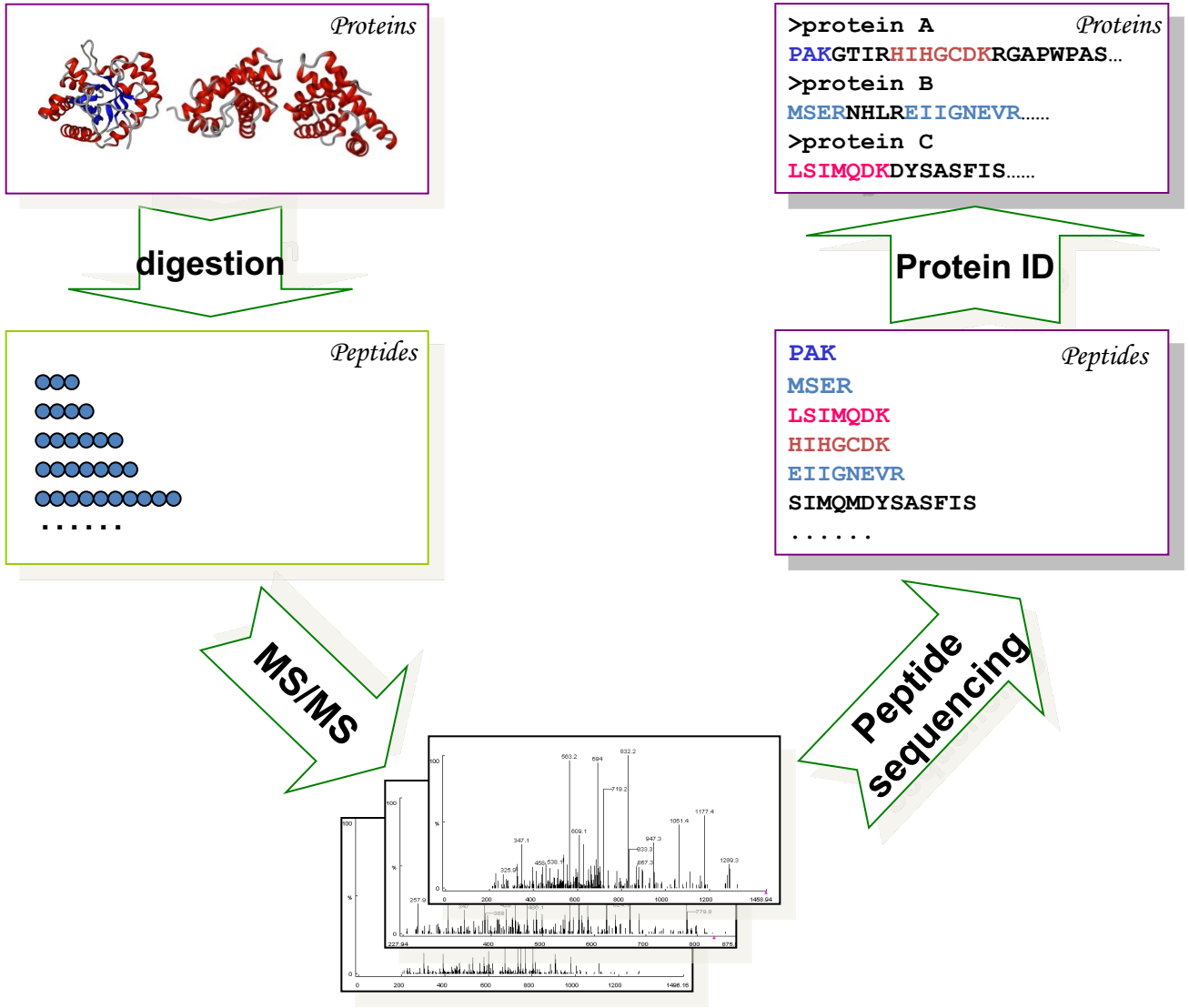
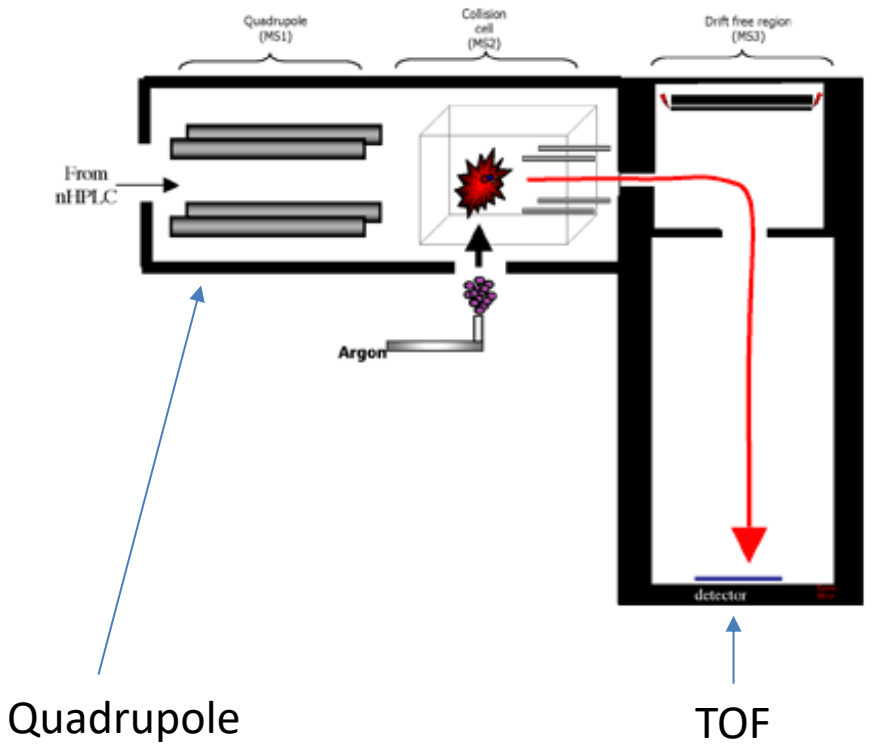
Database Search Method for Peptide Identification with MS/MS

Bottom Up Proteomics



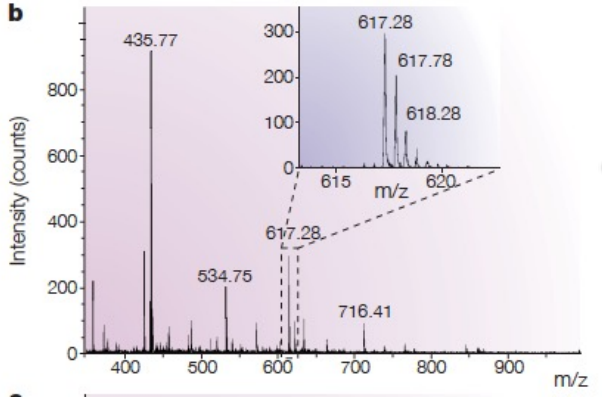
Tandem Mass Spectrometry

- Tandem MS combines different mass analyzers. E.g. Q-Tof.
- Quadrupole can run in either ion guide or ion filter modes.
- To measure the precursor ions
  - Quadrupole in ion guide mode
  - Collision off
- To measure the fragment ions of a precursor ion
  - Quadrupole to select the target m/z
  - Collision on

