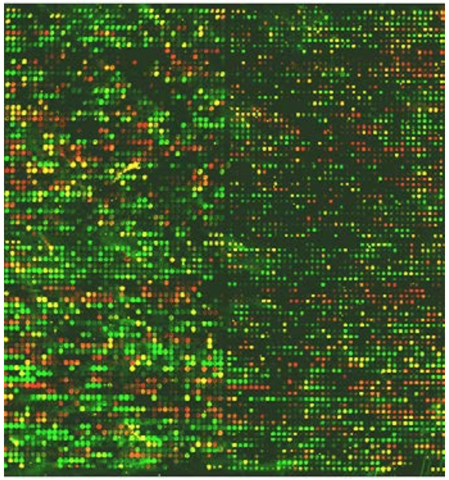
Proteomics versus Transcriptomics

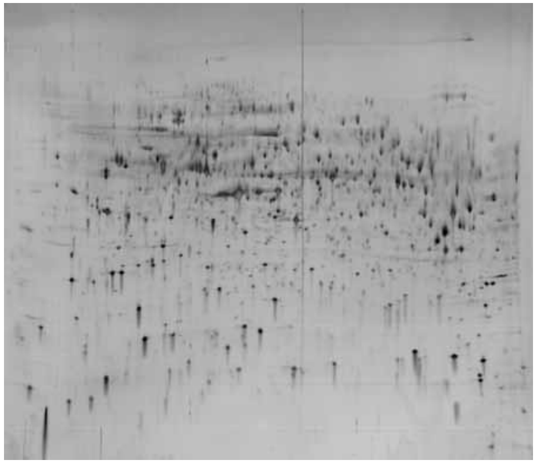




- Advantages
  - Proteins make you live, not mRNA
  - mRNA is only indirect evidence with little correlation with proteome
    - Regulation by miRNA, post-translation modifications, decay, …
  - Protein survive (some time), mRNA is (mostly) transient
  - Proteins are favorite drug targets

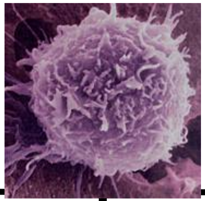
Proteomics versus Transcriptomics

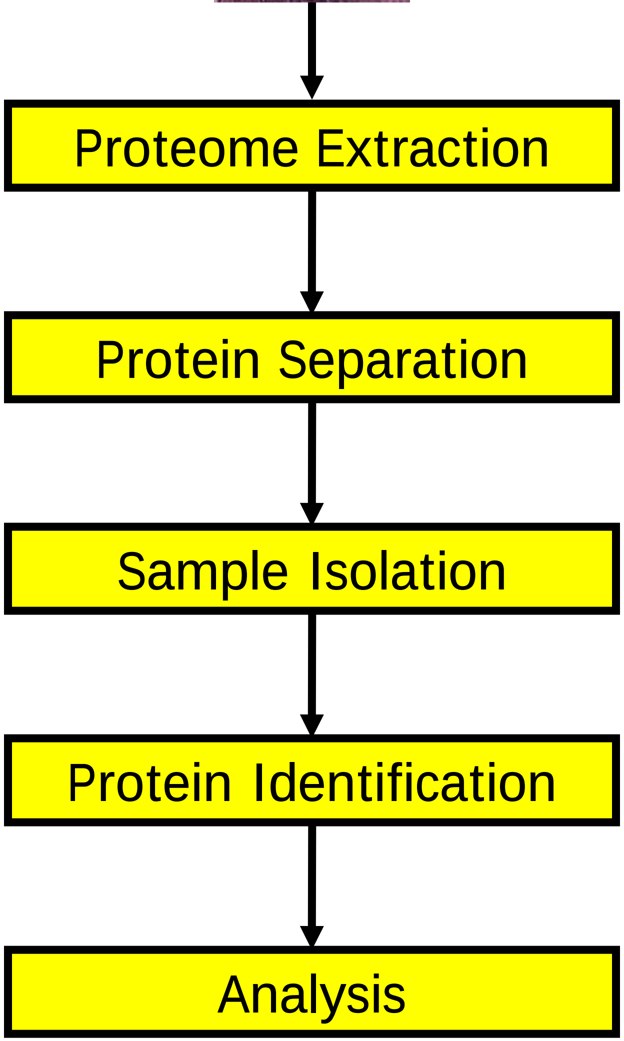




- Disadvantages
  - Scale: ~20K genes, ~300K proteins, ~1M protein forms
  - Handling: No PCR, no hybridization, no simple synthesis, no sequencing, no long-term „storage“ as clones, high reactivity, ...
  - Behavior highly context-dependent: Temperature, solution, pH, ...

Typical Proteomics Workflow





Proteome Extraction

Protein Separation

Sample Isolation

Protein Identification

Analysis

From a cell mixture

2D gel electrophoresis / LC/GC

From the gel / from the flow

Mass spectrometry

Quantification, clustering, ...

