Bioinformatics 2

Lecture 2

Proteomics

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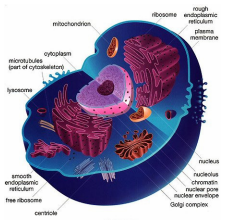
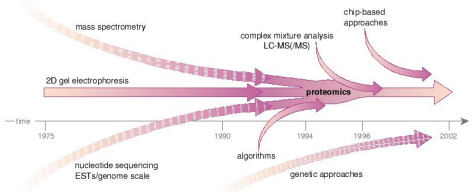
Key questions of proteomics

- **What proteins are there**?
- **How much** is there of each of the proteins?
  - Absolute quantitation
  - Stoichiometry
- What (modification/splice) **state** are the proteins in?
- Which proteins **interact** with each other or with other molecules (DNA, RNA)?
- How does all of the above **change** with time/stimulation/mutation of a key protein/… ?



Foundation of proteomics

- **M**ass spectrometry
- **A**lgorithms
- **D**NA sequencing

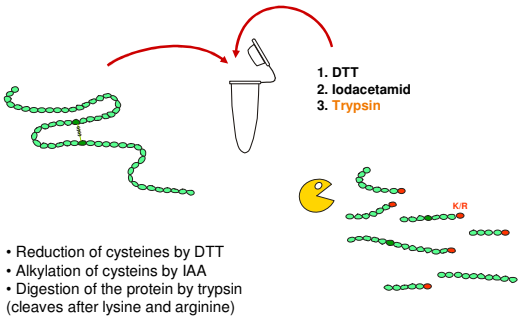


What proteins are there?

Protein identification is achieved by

- Proteolysis of the proteins into peptides
- Mass spectrometric detection of the peptides
(shortcut to protein identification: peptide mass fingerprinting)
- Mass spectrometric fragmentation of the peptides
- Database search to identify the peptides

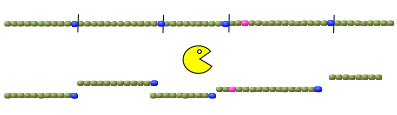
Protein digestion



- Reduction of cysteines by DTT
- Alkylation of cysteins by IAA
- Digestion of the protein by trypsin
(cleaves after lysine and arginine)

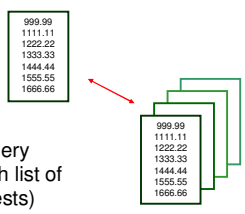
Peptide mass fingerprinting

Isolated protein



Digestion

MALDI MS

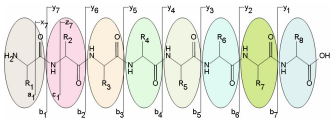


Database Query
(compare with list of 'in-silico' digests)

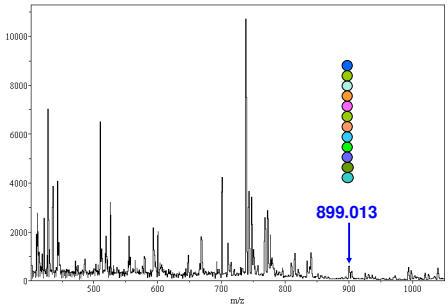
Peptide Fragmentation

(Low-Energy Collision induced fragmentation)

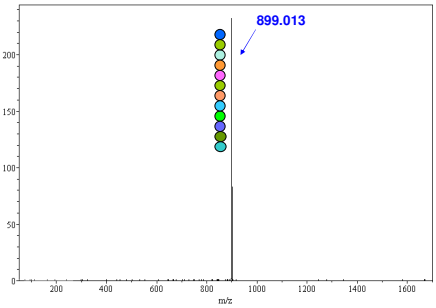
- Peptides fragment preferentially between amino acids
- The chemical bond that cleaves depends on the fragmentation method.
- Low-Energy Collision Induced Dissociation (CID) is most common. Leads to b and y ions
- Electron Transfer Dissociation (ETD) is up and coming. Leads to c and z ions.