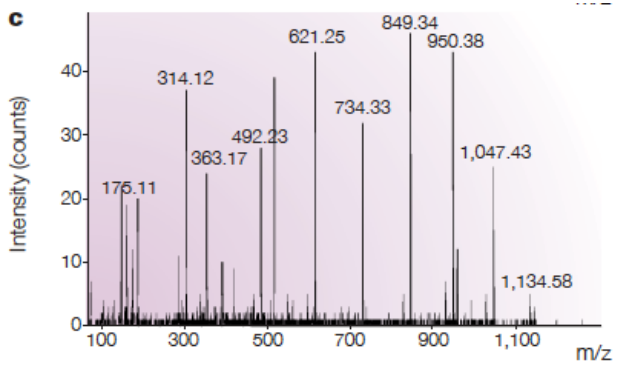


Tandem Mass Spectrometry Procedure

MS

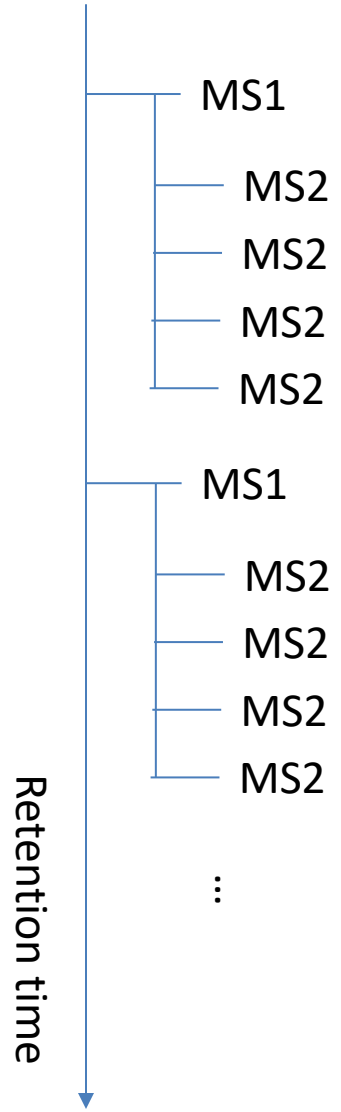


MS/MS

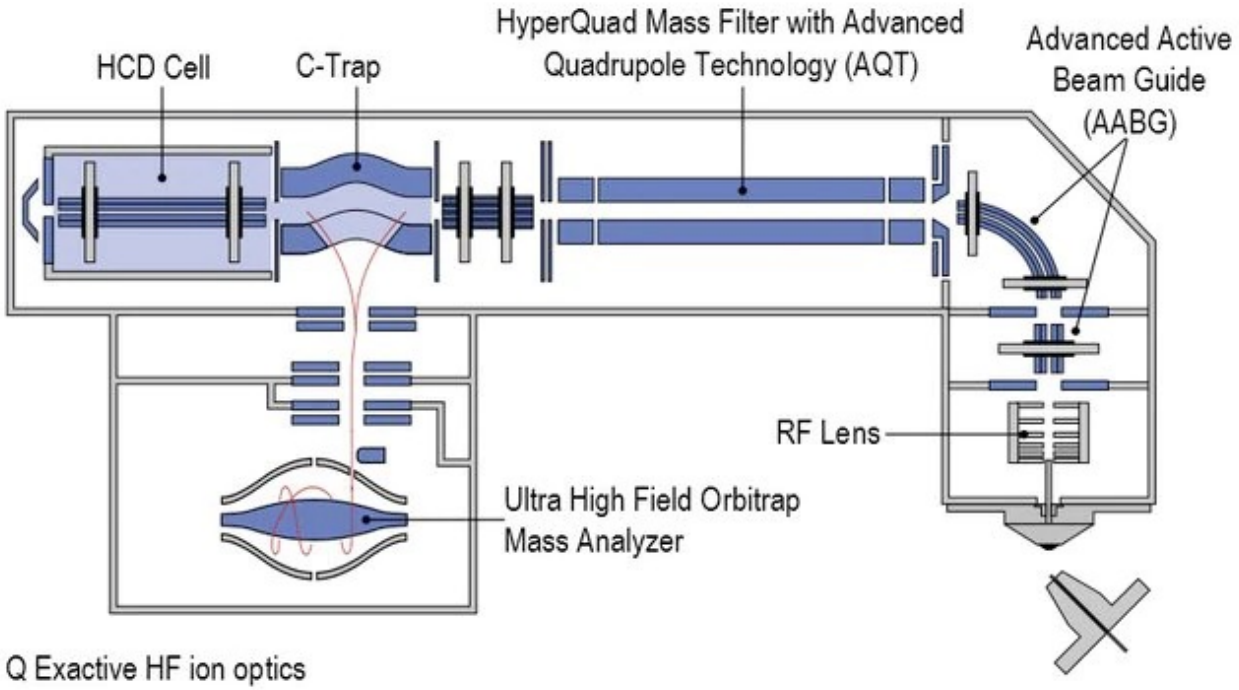


- Step 1. All precursor ions are measured to produce the survey scan.
- Step 2. A precursor ion is selected (by m/z) and fragmented. All fragment ions are measured to produce the tandem MS scan (also called as MS/MS or MS2 scan).
- Repeat Step 2 a few times. Then go back to Step 1.

Note: For each MS2 spectrum, we additional know the precursor m/z.

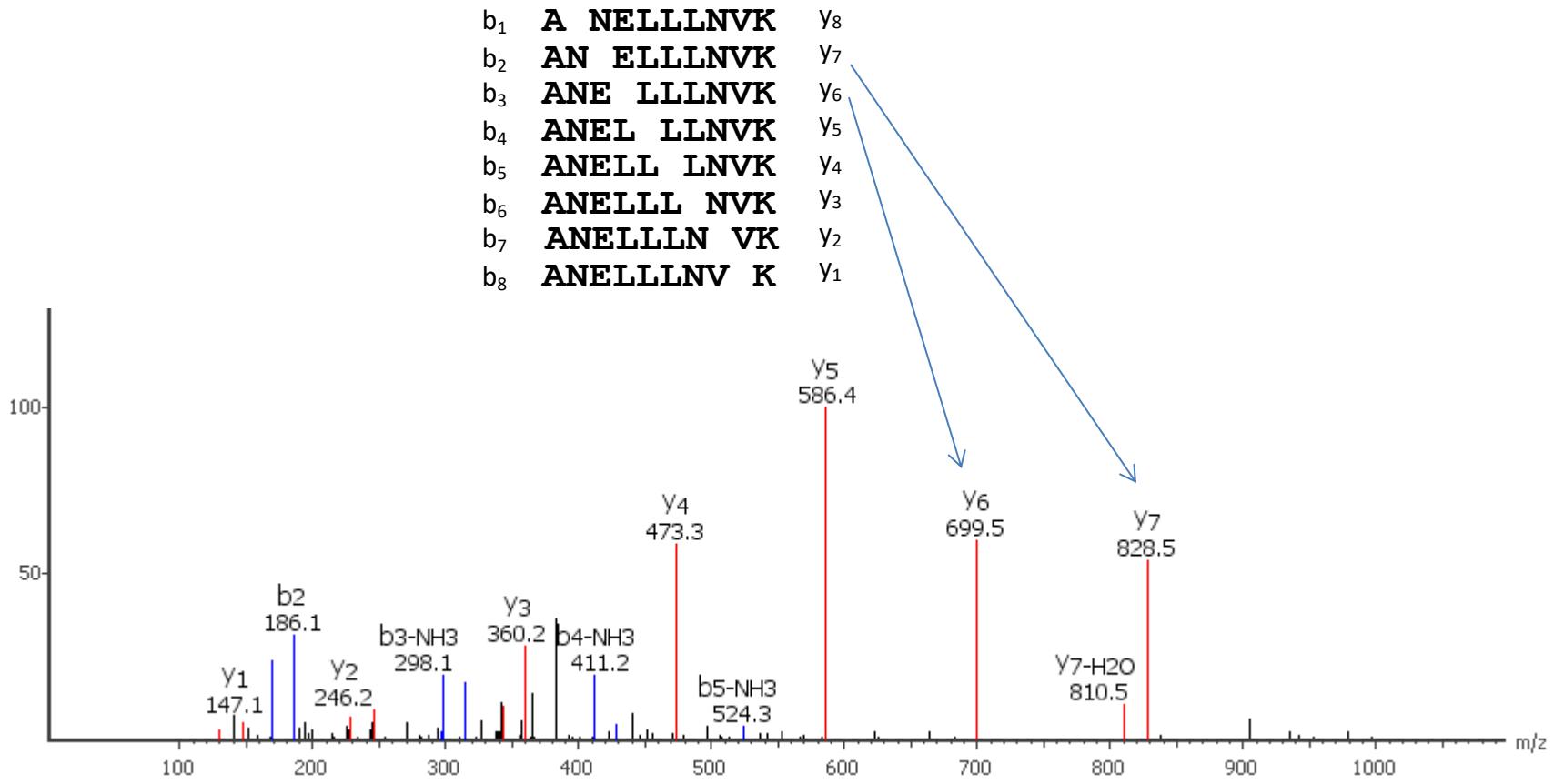


Orbitrap QE-HF



An actual instrument may consist many components for better sensitivity, accuracy, throughput and robustness. The figure illustrate the main components of an Orbitrap QE-HF instrument made by ThermoFisher Scientific.