This Lecture

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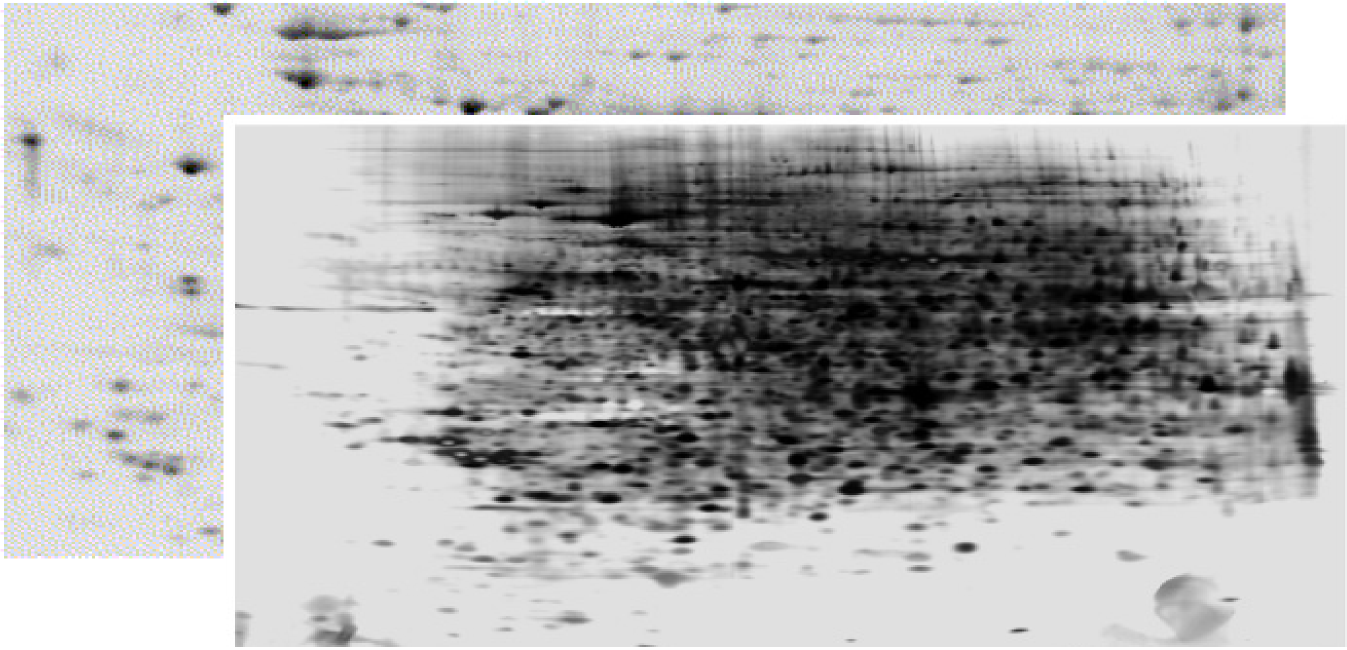
2D Gel Elektrophoresis

- Separation of proteins in two dimensions
  - Mass
  - Charge
- Every spot one protein (hopefully)