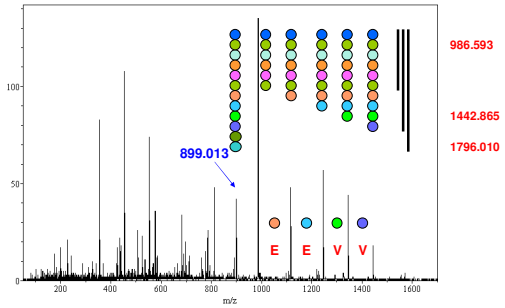
MS of a Peptide Mixture



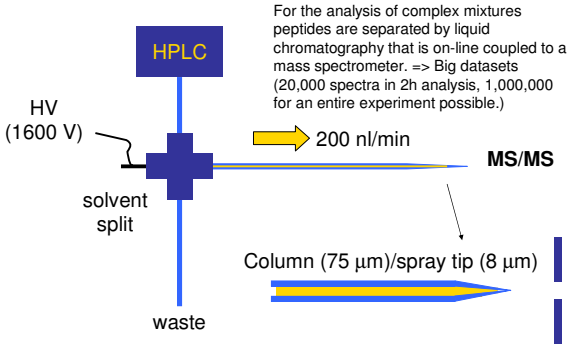
MS/MS of a Peptide (low collision energy)



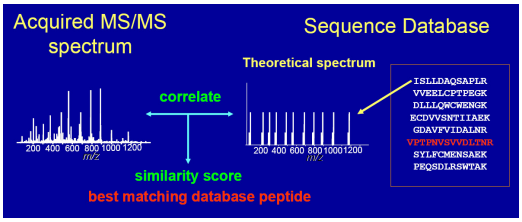
MS/MS of a Peptide (high collision energy)



LC-MS interface

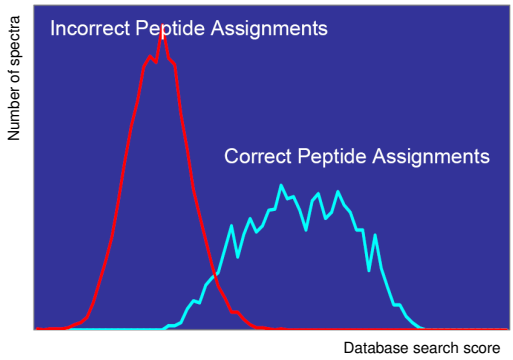


For the analysis of complex mixtures peptides are separated by liquid chromatography that is on-line coupled to a mass spectrometer. => Big datasets (20,000 spectra in 2h analysis, 1,000,000 for an entire experiment possible.)

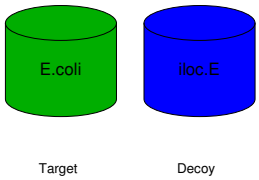


- Many programs available for this matching of fragmentation spectra with peptide sequences from databases (Mascot, Sequest, OMSSA, XTandem!)
- Each program has its own score.
- None of the scores is truly statistical.
- Results for the same dataset vary (overlap between any two ca. 50-60%).

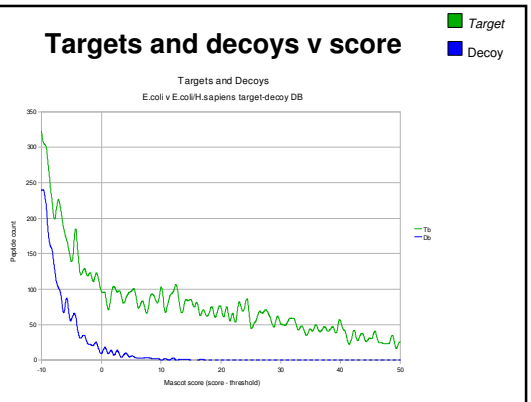
How to find the rate of incorrect assignments => confidence?



