Applications for Peptide Mass Spectral Libraries in Proteomics

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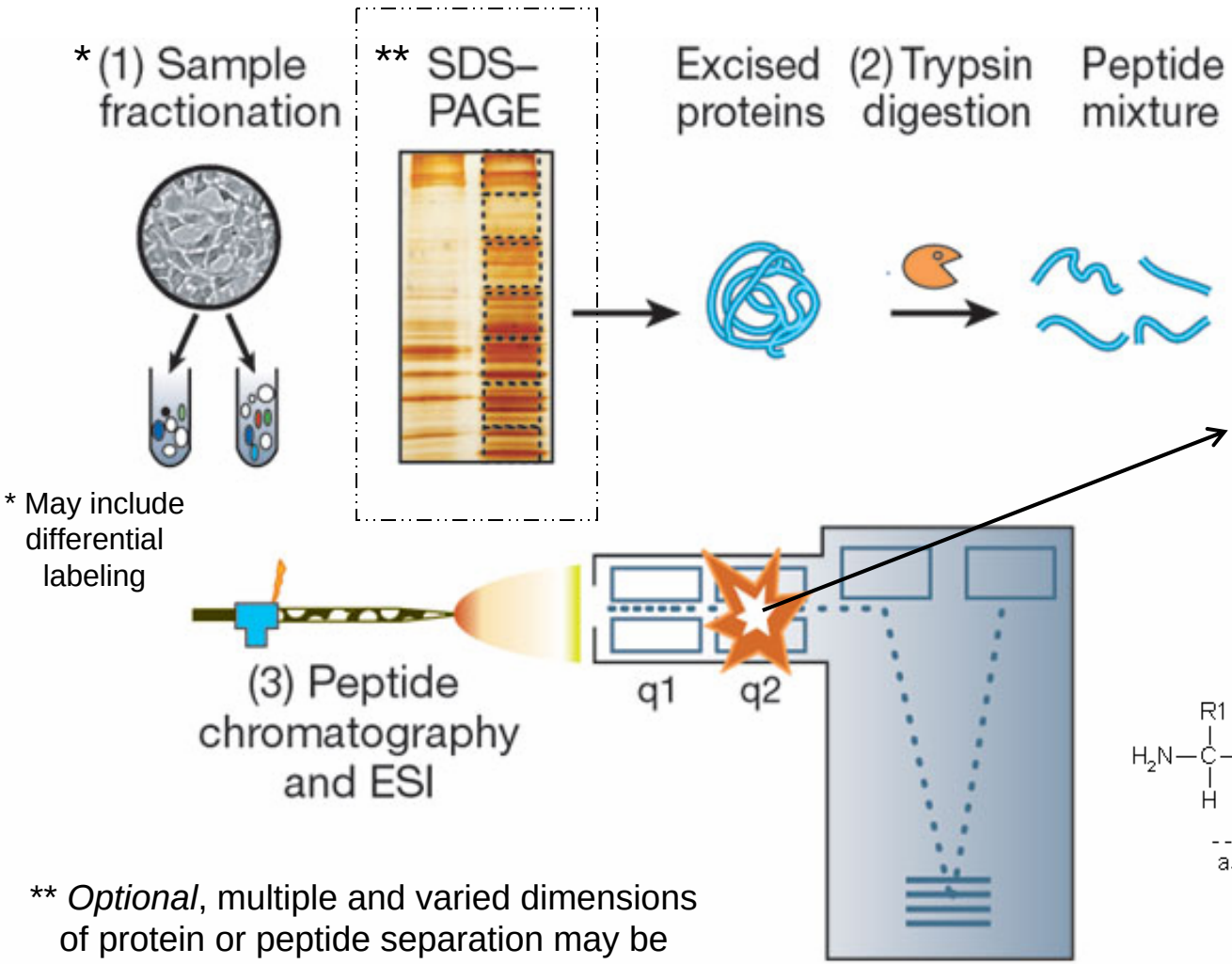
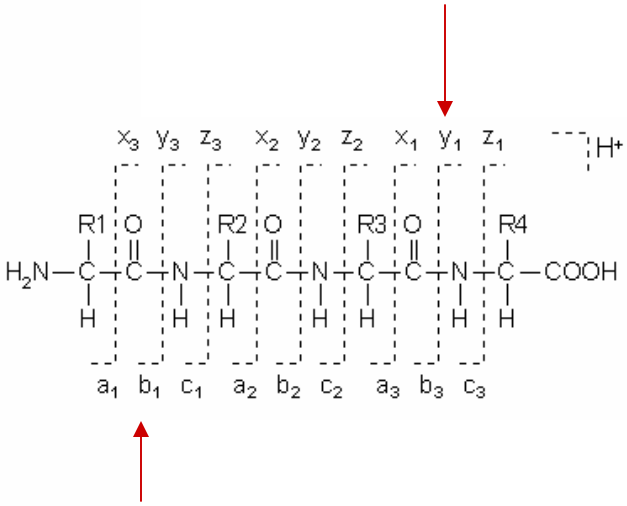
Overview