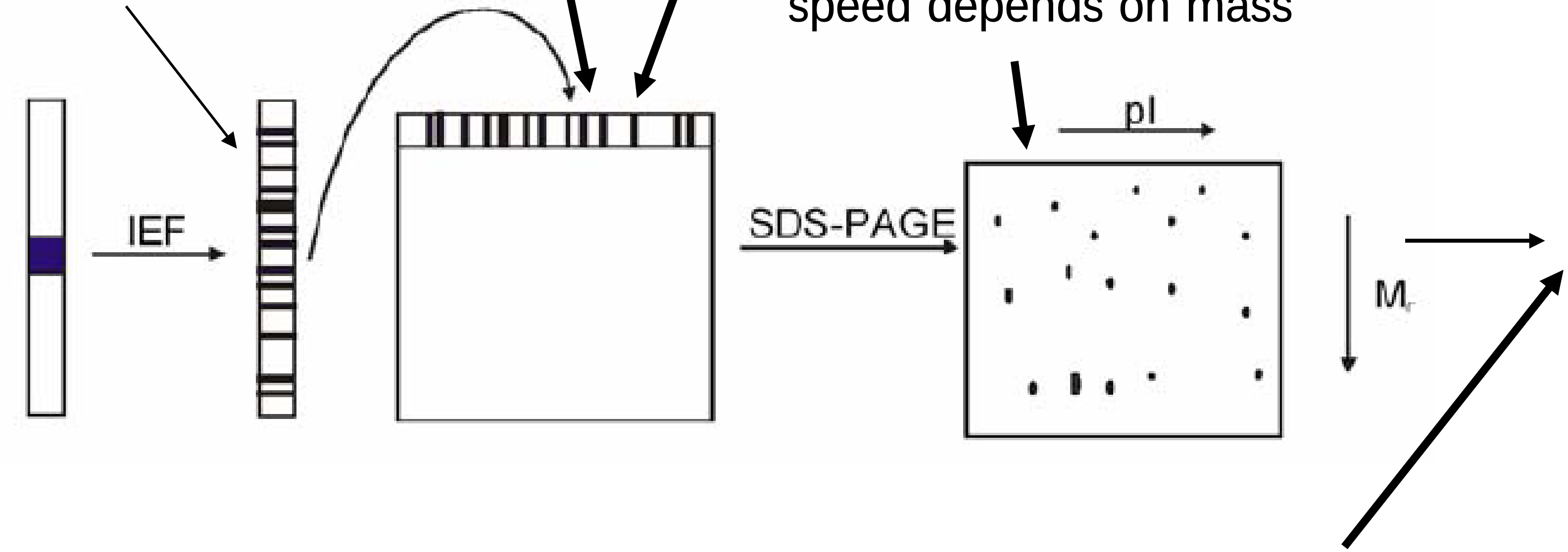
Method

2. Charging of proteins with SDS (Sodiumdodecylsulphate)

3. Place on slide of polyacrylamide gel (PAGE)

1. Separation in pH-gradient: Proteins move to their isoelectric points

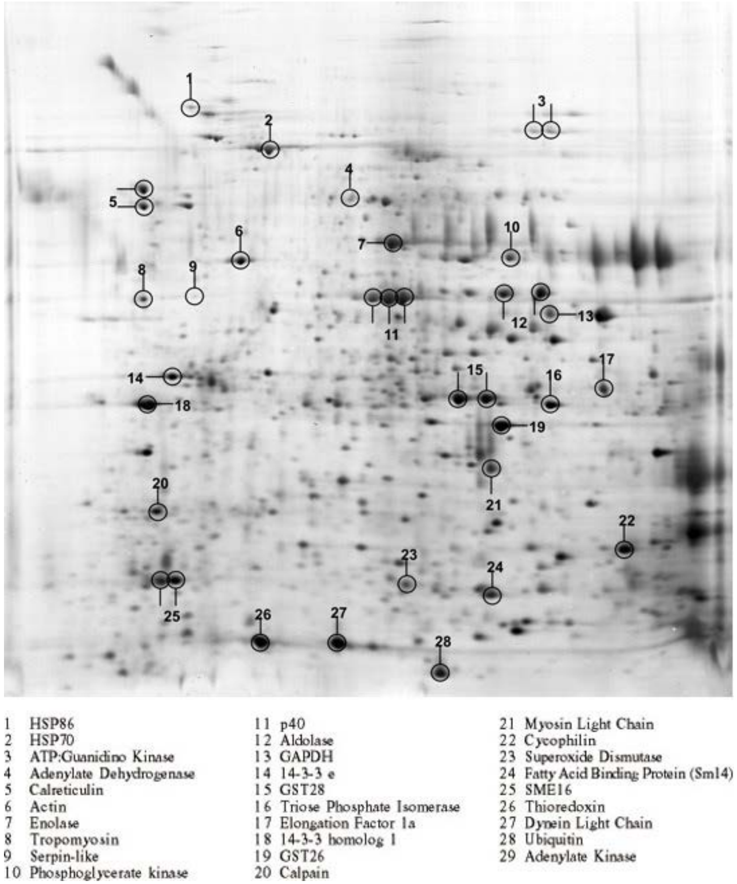
4. Proteins move in an electric field: speed depends on mass



5. Staining; photo; image analysis; excision

Analysis

- 2D-Page may separate up to 10.000 proteins
- Under identical conditions, the position of a particular protein is fairly stable
- Software for identification of proteins by position
  - After photo and image analysis
  - Align image to reference
- Various databases of 2D-Gels



1 HSP86
2 HSP70
3 ATP:Guanidino Kinase
4 Adenylate Dehydrogenase
5 Calreticulin
6 Actin
7 Enolase
8 Tropomyosin
9 Serpin-like
10 Phosphoglycerate kinase

11 p40
12 Aldolase
13 GAPDH
14 14-3-3 e
15 GST28
16 Triose Phosphate Isomerase
17 Elongation Factor 1a
18 14-3-3 homolog 1
19 GST26
20 Calpain

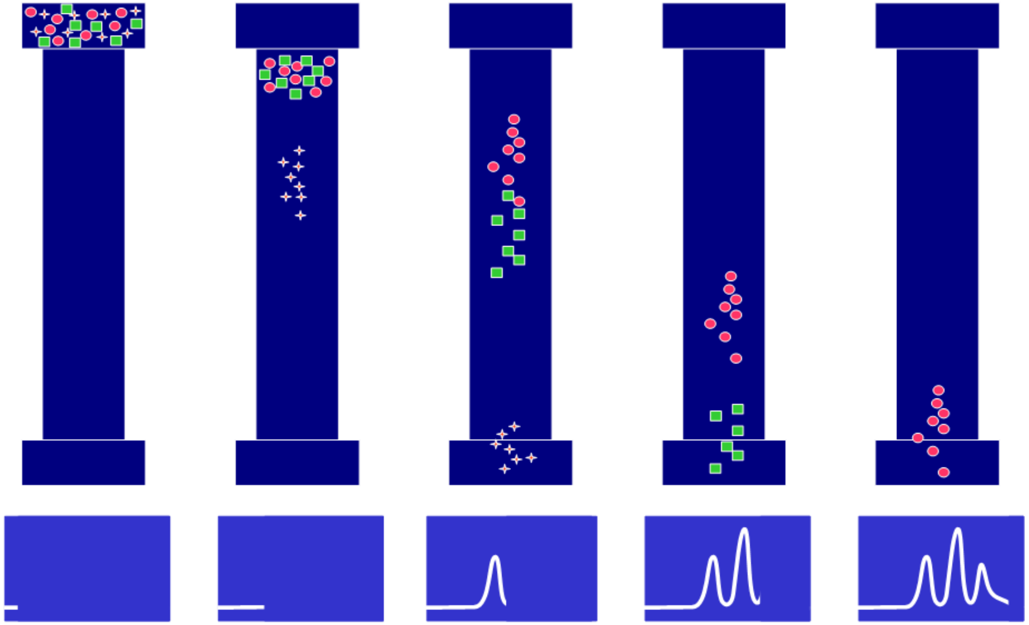
21 Myosin Light Chain
22 Cycophilin
23 Superoxide Dismutase
24 Fatty Acid Binding Protein (Sm14)
25 SME16
26 Thioredoxin
27 Dynein Light Chain
28 Ubiquitin
29 Adenylate Kinase