Peptide-Spectrum Match



y-ion m/z = (total of amino acid residue mass + 18.011 + z * 1.007) / z

b-ion m/z = (total of amino acid residue mass + z * 1.007) / z

H2O

proton

charge state

Mass Error Tolerance

- Peak matching allows certain mass error tolerance (due to instrument measurement errors).
- Error can be specified either in Da or in ppm (part-per-million).
- ppm error = 1e6 * (observed mass – theoretical mass)/theoretical mass
- Different instrument has different error tolerance:
  - Low resolution: often 0.5-1 Da
  - High resolution: often 1-20 ppm
- Precursor ions and fragment ions often have slightly different error tolerances.

Search Through Database

>sp|P02769|ALBU_BOVIN Serum albumin OS=Bos taurus GN=ALB PE=1 SV=4
MKWVTFISLLLLFSSAYSRGVFRRDTHKSEIAHRFKDLGEEHFKGLVLIAFSQYLQQCPFDEH
VKLVNELTEFAKTCVADESHAGCEKSLHTLFGDELCKVASLRETYGDMADCCEKQEPERNEC
FLSHKDDSPDLPKLKPDPNTLCDEFKADEKKFWGKYLYEIARRHPYFYAPELLYYANKYNGVF
QECCQAEDKGACLLPKIETMREKVLASSARQRLRCASIQKFGERALKAWSVARLSQKFPKA
EFVEVTKLVTDLTKVHKECCHGDLLECADDRADLAKYICDNQDTISSKLKECCDKPLLEKSHC
IAEVEKDAIPENLPPLTADFAEDKDVCKNYQEAKDAFLGSFLYEYSRRHPEYAVSVLLRLAKEY
EATLEECCAKDDPHACYSTVFDKLKHLVDEPQNLIKQNCDQFEKLGEYGFQNALIVRYTRKV
PQVSTPTLVEVSRSLGKVGTRCCTKPESERMPCTEDYLSLILNRLCVLHEKTPVSEKVTKCCTE
SLVNRRPCFSALTPDETYVPKAFDEKLFTFHADICTLPDTEKQIKKQTALVELLKHKPKATEEQL
KTVMENFVAFVDKCCAADDKEACFAVEGPKLVVSTQTALA

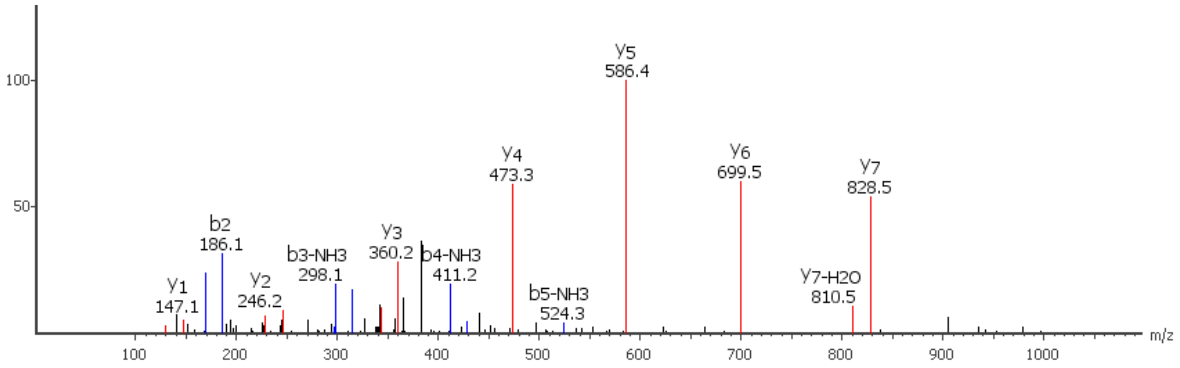
1. Use the enzyme digestion rule to cut each protein into peptides

MK|WVTFISLLLLFSSAYSR|GVFR|R|DTHK|SEIAHR|FK|DLGEEHFK|GLVLIAFSQYLQQCPFDEHVK|L

2. For each peptide, compare with the spectrum to see how well they match.

An Empirical Score

- y-ion m/z at charge one = total residue mass + 19.0178.
- Find approximate matching peak. Assume relative intensity = x.
- Relative intensity = current_peak_intensity / max_peak_intensity.
- Score contribution = $\max \begin{cases} \log_{10} 100 \cdot x \,, if\ x > 0.01 \\ 0, otherwise \end{cases}$
- Add up all score contributions of all y-ions.
- Better score functions will be discussed later.



Database Search

- Input:
  - A list of MS/MS spectra
  - A protein sequence database
- Algorithm 1:

```
For each MS/MS spectrum
    For each protein in the database
        In-silico digest the protein into peptides
        For each peptide
            Evaluate the peptide-spectrum match
Assign the highest-scoring peptide to the spectrum
```

Speed Optimization

- Algorithm 2:

  For each MS/MS spectrum

    For each protein in the database

      In-silico digest the protein into peptides

      For each peptide

        if (precursor mass error < allowed error tolerance)

          Evaluate the peptide-spectrum match

    Assign the highest-scoring peptide to the spectrum

  - Precursor mass error = | theoretical precursor mass – observed precursor mass |
  - The mass filtration reduces the number of PSM evaluation.

Speed Optimization

- Algorithm 3:

  Sort the spectra according to precursor mass.