1. A typical “shotgun” proteomics workflow
2. Peptide identification by searching sequences (e.g. Sequest, MASCOT) – ‘current practice’
3. Libraries of peptide fragmentation spectra– a “new” alternative
   - Description
     - History – GC/MS, EI spectra
     - Construction of high-quality, consensus libraries
     - Structure/format of the library entries
   - Searching
     - NIST algorithms, Windows software
     - Other algorithms, publications
     - Compared to ‘current practice’
4. Integrating the two search methods (OMSSA/NISTMS)
5. Current status of peptide library projects and availability
6. What’s next

“Shotgun” Proteomics



backbone fragmentation from CID gives rise to <u>reproducible</u> ***b*** and ***y*** ions series spectra MS/MS



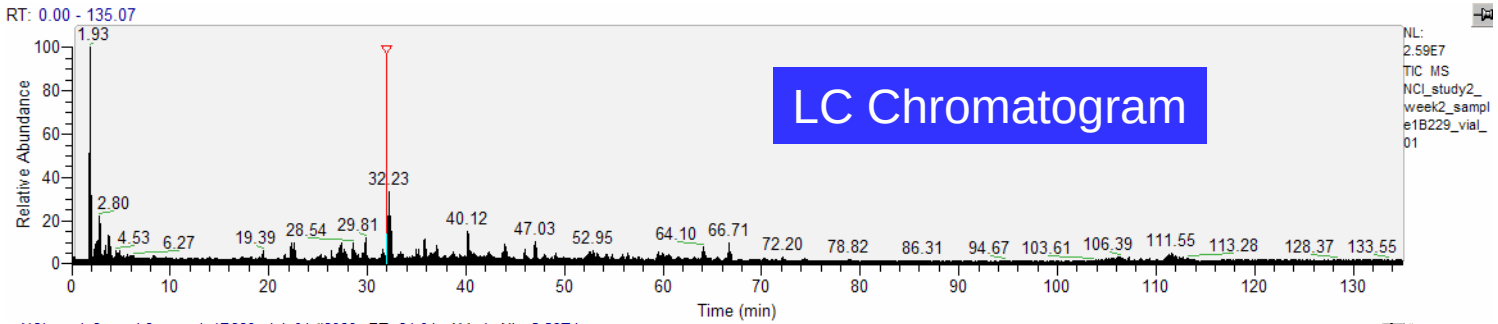
** *Optional*, multiple and varied dimensions of protein or peptide separation may be added to extend “depth” of analysis

