Lecture 17: Strategies for protein identification

Protein identification may be done by various methods:

1. Peptide mass fingerprinting
2. Protein sequencing by mass spectrometry
3. Protein sequencing by chemical methods

Peptide mass fingerprinting

We have studied two-dimensional gel electrophoresis in earlier lectures. We have also studied how over or under expression of a protein spot is identified. Once protein spot of interest is identified, the same is excised and digested by specific protease such as trypsin. As trypsin is very specific in the cleavage each protein sequence will result in specific set of peptides of varying masses that are characteristic of that protein. The mass of each peptide will be the sum of the amino acids present including any modifications that those amino acids might have undergone. The masses of these peptides are measured by mass spectrometry and then *in silico* compared to either a database containing known protein sequences or even the genome. Computer programs translate the known genome of the organism into proteins, then theoretically cut the proteins into peptides with the same protease (for example trypsin), and calculate the absolute masses of the peptides from each protein. They then compare the masses of the peptides of the unknown protein to the theoretical peptide masses of each protein encoded in the genome. The results are statistically analyzed to find the best match (Fig. 1)

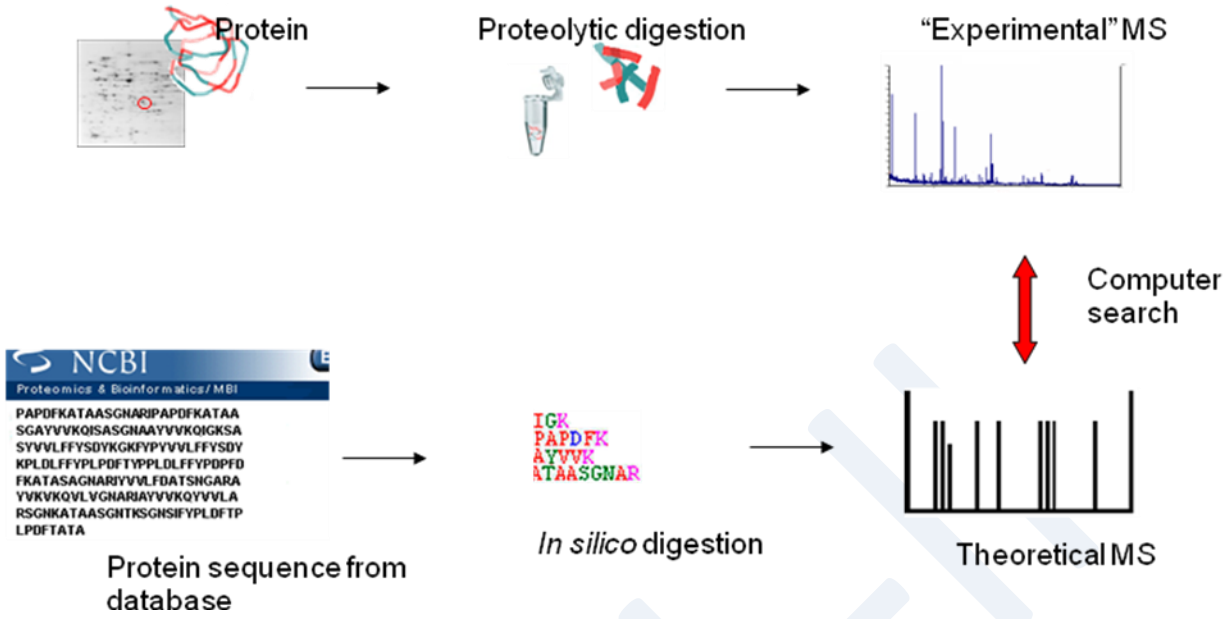


Figure 1: Various steps of Peptide mass fingerprinting

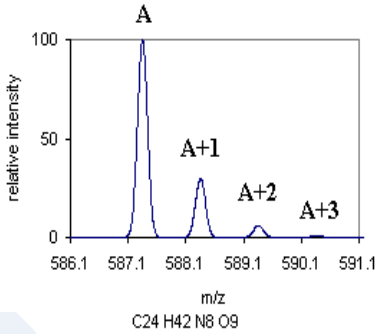
Although, the method looks very simple, there are certain limitations

- Peptide mass fingerprinting algorithms assumes the peptides come from a single protein. If there is any impurity the search result will not work.
- If protein has post translational modification, identification by Peptide mass fingerprinting does not work. As the methods assumes that the peptide mass is coming from amino acids only. Moreover, when genome data is virtually converted to protein, post-translational modification is difficult to precisely predict to match with experimental data.
- Although, specific proteases are available for protein cleave but sometime they cleave at wrong site (Mis-cleavage) which results in false prediction.