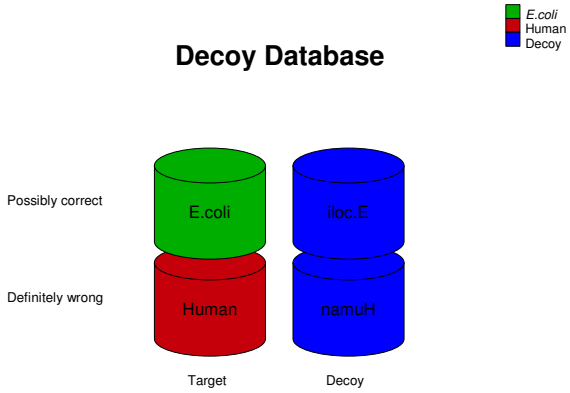
Decoy Database



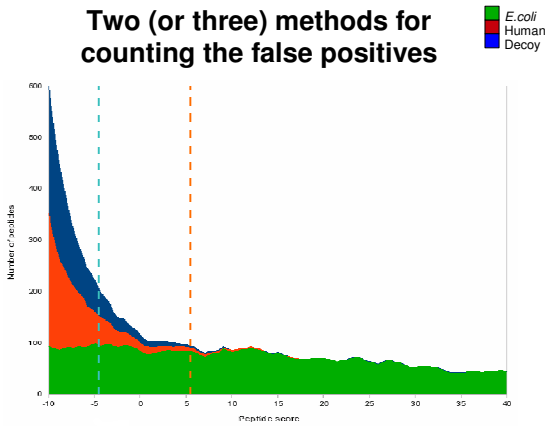
Targets and decoys v score



Decoy Database



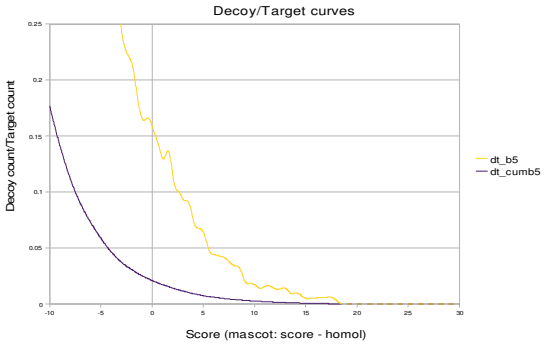
Two (or three) methods for counting the false positives



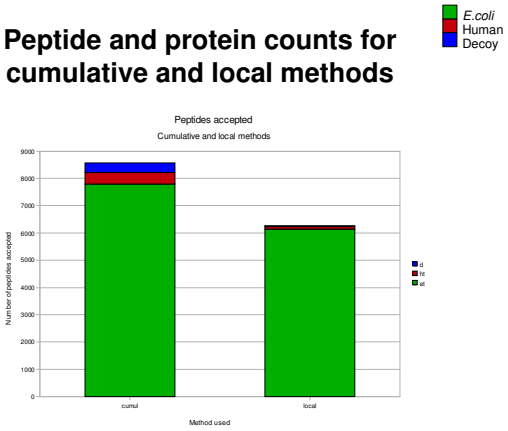
Decoy calculation methods

- Locally (within a local window)
  - Decoy count / Target count
- Globally (everything above the score)
  - Decoy count / Target count
  - Decoy count x 2 / (Target count + Decoy count)

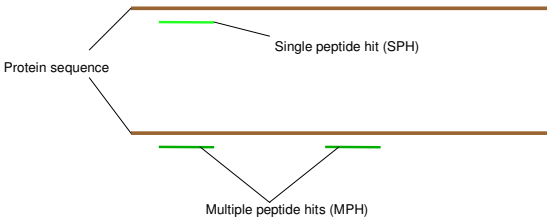
Cumulative and Local D/T v score



Peptide and protein counts for cumulative and local methods



Single and multiple peptide hits



Significance of SPH and MPH

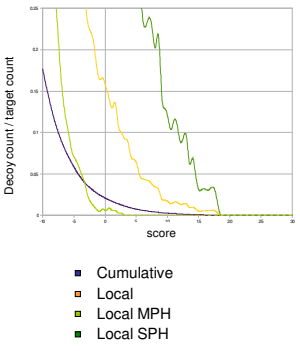
- MPHs confer additional corroboration to each-other
- SPH are often disregarded in practice
- What if we treat MPH and SPH separately?

Improved confidence by SPH/MPH

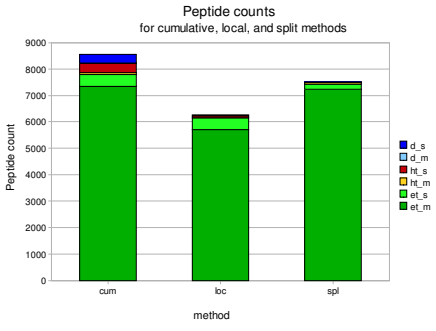
• MPH and SPH show very different curves.

• Local MPH cutoff is similar to cumulative cutoff.

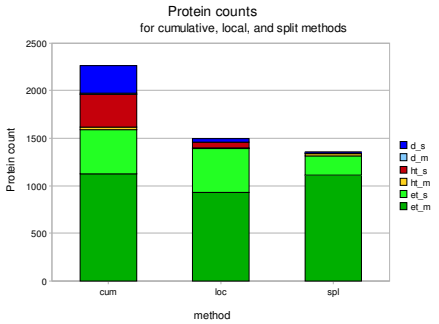
• Local SPH cutoff is much higher than cumulative cutoff => cumulative method overestimates confidence in SPH leading to high false discovery rates for SPH proteins and their rejection.