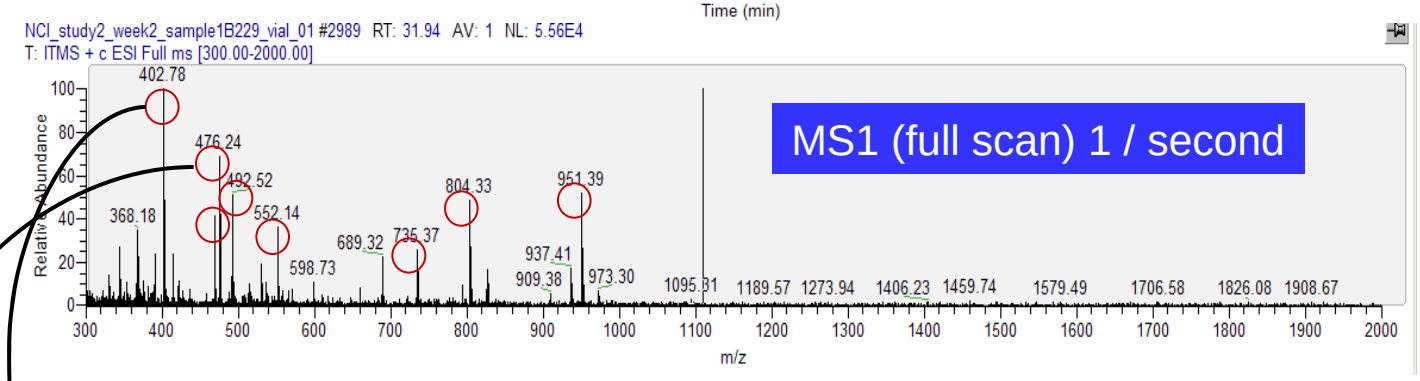
Aebersold & Mann, *Nature* **422**, 198-207 (13 March 2003)

Why do these experiments?

- Confirmation of gene products (protein sequencing)
- Differential expression studies (i.e.. global or targeted)
- Discovery of “biomarkers”
- Validation of candidate markers
- Discovery / mapping / validation of PTMs

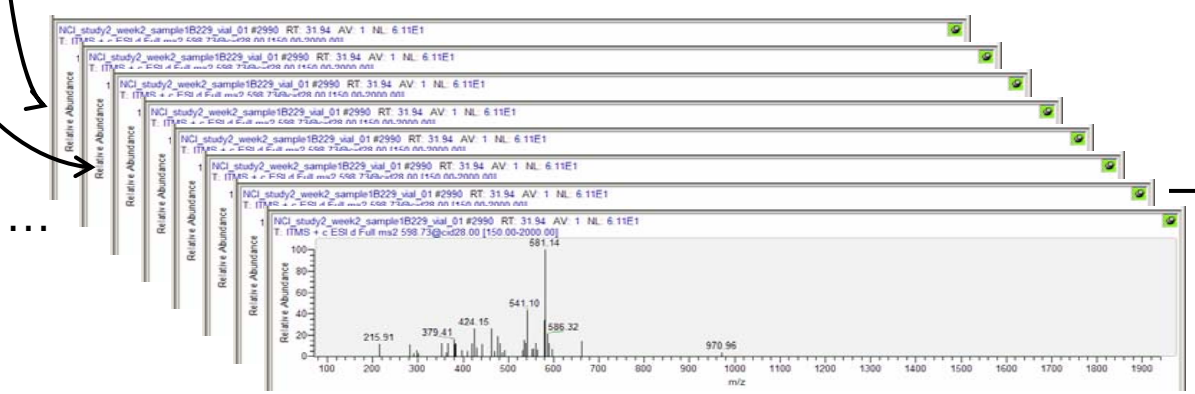
LC-ESI-MS/MS Data Acquisition





CID

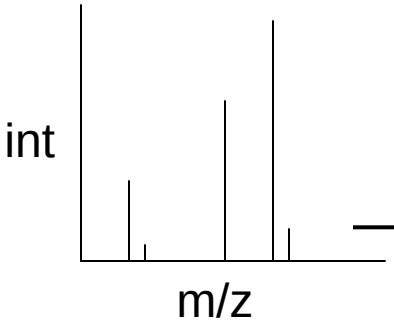
~8 MS/MS sampled from most intense MS1 ions (8-1)



…

Raw data passed to analysis

Data Analysis (Bioinformatics "Pipeline")



[~10,000 MS/MS spectra / 60 min. gradient, complex sample]

MS/MS spectra + monoisotopic precursor m/z extracted from raw data to ASCII text

extract

Peak list (e.g. dta, mgf, mzXML)