Each data peak in mass spectra may have more than one peak as atoms have several isotopes (Table 1). These peaks called isotopic peaks. For example a fragment of protein with mass M, when analyzed by mass spectrometry will give a major peak corresponding to mass M (Monoisotopic peak) but a small fraction will show M+1 mass because a small fraction of protein molecule may have 13C isotope. While searching database for protein identification (Fig. 2), one must mention the peak corresponds to monoisotopic or average. Some additional details about the protein, if known, like molecular mass, modifications etc may further help in protein identification.

Table 1: Isotopes of few common atoms

Isotope (A)	mass	Abundance, %	Isotope (A+1)	mass	Abundance, %	Isotope (A+2)	mass
12C	12	98.93	13C	13.0033548378	1.07	C14	14.003241988
1H	1.0078250321	99.9885	2H	2.0141017780	0.0115	3H	3.0160492675
14N	14.0030740052	99.632	15N	15.0001088984	0.368		
16O	15.9949146221	99.757	17O	16.99913150	0.038	18O	17.9991604



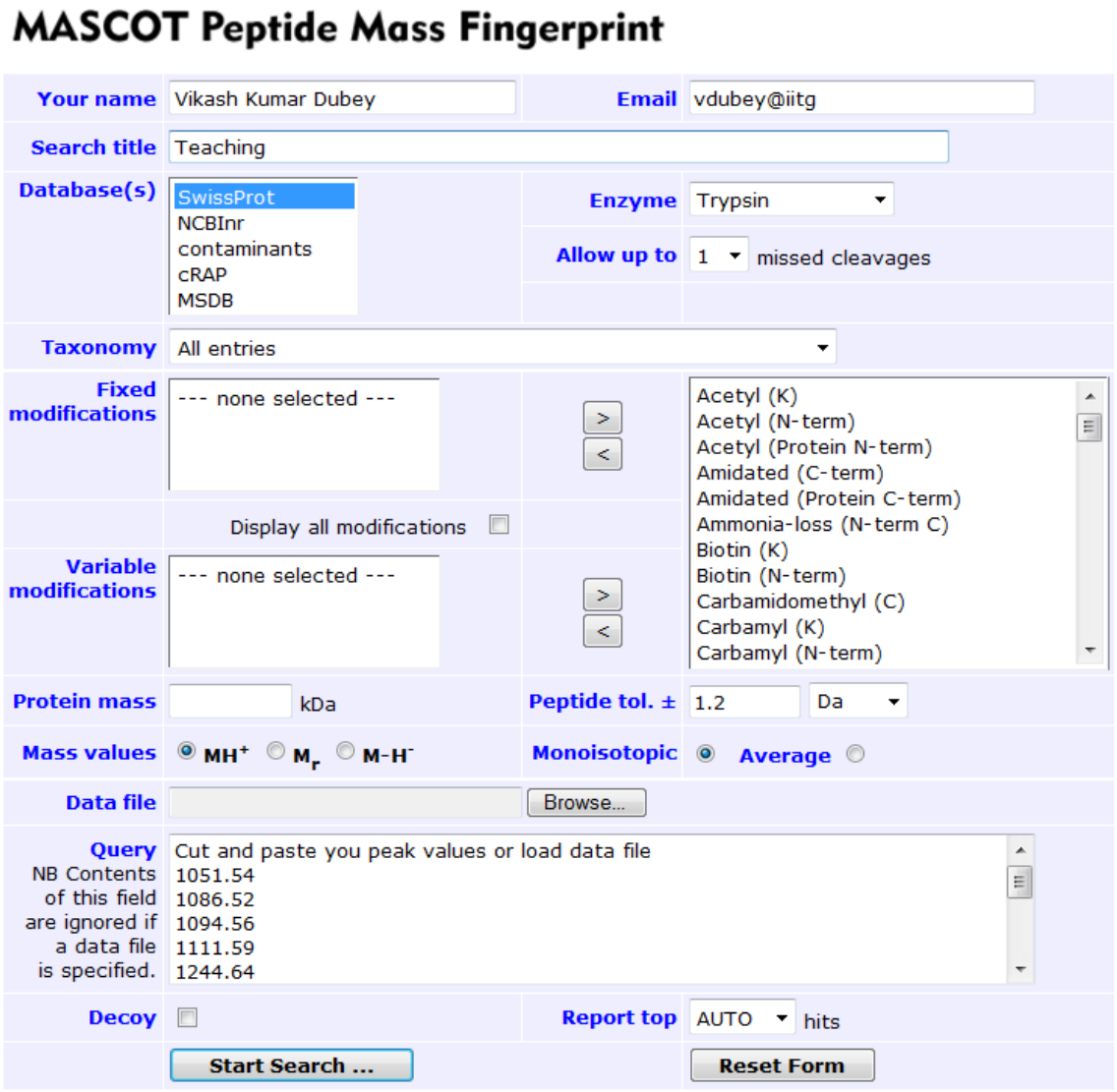


Figure 2: A snapshot of MASCOT search window

(http://www.matrixscience.com/cgi/search_form.pl?FORMVER=2&SEARCH=PMF)