  For each protein in the database

    In-silico digest the protein into peptides

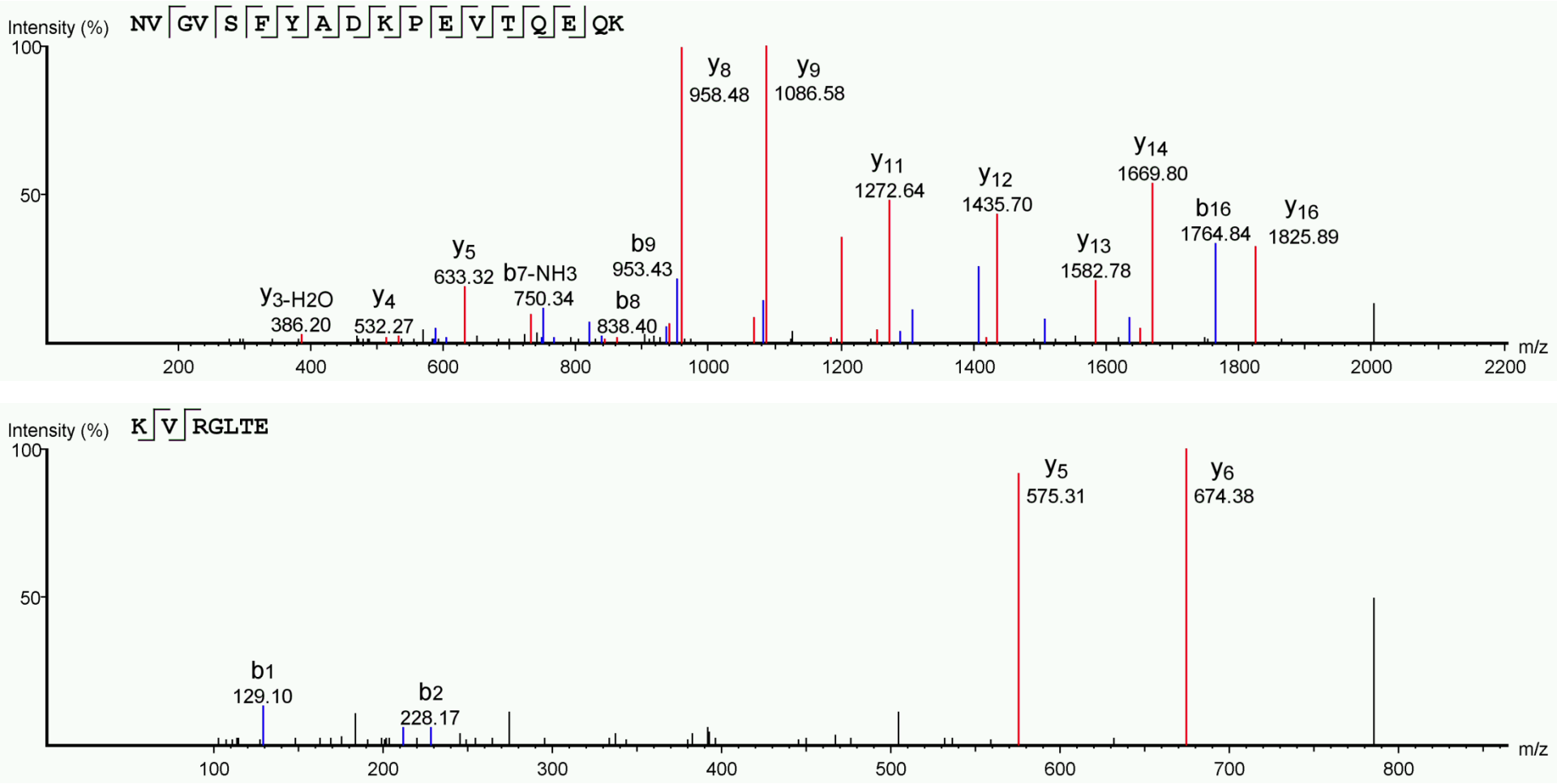
      For each peptide

        For each spectrum with matching precursor mass

          Evaluate the peptide-spectrum match

          Keep the highest scoring peptide for the spectrum

Result Validation

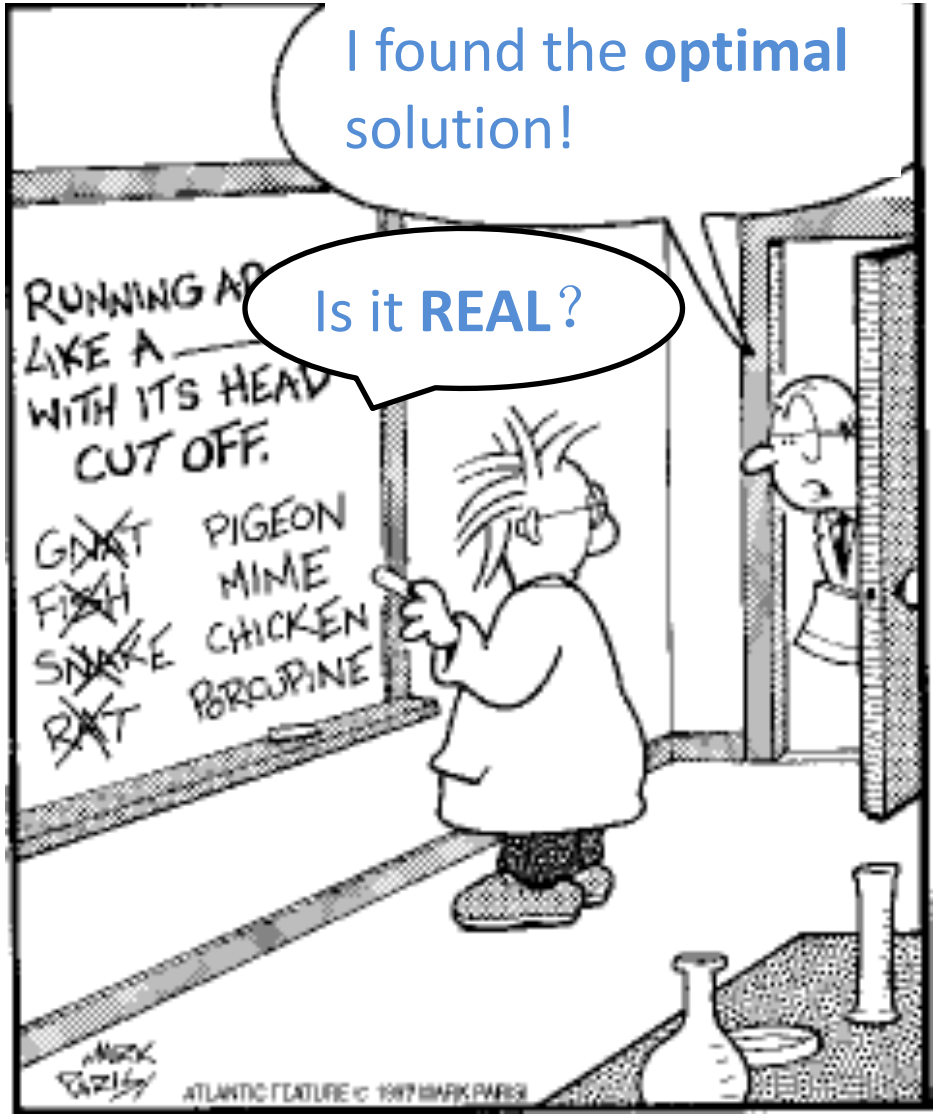


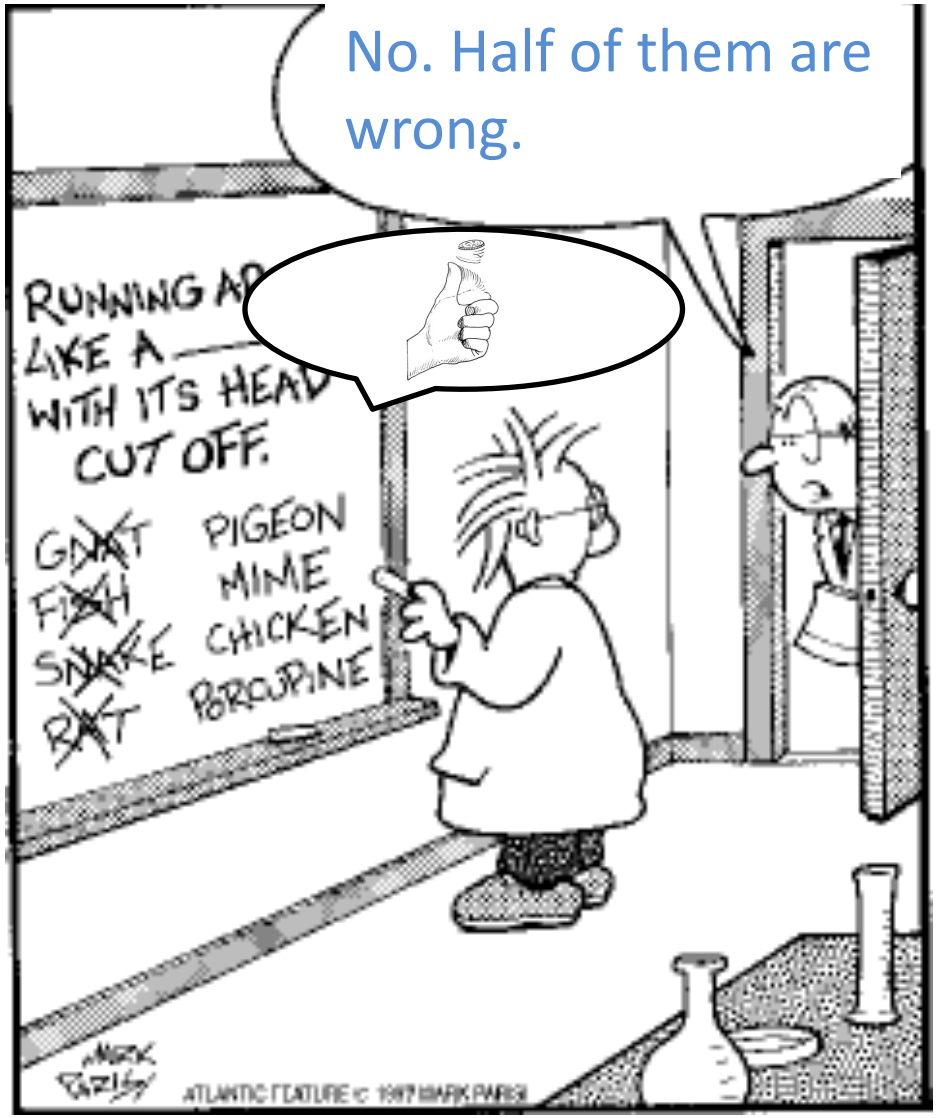
These two peptide-spectrum maches (PSMs) are all the best match from database for two different spectra. Their confidences are clearly different.