Sequest, MASCOT, X!Tandem, OMSSA, Spectrum Mill, others

searching / identification

Search Results – peptide identifications and raw scores

Reverse (decoy) database search results, PeptideProphet, distribution analysis

compute confidence

Discriminant scores, E-values, FDRs

User-defined threshold (e.g. 1%, 5%)

filter

Filtered peptide list with probabilities

Protein mapping, ProteinProphet, parsimony analysis

map proteins

Matched Protein – Peptide List

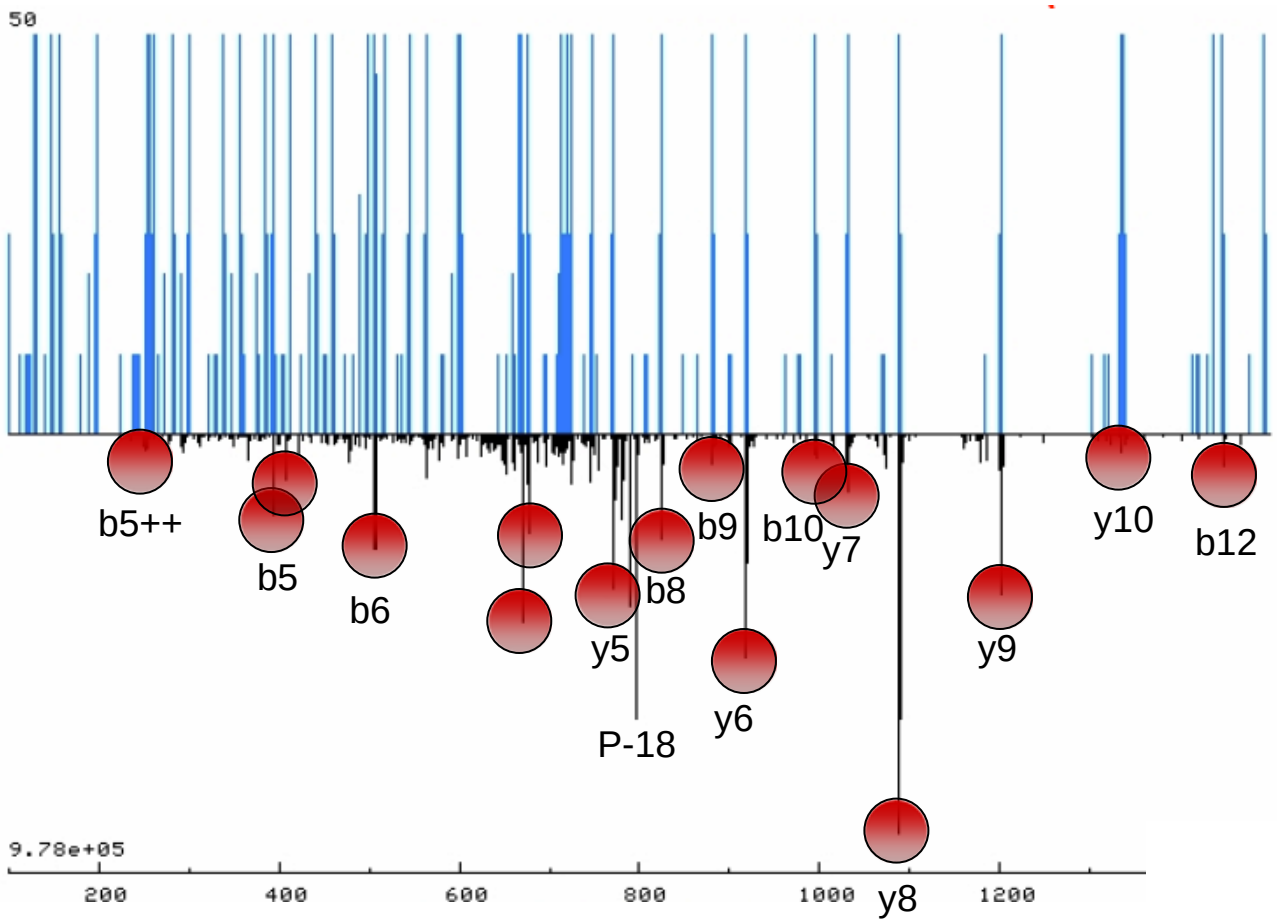
Peptide Identification – ‘Current Practice’

1. *In silico* digestion protein sequences
2. Predict sequence peaks (*b* and *y* ions)
3. Predict peaks due to minor isotopes and neutral losses
4. Normalize intensities
5. Find peaks in common within tolerance constraints (precursor & fragment)
6. Weight and score the correlation

Peptide Identification – ‘Current Practice’