Final comparison: peptide counts

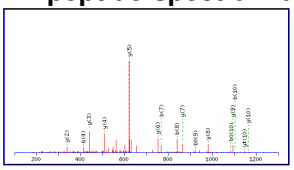


Final comparison: protein counts



With current approach (cum) proteins cannot be identified reliably with a single peptide. This is now possible using local split method (spl).

Non-statistical component of peptide-spectra matches



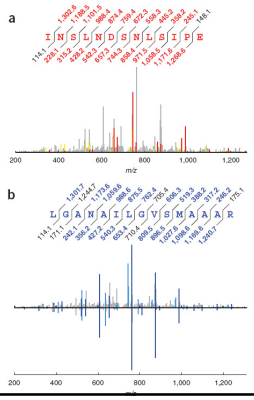
E.g.: Observed fragments do not scatter randomly among the calculated fragments.

Monoisotopic mass of neutral peptide Mr(calc): 1276.7027
Fixed modifications: Carbamidomethyl (C)
Ions Score: 64 **Expect:** 1.2e-05
Matches (Bold Red): 16/90 fragment ions using 30 most intense peaks

#	b	b^{++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y^{*}	y^{*++}	y^{0}	y^{0++}	#
1	114.0913	57.5493			L							11
2	185.1284	93.0679			A	1164.6259	582.8166	1147.5993	574.3033	1146.6153	573.8113	10
3	298.2125	149.6099			L	1093.5888	547.2980	1076.5622	538.7848	1075.5782	538.2927	9
4	413.2394	207.1234	395.2289	198.1181	D	980.5047	490.7560	963.4782	482.2427	962.4942	481.7507	8
5	526.3235	263.6654	508.3129	254.6601	L	865.4778	433.2425	848.4512	424.7293	847.4672	424.2372	7
6	655.3661	328.1867	637.3555	319.1814	F	752.3937	376.7005	735.3672	368.1872	734.3832	367.6952	6
7	768.4502	384.7287	750.4396	375.7234	I	623.3511	312.1792	606.3246	303.6659	605.3406	303.1739	5
8	839.4873	420.2473	821.4767	411.2420	A	510.2671	255.6372	493.2405	247.1239	492.2565	246.6319	4
9	940.5349	470.7711	922.5244	461.7658	T	439.2300	220.1186	422.2034	211.6053	421.2194	211.1133	3
10	1103.5983	552.3028	1085.5877	543.2975	Y	338.1823	169.5948	321.1557	161.0815			2
11					R	175.1190	88.0631	158.0924	79.5498			1

SVM approach

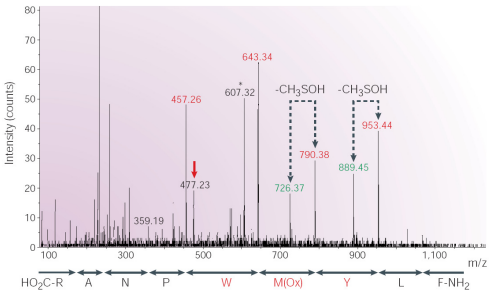
- Collect long list of features characterizing the peptide-spectrum match (this includes the score but also other parameters)
- Use decoy matches as false positives
- Train the SVM with each dataset new
- Gives significant improvement (20-400%) over search program alone or alternative procedures.



Käll L, Canterbury JD, Weston J, Noble WS, MacCoss MJ.
Semi-supervised learning for peptide identification from shotgun proteomics datasets.
Nat Methods. 2007 Nov;4(11):923-5. Epub 2007 Oct 21.

Modified peptides

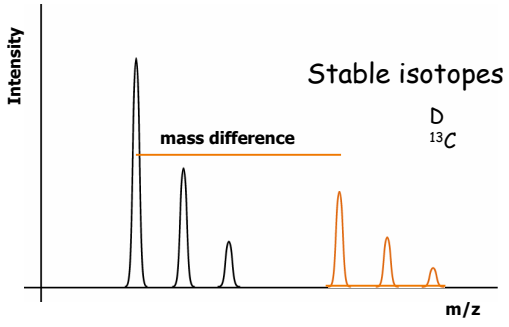
- Include modification as possibility in the database search
- For informatics the same problem as peptide identification



Steen H, Mann M. The ABC's (and XYZ's) of peptide sequencing. Nat Rev Mol Cell Biol. 2004 Sep;5(9):699-711. Review.