Result Validation

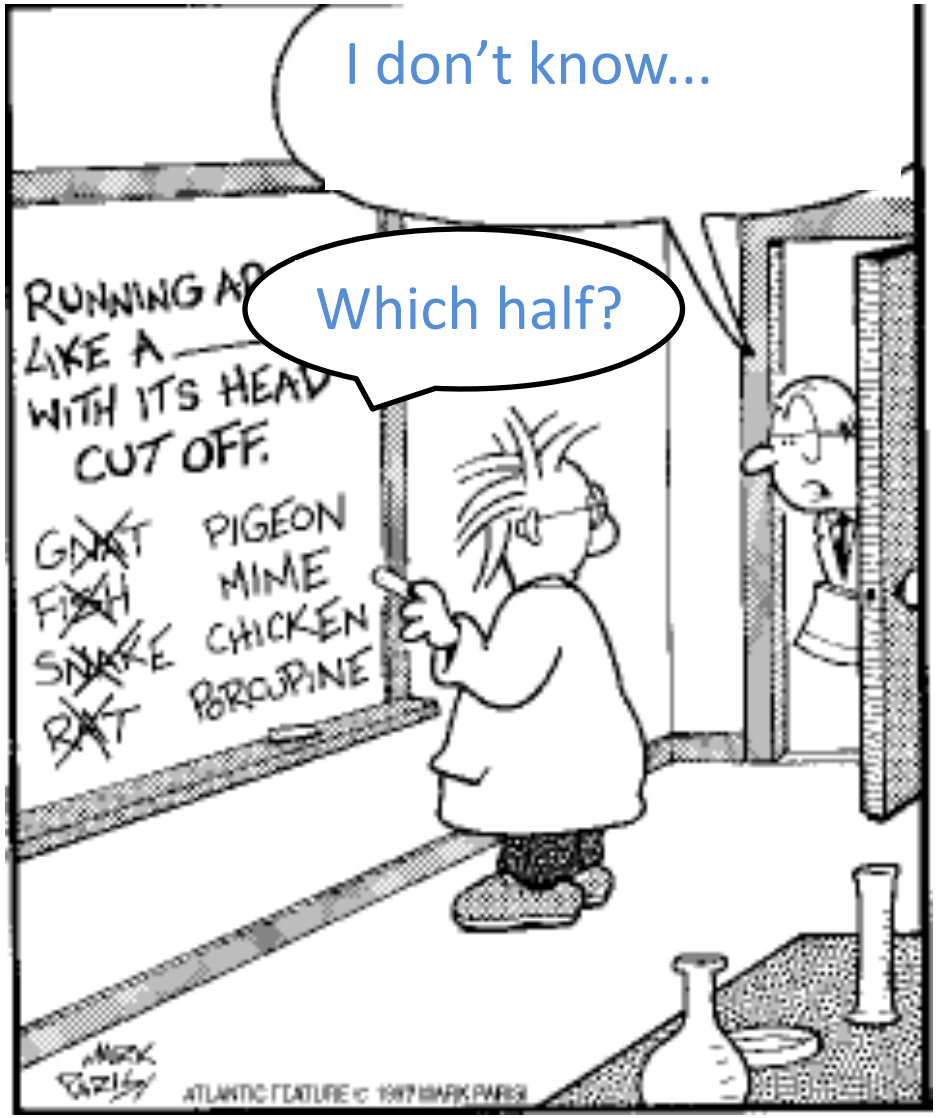
- Some spectra are of lower quality.
  - Peptides do not fragment well
  - Peptides fragment too much
  - Peptides do not get charged
  - Peptides are of low concentration
  - Etc.
- Some spectra's true peptides are not in database.
- Therefore, search results of some spectra are junk.
- Computer scientists may think these are not their problems. After all, they've reported the "optimal" peptide for each spectrum.