Predicted spectrum

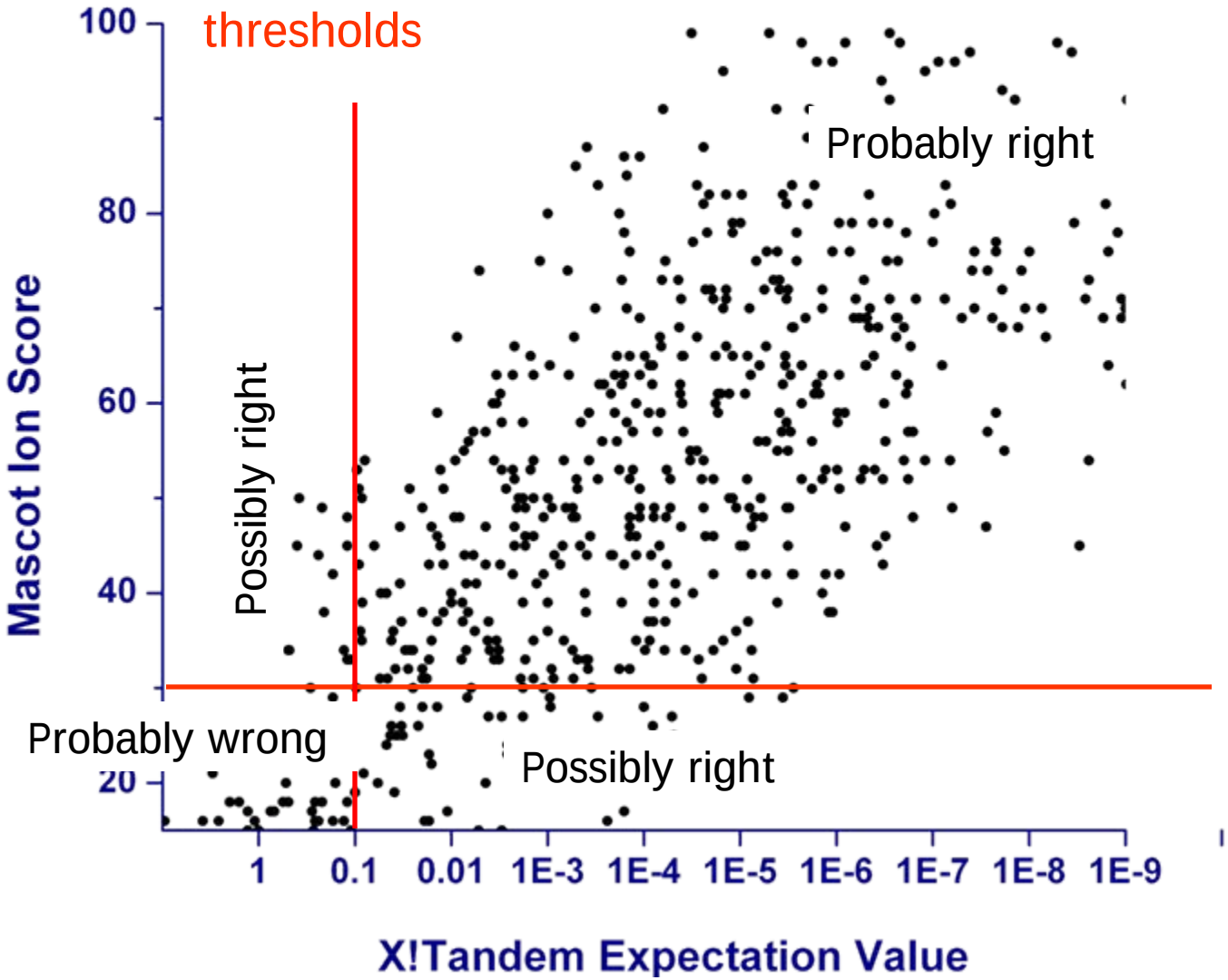
Measured spectrum



Good m/z matches. Not good predictions of relative intensities. Misses some major features.