Pro / Contra

- Comparably simple and cheap
- Disadvantages
  - No high-throughput – much manual work
  - No robust quantification (spot intensity depends on staining)
  - Similar proteins (e.g. protein forms) build overlapping spots
  - Many restrictions
    - No proteins with <20KD or >200KD
    - No highly charged proteins
    - No detection of low concentrations
    - No membrane proteins (depending on method)
    - ...
  - No de-novo protein identification
  - Limited accuracy in comparative identification

Liquide / Gas Chromatography

- Other option: GC/LC
  - Chamber contains two phases (liquid / liquid, liquid/gas)
  - Different speeds depending on mass/charge ratio
  - Separation by retention times



This Lecture

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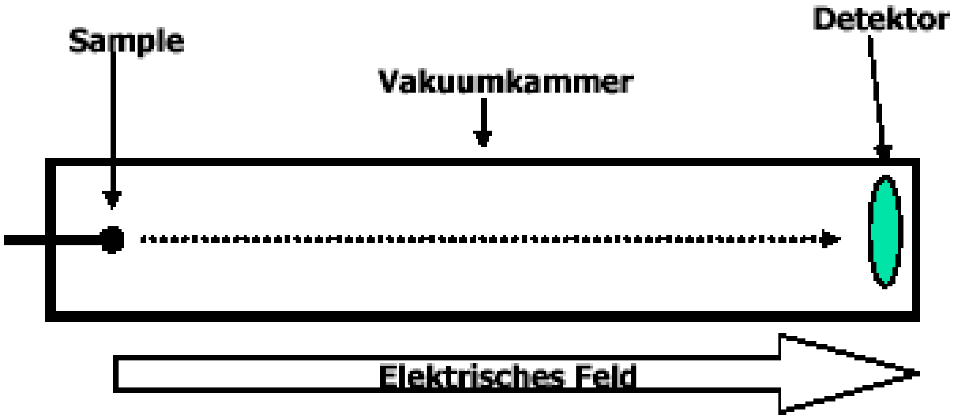
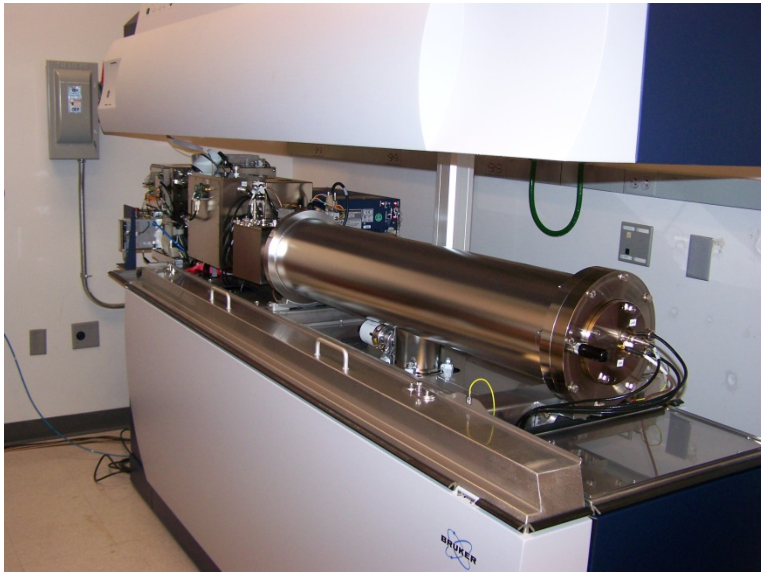
- Identification: Mass Spectrometry
  - Method
  - Algorithms: Naïve, probabilistic

Mass Spectrometry

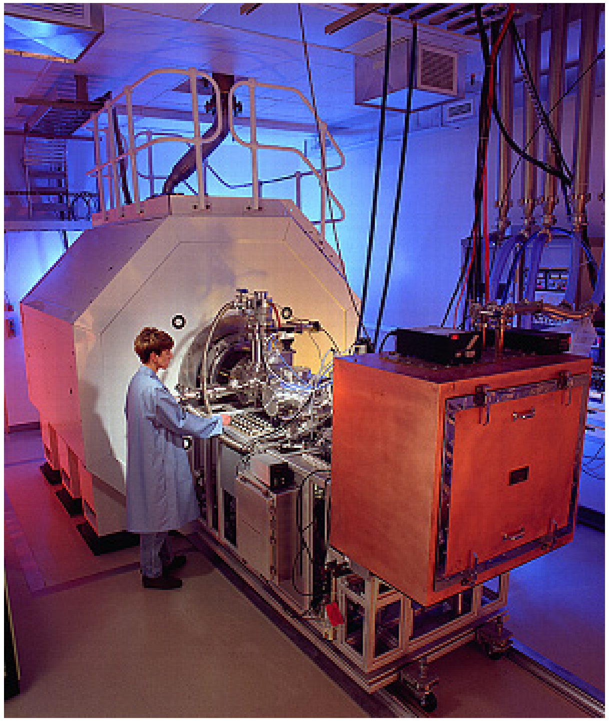
- Accelerate particles (must be charged) in an electric field
- Detector measures hits at back wall
- Time of flight (ToF) proportional to mass
  - Other techniques exist (magnetic drift, ...)
- Spectrum of mass peaks is used to identify particle