Biologists vs. Computer Scientists





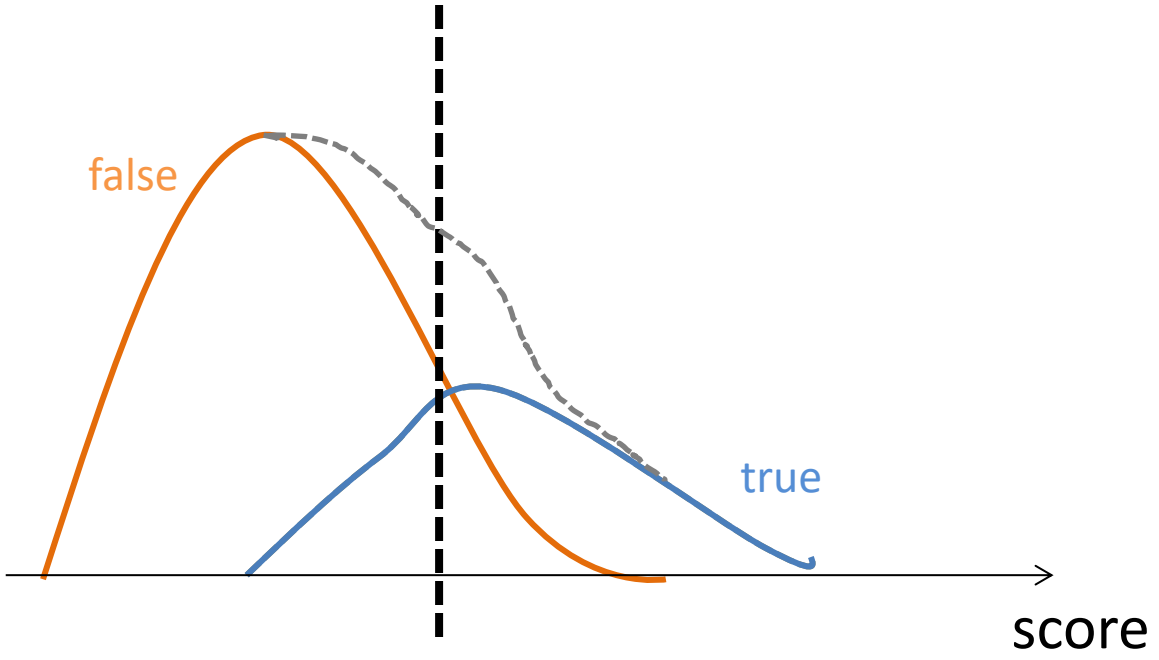
Biologists vs. Computer Scientists



Solution to Noisy Input

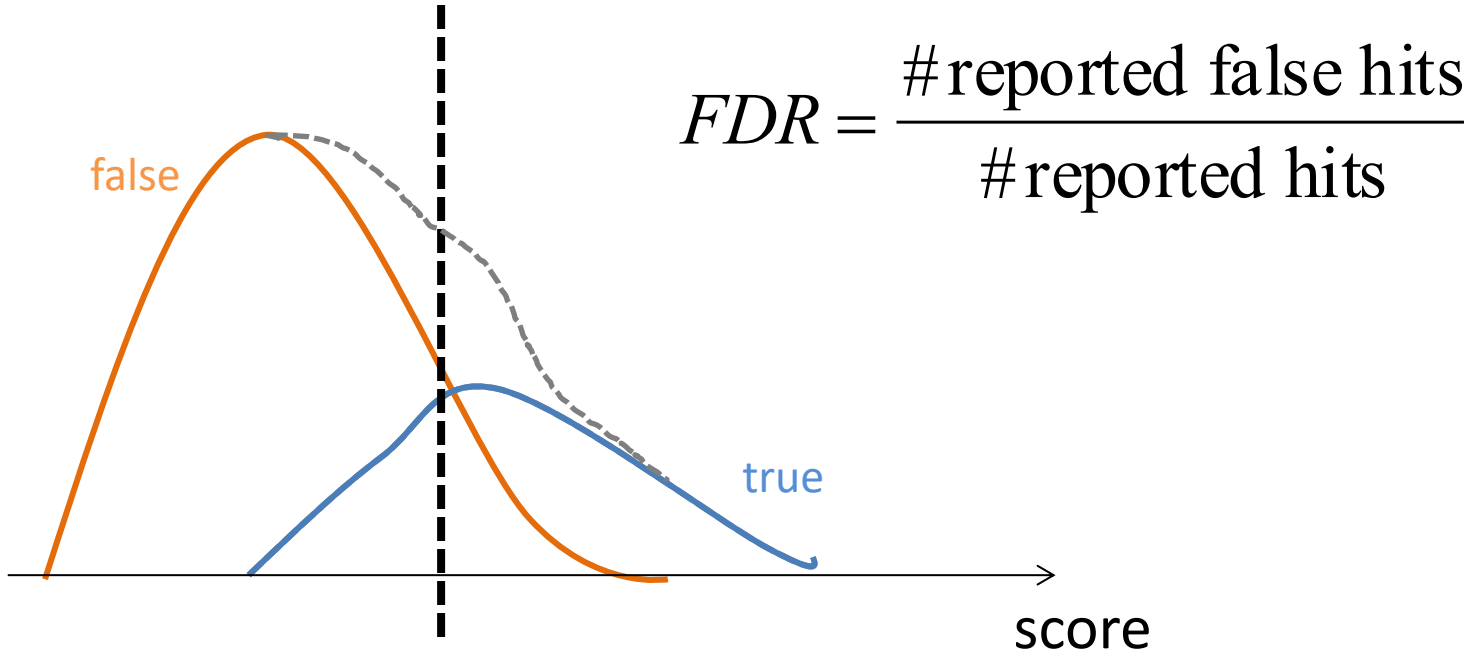
- Only report results if you're confident.
- Discard the less confident ones.
- This increases accuracy to make the results useful at the price of discarding some data.

Only Report Highly Confident Results



- Grey curve is the aggregated distribution for true and false matches.
- By reporting only the results (PSMs) above a score threshold, one can reduce the error rate.
- Trade sensitivity for accuracy.

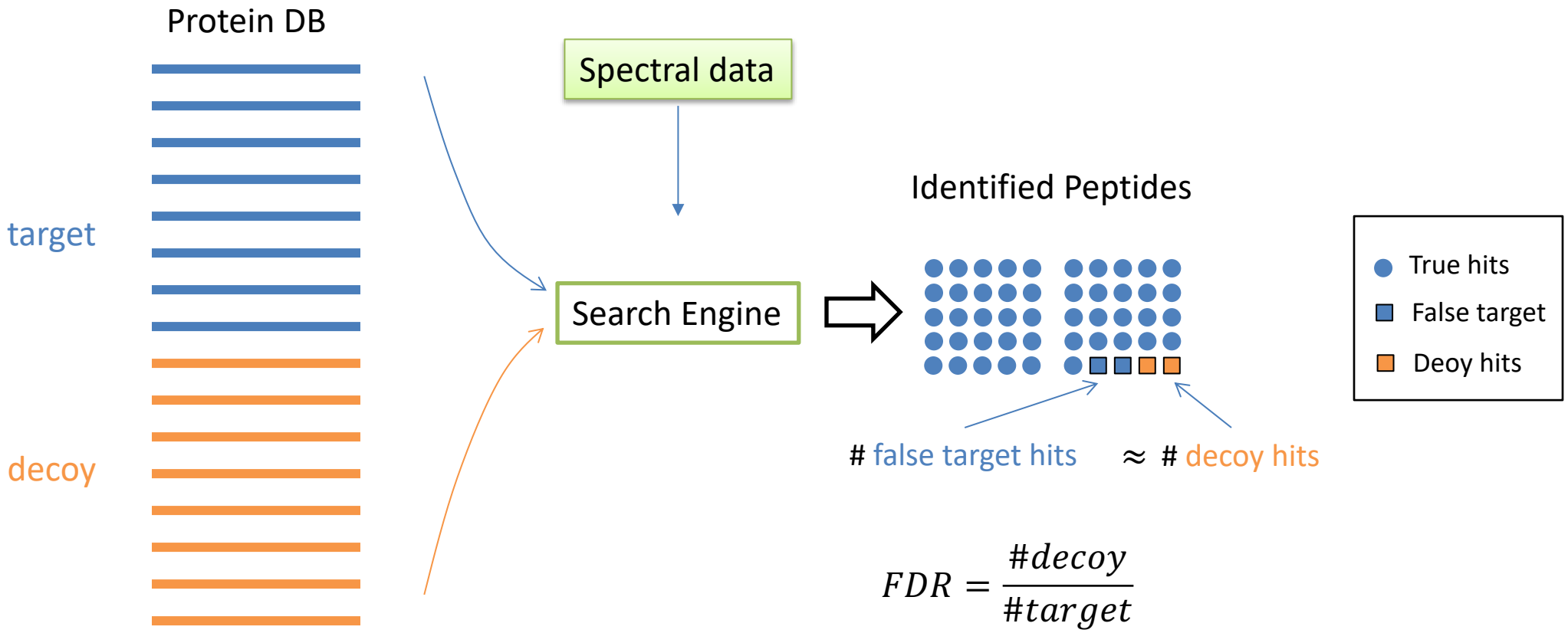
False Discovery Rate



$$FDR = \frac{\#\text{reported false hits}}{\#\text{reported hits}}$$

- By choosing different score threshold, one can calculate the FDR for all target PSMs above the threshold. Or conversely, one can choose a proper threshold to meet a FDR requirement.
- As of today, a typical FDR requirement is 1%.
- Unfortunately, we only know the aggregated distribution (grey curve)