MATRIX SCIENCE Mascot Search Results

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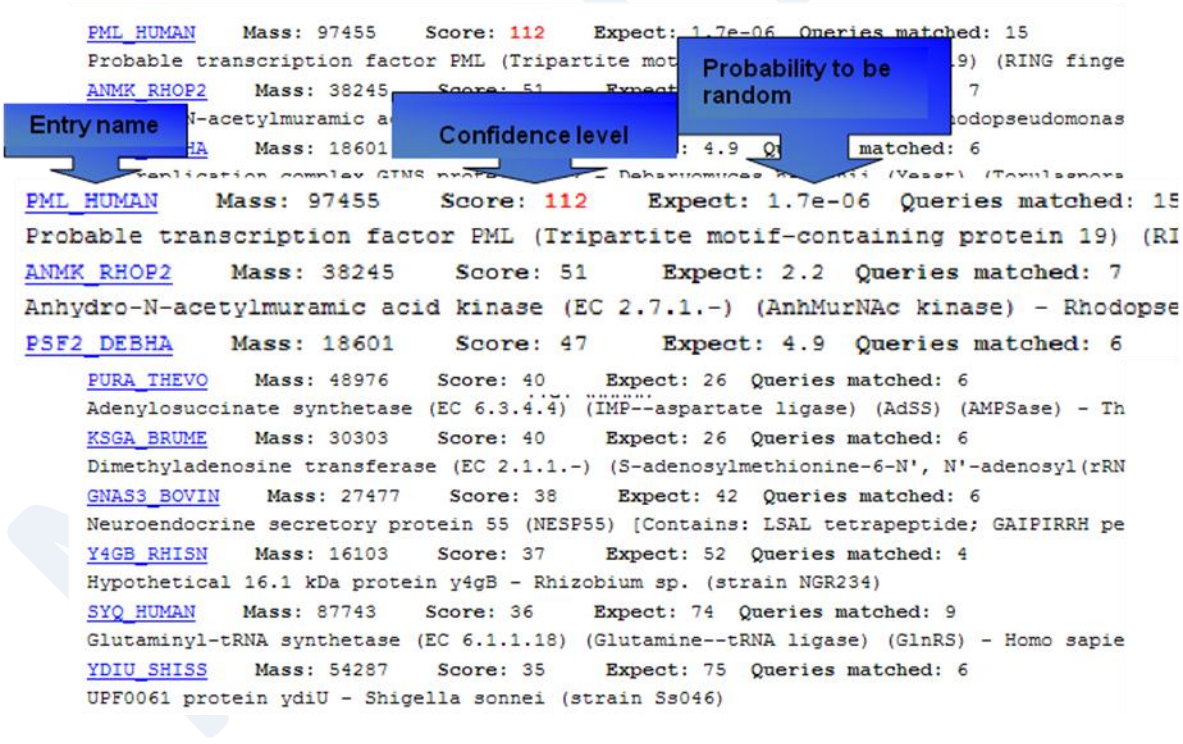
User            : Proteomics & Bioinformatics
Email           : MBI@Helsinki.fi
Search title    : Peptide Mass Fingerprint Example
Database        : SwissProt 52.1 (261513 sequences; 95638062 residues)
Timestamp       : 29 Mar 2007 at 12:10:41 GMT
Top Score       : 112 for PML_HUMAN, Probable transcription factor PML (Tripart
```

Probability Based Mowse Score

Protein score is -10*Log(P), where P is the probability that the observed match is a random event.
Protein scores greater than 67 are significant (p<0.05).

Mascot PMF results



Mascot protein view

Protein View

Match to: PML_HUMAN Score: 112 Expect: 1.7e-06
Probable transcription factor PML (Tripartite motif-containing protein 19)

Nominal mass (M_r): 97455; Calculated pI value: 5.88
NCBI BLAST search of PML_HUMAN against nr
Unformatted sequence string for pasting into other applications

Taxonomy: Homo sapiens

Cleavage by Trypsin: cuts C-term side of KR unless next residue is P
Number of mass values searched: 18
Number of mass values matched: 15
Sequence Coverage: 22%

coverage

Matched peptides shown in Bold Red

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    1 MEPAPARSPR PQQDPARPQE PTMPPPETPS EGRQPSPSPS PTERAPASEE
   51 EFQFLRCQQC QAEAKCPKLL PCLHTLCSGC LEASGMQCPI CQAPWPLGAD
  101 TPALDNVFFE SLQRRLSVYR QIVDAQAVCT RCKESADFWC FECEQLLCAK