What does the peptide based analysis mean for identifying proteins?

Quantitation in MS

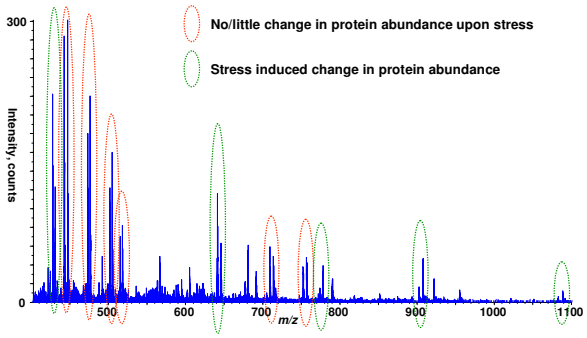


Quantitation in MS

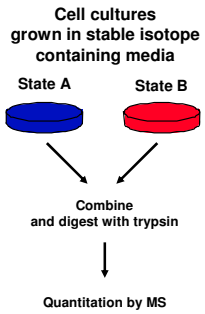
• Absolute quantitation possible by using a labelled peptide as reference standard.

• Differential analysis possible by labelling on sample and not labelling the other. Both can then be mixed and analyzed together.

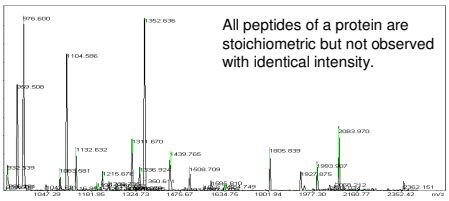
Analysis of proteins from stressed cells



In vivo labeling with *SILAC*



Stoichiometry



All peptides of a protein are stoichiometric but not observed with identical intensity.

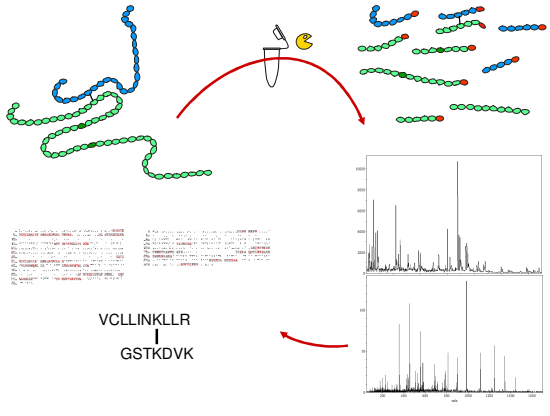
Intensity in mass spectrum not direct consequence of abundance but influenced by many molecule-specific factors
=> Apple-orange problem

Approximation possible by summing up the mass spectrometric evidence gathered for a protein and normalizing this by the expected volume of evidence
Example: number of observed peptides / number of observable peptides

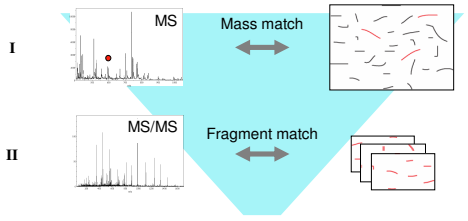
Protein-protein interactions

• Can be analyzed using same tools as for protein identification (mass spectrometry and database searching).

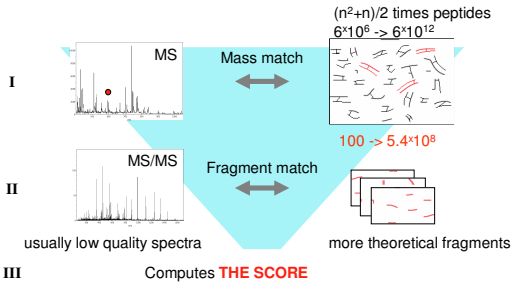
• Need to cross-link proteins to maintain their proximity also after proteolysis.



Normal peptide identification



~~Normal~~ peptide identification
Cross-linked



Maiolica A, Cittaro D, Borsotti D, Sennels L, Ciferri C, Tarricone C, Musacchio A, Rappsilber J.
Structural analysis of multiprotein complexes by cross-linking, mass spectrometry, and database searching.
Mol Cell Proteomics. 2007 Dec;6(12):2200-11. Epub 2007 Oct 5.