FDR Estimation with Target-Decoy



$$FDR = \frac{\#decoy}{\#target}$$

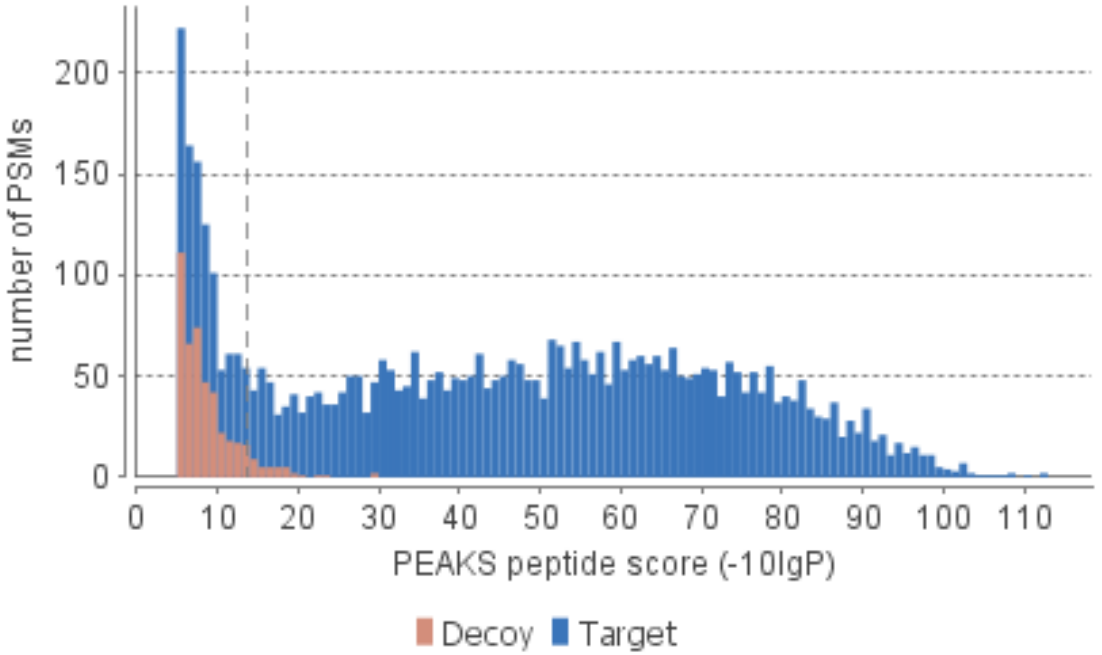
Target-decoy search strategy for increased confidence in large-scale protein identifications by mass spectrometry

Joshua E Elias[1] & Steven P Gygi[1,2]

Nature Methods 4, 207 - 214 (2007)

FDR Estimation

Distribution of PSM scores



Question

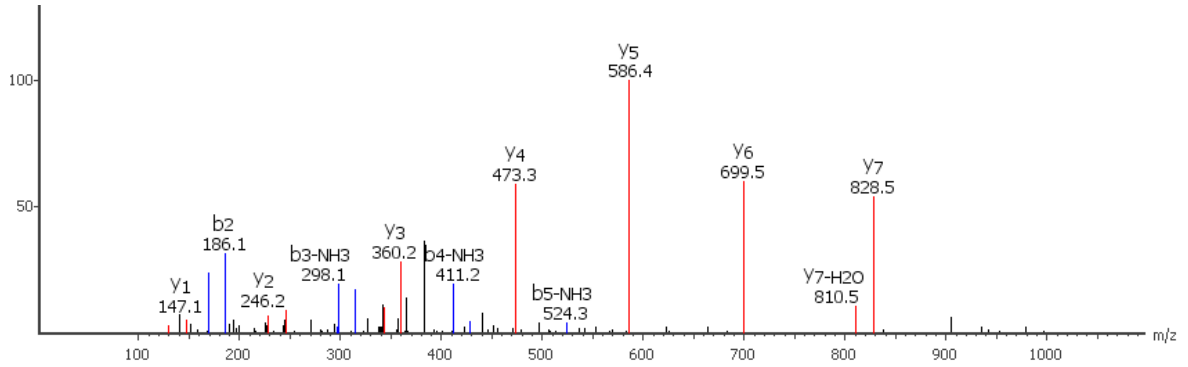
- Consider in assignment 2, we mix N true and N random peptides together.
- By using your score, from the N top-scoring peptides, there are about 0.6N true peptides and 0.4N random peptides.
- How many of the “true” peptides are selected because your scoring function is truly amazing, and how many are pure luck?

Better Scoring Function

- The empirical score is only good for start up.
- Soon competition will get fierce and you'll need a better scoring function.

Likelihood Ratio

- Let m be the m/z of a y-ion, and indeed, we see a peak with m/z = m in the spectrum.
- Two assumptions:
  - The peptide is the real peptide so peak is caused by the y-ion.
    - Pr(observe a peak at m|m is a y-ion m/z of the real peptide)
  - The peptide is a random peptide so the match is purely by chance.
    - Pr(observe a peak at m|m is a random mass)

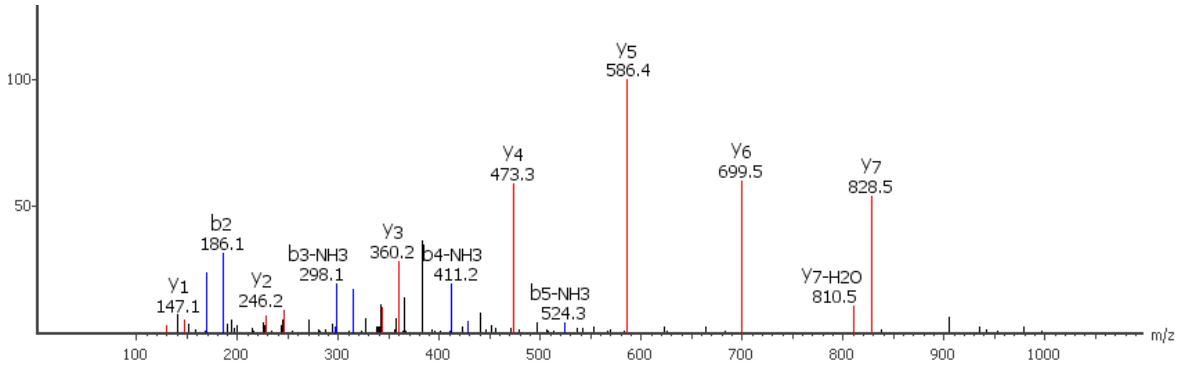


Log Likelihood Ratio

- Learn two probabilities from large training data
  - p : Prob(a peak is observed at a y-ion m/z).
  - q : Prob(a peak is observed at a random m/z).
  - Usually $p > q$.
- Given a peptide sequence, calculate m/z of all possible y-ions. For each y-ion,
  - If a peak observed, $\log \frac{p}{q}$ is added to score.
  - If no peak is observed, $\log \frac{1-p}{1-q}$, is added to score.
- Thus, matching ion is rewarded and missing ion is penalized.
- Other fragment ion types can be considered similarly, and added to the score.