Mass Spectrometry



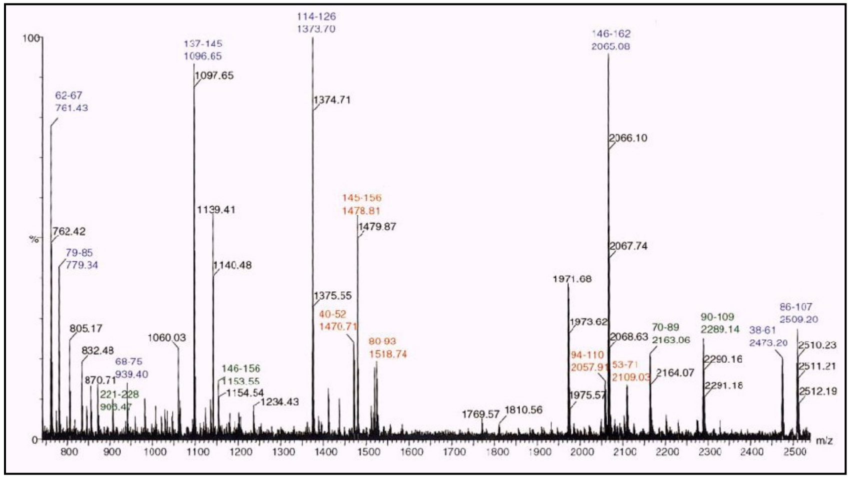
Source: http://imr.osu.edu



Source: http://www.sysbio.org

MS for Protein Identification

- Problem: Proteins are fragile and break during acceleration
- Solution
  - Break proteins at defined points before acceleration (digestion)
  - Measure peptides (each peptide one signal – time of flight)
  - Identify protein based on spectrum of peptide hits
- In theory, every protein has an almost unique spectrum
  - Using modern MS/MS, even different forms of the same protein are separable