  151 CFEAHQWFLK HEARPLAELR NQSVREFLDG TRKTNNIFCS NPNHRTPTLT
  201 SIYCRGCSKP LCCSCALLDS SHSELKCDIS AEIQQRQEEL DAMTQALQEQ
  251 DSAFGAVHAQ MHAAVGQLGR ARAETEELIR ERVRQVVAHV RAQERELLEA
  301 VDARYQRDYE EMASRLGRLD AVLQRIRTGS ALVQRMKCYA SDQEVLDMHG
  351 FLRQALCRLR QEEPQSLQAA VRTDGFDEFK VRLQDLSSCI TQGKDAAVSK
  401 KASPEAASTP RDPIDVDLPE EAERVKAQVQ ALGLAEAQPM AVVQSVPGAH
  451 PVPVYAFSIK GPSYGEDVSN TTTAQKRKCS QTQCPRKVIK MESEEGKEAR
  501 LARSSPEQPR PSTSKAVSPP HLDGPPSPRS PVIGSEVFLP NSNHVASGAG
  551 EAEERVVVIS SSEDSDAENS SSRELDDSSS ESSDLQLEGP STLRVLDENL
  601 ADPQAEDRPL VFFDLKIDNE TQKISQLAAV NRESKFRVVI QPEALFSIYS
  651 KAVSLEVGLQ HFLSFLSSMR RPILACYKLW GPGLPNFFRA LEDINRLWEF
  701 QEAISGFLAA LPLIRERVPG ASSFKLKNLA QTYLARNMSE RSAMAAVLAM
  751 RDLCRLLEVS PGPQLAQHVY PFSSLQCFAS LQPLVQAAVL PRAEARLLAL
  801 HNVSFMELLS AHRRDRQGGL KKYSRYLSLQ TTTLPPAQPA FNLQALGTYF
  851 EGLLEGPALA RAEGVSTPLA GRGLAERASQ QS
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% of protein length covered by the experimental peptides

Home Assignment

From a peptide mass fingerprinting experiment you have following monoisotopic peaks. Using peptide mass fingerprinting programs identify the protein. What is the confidence level of your identification (with explanation)?

1051.54
1086.52
1094.56
1111.59
1244.64
1421.7
1476.67
1542.84
1613.88
1664.97
1763.79
1777.82.

Submit your assignment to course developer by e-mail; you shall get e-mail rely with grading and feedback in a week time.