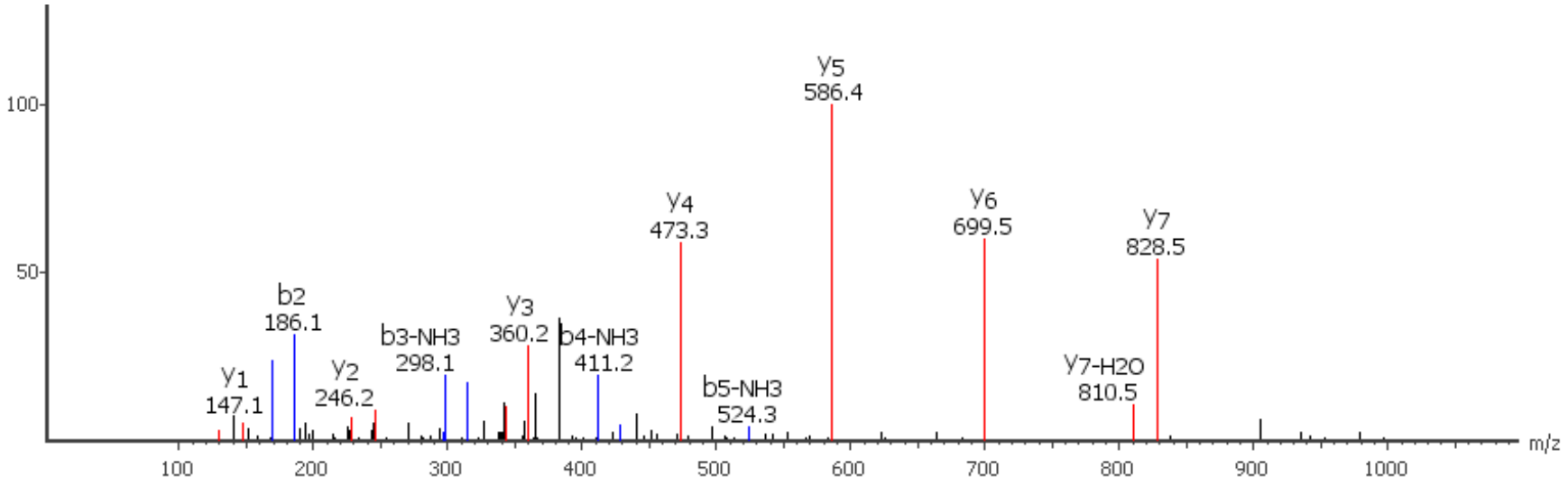
Ideas of Even Better Scores

- Machine learning that combines many factors
  - Log likelihood ratio score
  - Empirical score (log of relative intensity)
  - Precursor error tolerance
  - Number of matching peaks
  - Number of unmatched peaks
  - Number of unmatched y-ions
  - Include b-ions.
  - Include charge 2 fragment ions.
  - Etc.



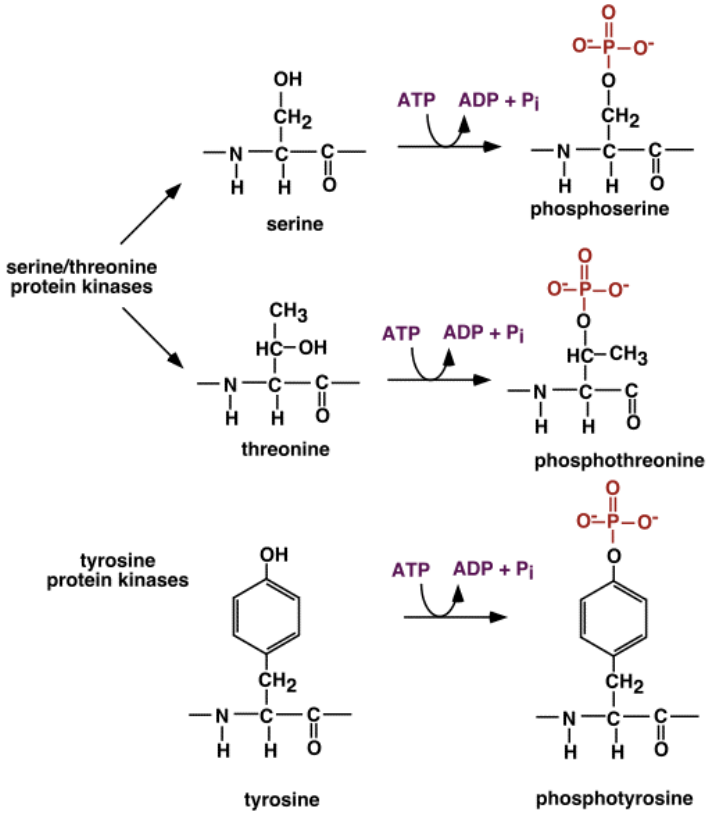
Ideas of Even Better Scores

- Deep Neural Network can be used too.
- E.g. Encode the peaks in the PSM and use a TransformerEncoder to distinguish target and decoy PSMs.



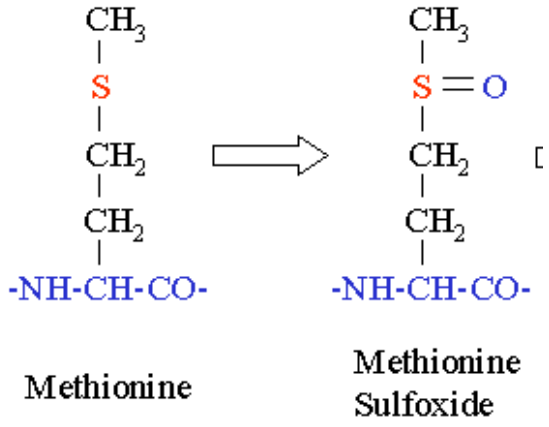
OTHER PRACTICAL CONCERNS

Post-Translational Modifications (PTM)



Phosphorylation (Δm = +80)

- PTM important to protein functions.
- Hundreds of different types of PTMs
- PTM normally change the mass of an amino acid.
- Some PTMs can be on and off.
- The figure shows two common types of PTMs.



Oxidation (Δm = +16)

Post-Translational Modifications

- There are many hundreds of different types of PTMs included in the unimod PTM database (unimod.org)
- 30% of human proteins are phosphorylated, 50% are glycosylated.
- PTMs are important to the functions of proteins.
  - For example: Reversible phosphorylation of proteins is an important regulatory mechanism. Many enzymes are switched "on" or "off" by phosphorylation and dephosphorylation. The structural change caused by the PTM changes the function of the protein.
- For data analysis purposes, one can treat a modified amino acid as a new amino acid in the alphabet.

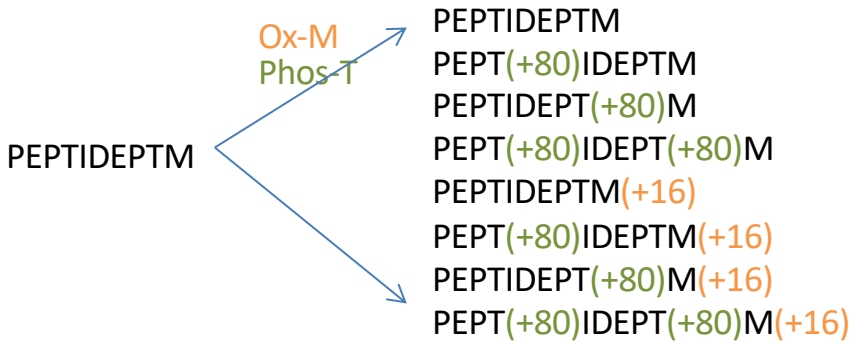
Fixed PTMs

- Certain modifications are deliberately added during the sample preparation and is (almost) 100%. These are called fixed PTMs.
- The most common one is that cysteines are usually modified chemically. And the most common modification changes the mass from 103.00919 to 160.03065. Roughly 57.02 Da were added.
- Fixed modification changes the amino acid residue mass table, but does not affect the database search speed.

- For curiosity only, cysteines are modified to avoid the formation of “disulphide bonds”.

Variable PTMs

- If user selects some PTMs as “variable”, all possible modification forms of a database peptide need to be tried to match the spectra. This results in exponential growth of search space. E.g.



- Consequently, one can only search with very few variable PTMs.

Missed and Nonspecific Cleavages

- The proteolyses may not be 100% efficient.
  - Assuming Trypsin digests the following protein with 100% efficiency
  - SSAYSR/GVFR/R/DTHK/SEIAHR/F
- Missed cleavages: a digestion site is not cut.
  - E.g. peptide GVFRR
- Non-specific cleavages: a non-digestion site got cut.
  - E.g. peptide SEIAH
- Allowing them will both affect the algorithm's time complexity.
  - Which one has a bigger impact?

Summary

- MS/MS data includes survey scans and MS/MS scans.
- Database search to assign peptides to MS/MS scans.
- Scoring functions.
- Target-decoy for FDR estimation.
- Practical issues:
  - Fixed and variable PTMs
  - Nonspecific cleavages