Digestion

Trypsin:
Cleaves after Arginine und Lysine if next AA is not Proline

N-Asp-Ala-Gly-Arg-His-Cys-Lys-Pro-Lys-Ser-Glu-Asn-Leu-Ile-Arg-Thr-Tyr-C

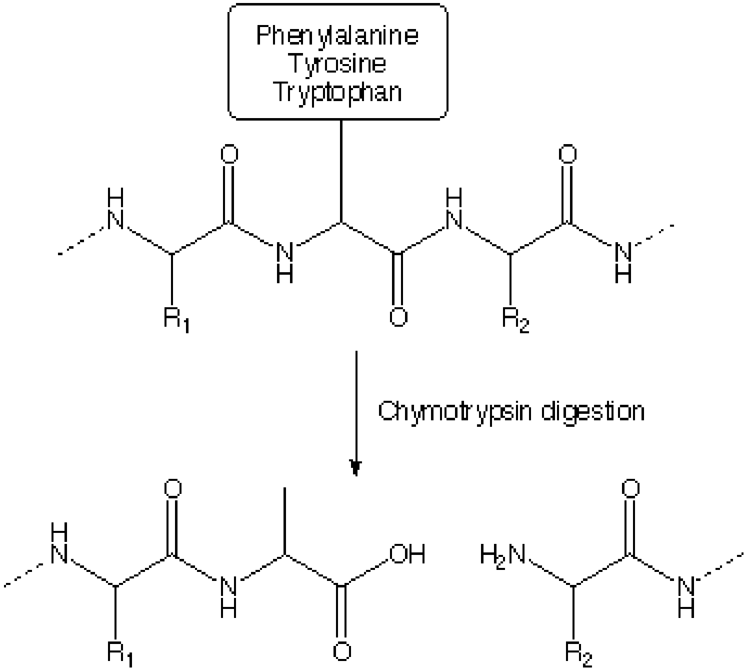
↓ *Trypsin*

N-Asp-Ala-Gly-Arg

Ser-Glu-Asn-Leu-Ile-Arg

His-Cys-LysPro -Lys

Thr-Tyr-C



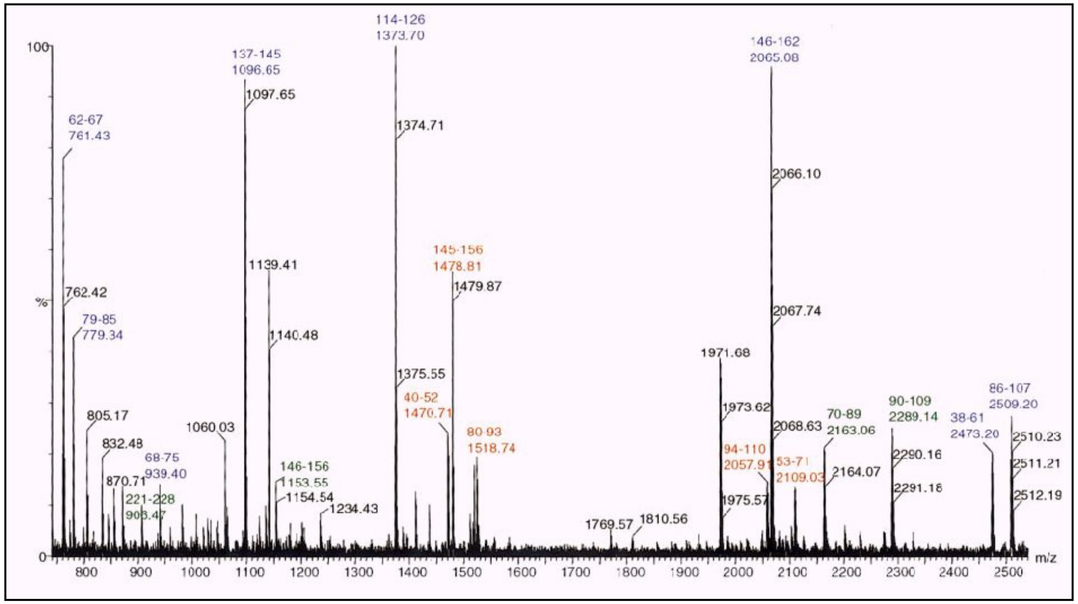
Chymotrypsin:
After Tyr, Trp, Phe, Met

Ionization

- Problem: Peptides often are uncharged – no acceleration
- Solution
  - MALDI – Matrix Assisted Laser Desorption / Ionization
  - Peptide are embedded in a „matrix“
    - Crystallization with charged, light-sensitive molecules
  - Fire on crystal with laser
  - Light-sensitive molecules vaporize and carry peptides with them
  - Accelerate
- Other techniques known
  - E.g. ESI: electrospray ionization

From Spectra to Peaks

- Detecting peaks and assigning them to peptides is difficult
  - Technical bias in runs / machines
  - Inaccuracies of measurement
  - Inhomogeneous sample preparation
    - Matrix etc.
  - Different quantities of peptides



- Creating a spectrum: Signal processing (not covered here)
  - Peak detection, peak disambiguation, noise filtering, ...

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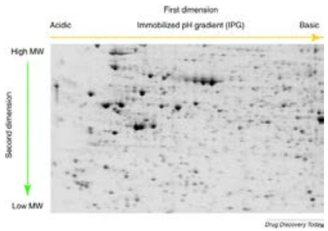
  - Method
  - Algorithms: Naïve, probabilistic

Algorithms for Protein Identification from Spectra

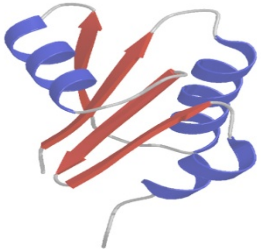
- We focus on database-based identification
- Idea
  - We have a database D of protein sequences d_1, d_2, ...
    - Each d_i is subjected to electronic digestion – peptide set / protein
    - For each peptide, we know its theoretical ToF
    - Compute a theoretical spectrum s_i for each d_i
  - Measure real spectrum s of unknown protein k
  - Compare empirical spectrum s with all theoretical spectra s_i
- We can only find what we already know

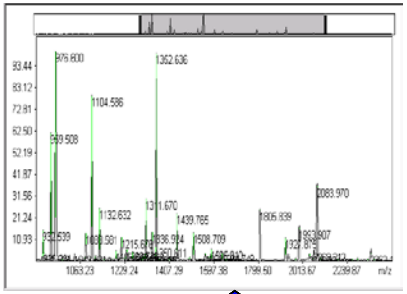
Illustration

Real experiment



Drug Discovery Today



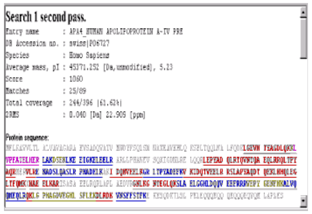


Comparison

Theoretical experiment

```
ttctcatcaaagaaatcaaagggaagc
ttattaccgagtggagttcaaggtcttgg
caaaaggtttccaaagaggatcatgttg
tcaaaggattggaatccatttcttctaag
aaacaattcgaggatcttgctggatcta
```

```
ANSHTDDLDELLDSALDDFKDLNLSHQ
QGLGMGLPDMRSKKRGKQKVSKEDHV
LPASDDDGMVEDFLKQFEDLAGSKDLE
RYPKWLKENEASLSEDYKRYSQQYKLI
ECGQPPSDIVKEIDPGFDFASLGQISPEM
```

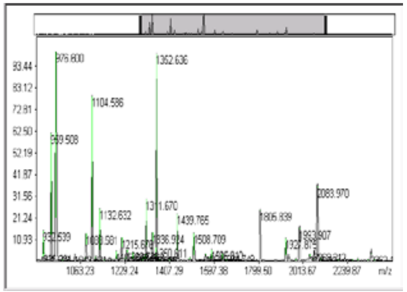


Search 1 second pass.

```
Entry name        : APA4_HUMAN APOLIPOPROTEIN A-IV PRE
DB Accession no.  : swiss|P06727
Species           : Homo Sapiens
Average mass, pI  : 45371.252 [Da,unmodified], 5.23
Score             : 1060
Matches           : 25/89
Total coverage    : 244/396 [61.62%]
2RMS              : 0.040 [Da] 22.909 [ppm]
```

Protein sequence:

```
MFLKAVVLTL ALVAVAGARA EVSADQVATV MWDYFSQLSN NAKEAVEHLQ KSELTQQLNA LFQDKLGEVN TYAGDLQKKL
VPFATELHER LAKDSEKLKE EIGKELEELR ARLLPHANEV SQKIGDNLRE LQQRLEPYAD QLRTQVNTQA EQLRRQLTPY
AQRMERVLRE NADSLQASLR PHADELKAKI DQNVEELKGR LTPYADEFKV KIDQTVEELR RSLAPYAQDT QEKLNHQLEG
LTFQMKKNAE ELKARISASA EELRQRLAPL AEDVRGNLKG NTEGLQKSLA ELGGHLDQQV EEFRRRVEPY GENFNKALVQ
QMEQLRQKLG PHAGDVEGHL SFLEKDLRDK VNSFFSTFKE KESQDKTLSL PELEQQQEQQ QEQQQEQVQM LAPLES
```



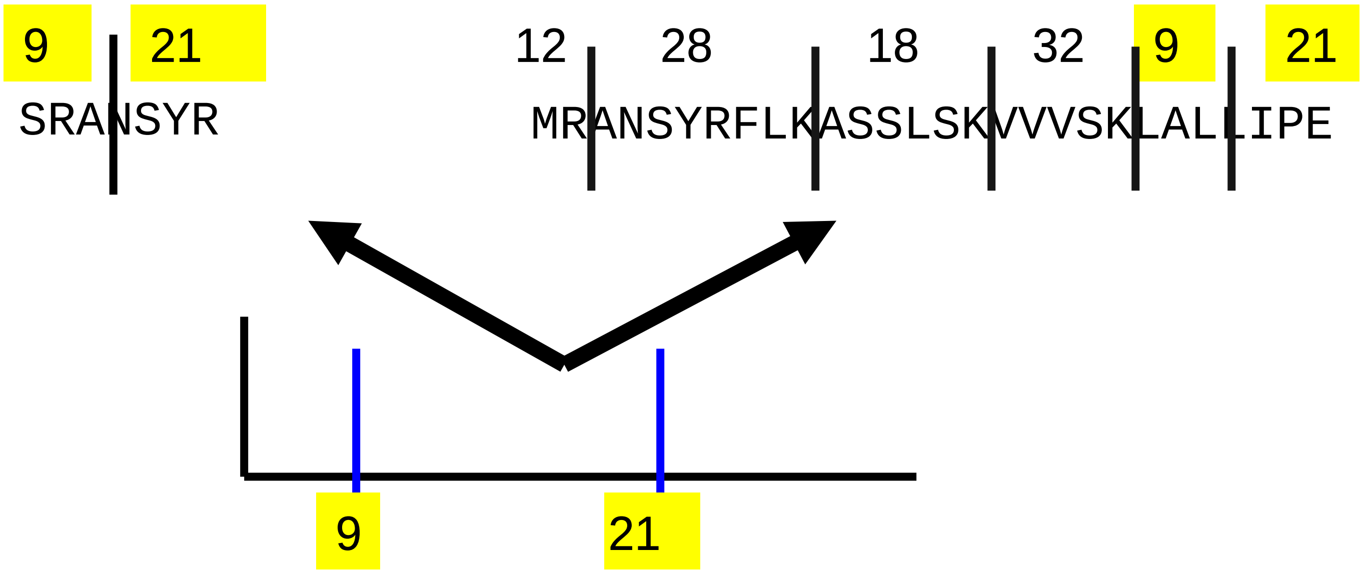
Naive Algorithm: Hitcount

- Compare measured s with all s_i in DB
- Protein d_i which has the most peaks in common wins
  - Input: $s=\{p_1,...p_m\}$, $s_i=\{q_1,...,q_j\}$
  - For each s_i: Compute $|s \cap s_i|$
  - Protein d_i where s_i has maximal overlap wins
- Complexity?
  - Keep peak lists s and s_i sorted
  - We need to compare |s| hits with |D| proteins in DB
  - Let q be the average number of peaks in a database spectrum
  - Together: $\sim(|s|+q)*|D|$ comparisons
  - Can be sped-up further (indexing)

Why “Naïve”?

- Peptide masses are not really equal (e.g. isotopes)
  - Small deviation – nearest peak; match might not be unique
- Some (short) peptides are more frequent than others
  - Some peptides appear in almost all proteins – little signal
  - Should have a lower impact
- Proteins have different lengths
  - Longer proteins have a higher a-priori chance for more peak matches

Example



- Which one would you prefer?

More Problems

- Enzymes don‘t work 100% correct
- Protein sequences in DB contain errors
  - Especially when directly translated from genome
  - Leads to theoretical spectra not existing in nature
- Posttranslational modifications
- MS is not perfect – spurious, shifted, missing peaks

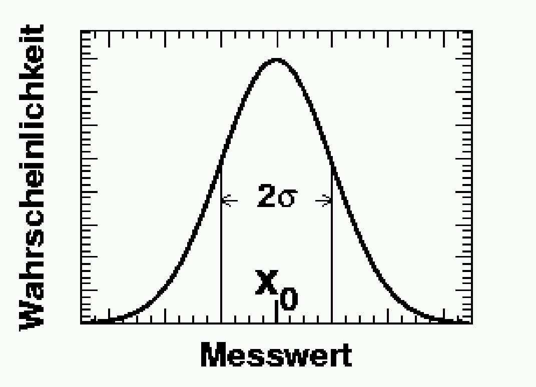
- All these issues lead to false positive and false negative peaks within the spectra
- Some protein always has the highest count – what if real sequence is not in the database?
  - No confidence scores

Practically Relevant Algorithms

- Heuristic: MOWSE (outdated)
  - Considers total protein mass and peptide frequencies
  - Generates a score
- Probabilistic algorithm: Profound
  - Copes with measurement errors, deviation in protein mass, and different peptide frequencies
  - Generates a probability of match for each protein (~ confidence)
- Many more (and newer) algorithms
  - MASCOT, PeptIdent, ProteinProspector, SEQAN, ...

Example of a Probabilistic Method: ProFound [ZC00]

- Computes, for a given spectrum D (s) and each protein k (s_i), the probability that D was produced by k
- The formula is complex; its derivation is even more complex and skipped
- Basic assumption: Measured peptide masses are normally distributed around the "canonical" value
  - Most probable isotope composition



- First step: Assign peaks from k to closest peak from D
  - A-priori assignment is a strong first filter; errors are propagated

ProFound Formula

$$\boxed{P(k|DI) \propto P(k|I)\,\frac{(N-r)!}{N!}\prod_{i=1}^{r}\left\{\sqrt{\frac{2}{\pi}}\,\frac{m_{\max}-m_{\min}}{\sigma_i}\times \sum_{j=1}^{g_i}\exp\left[-\frac{(m_i-m_{ij0})^2}{2\sigma_i^{\,2}}\right]\Bigg]\right\}F_{\text{pattern}}}$$

Legend

$$P(k|DI) \propto P(k|I) \frac{(N-r)!}{N!} \prod_{i=1}^{r} \left\{ \sqrt{\frac{2}{\pi}} \frac{m_{\max} - m_{\min}}{\sigma_i} \times \sum_{j=1}^{g_i} \exp\left[-\frac{(m_i - m_{ij0})^2}{2\sigma_i^2} \right] \right\} F_{\text{pattern}}$$

- p(k|D,I) = prob. that protein k was observed by spectrum D given the background information I
- p(k|I): A-priori probability of k in the given species / cell / tissue
- N: Predicted number of peptides of database protein k
- r: Number of hits between D and k (results from initial assignment)
- m_{max}, m_{min} – range of observed masses for current peak (background)
- σ_i – standard deviation of current peak (background)
- g_i: How often is the i'th peptide contained in k?
- m_i: Mean mass of the DB peak (background)
- m_{ij0}: Empirical mass of j'th occurrence of this peptide
- $F_{pattern}$: Heuristic factor dealing with "overlapping peaks"

ProFound Explanation

$$P(k|DI) \propto P(k|I)\frac{(N-r)!}{N!}\prod_{i=1}^{r}\left\{\sqrt{\frac{2}{\pi}}\frac{m_{\max}-m_{\min}}{\sigma_i}\times \sum_{j=1}^{g_i}\exp\left[-\frac{(m_i-m_{ij0})^2}{2\sigma_i^{\,2}}\right]\Big]\right\}F_{\text{pattern}}$$

- How many of the expected peptides for k did we observe?
- Multiply probabilities of all hits
- “Freedom” of measurements of hits for this peptide
- Many predicted peaks may create only one measured peak
- Probability of the deviation of the canonical mass to the measured mass (assuming normal distribution)

ProFound Intuition

$$P(k|DI) \propto P(k|I)\frac{(N-r)!}{N!}\prod_{i=1}^{r}\left\{\sqrt{\frac{2}{\pi}}\frac{m_{\max}-m_{\min}}{\sigma_i}\times \sum_{j=1}^{g_i}\exp\left[-\frac{(m_i-m_{ij0})^2}{2\sigma_i^{\,2}}\right]\right\}F_{\text{pattern}}$$

- Many hits (r ~ N) – score goes down (outweighs influence of more factors in the red product)
- Hits with a small stddev or a broad range – score goes up
- Many observed peaks match the predicted peaks – score goes up
- Observed peaks close to canonical peaks – score goes up
- Theoretical peak as high stddev – scores go down (also green)

Critique

- Score assumes that protein is in the database
  - Better: formulate „null“ hypothesis, compute prob. of the spectrum given the null hypothesis, and report the log-odds ratio as score
  - But this is not as simple done as said
- Assumes that every peak comes from “the” protein
  - But measurements might be contaminated with peptides from other proteins
- Assumes that observed peaks can be assigned clearly to predicted peaks
  - This problem is tried to be covered by $F_{pattern}$

Further Reading

- Basics on proteomics: Every Bioinformatics book
- Zhang, W. and Chait, B. T. (2000). "ProFound: an expert system for protein identification using mass spectrometric peptide mapping information." *Anal Chem 72(11): 2482-9.*
- Pappin, D. J. C., Hojrup, P. and Bleasby, A. J. (1993). "Rapid identification of proteins by peptide-mass fingerprinting." *Current Biology* ***3(327-332).***
- Survey: Colinge J, Bennett KL (2007) Introduction to Computational Proteomics. PLoS Comput Biol 3(7): e114