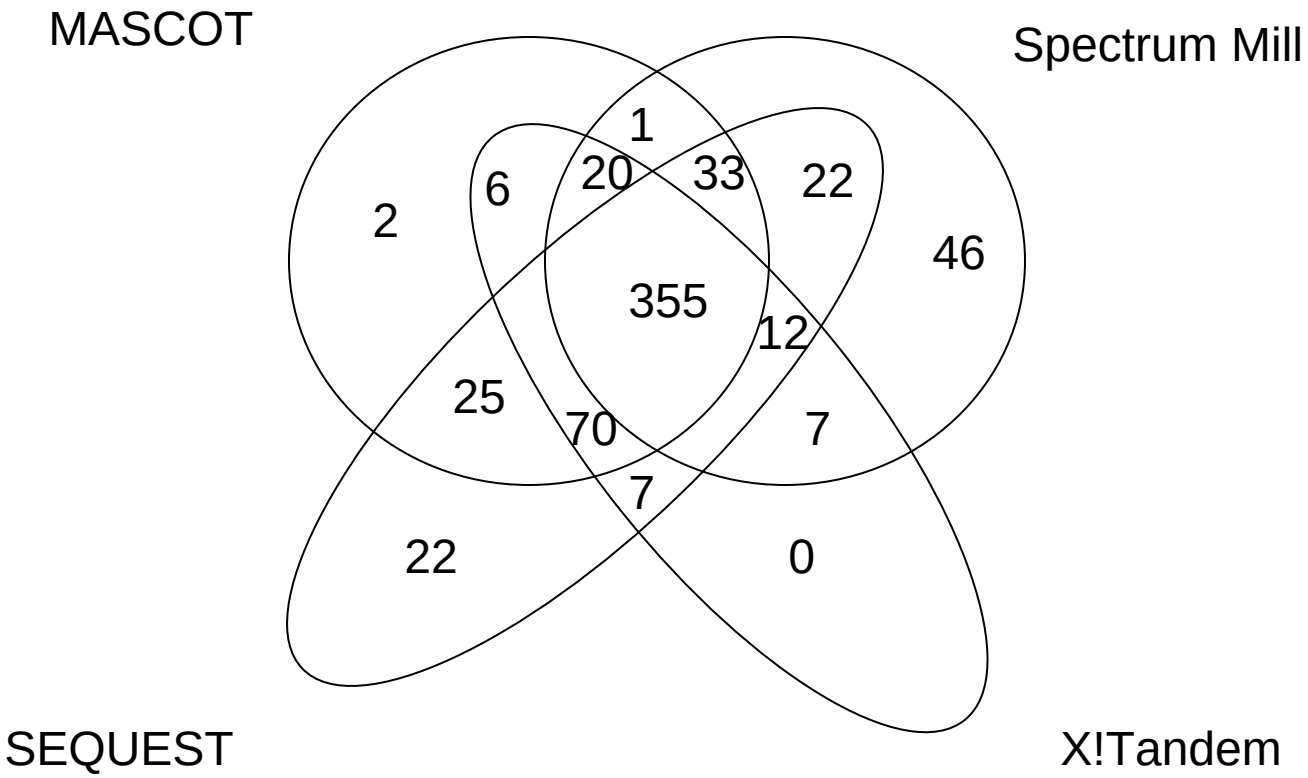
(Henry Lam, ISB)

Same Spectra - Different Scores



All Sequence Search Algorithms Report Different Matches

Kapp et al. *Proteomics*, 2005



Major algorithms are operating differently – 55% 4-way overlap

Drawbacks of ‘current practice’

- Unnatural use of peak intensities
- Little or no use of recurring, non-(b,y) fragmentation patterns, also higher charge states not accurate
- Scores for identical spectra vary between algorithms
- Time-consuming to compare results between algorithms
- Multiple commercial and ‘in-house’ data analysis systems create the appearance of variability – lack of ‘standardization’

MS Library Searching: a “New” Alternative for Peptide Ion Identification

- H.S. Hertz, R.A. Hites and K. Biemann *Anal. Chem.* **43** (1971)
- F.W. McLafferty, R.H. Hertel and R.D. Villwock *Org. Mass Spectrom.* **9** (1974)
- S. Sokolow, J. Karnofsky and P. Gustafson , The Finnigan Library Search Program. *Finnigan Application Report 2*, Finnigan Corp., San Jose, CA (March 1978).
- S.E. Stein, D.R. Scott J. Amer. Soc. Mass Spectrom., **5** (1994).

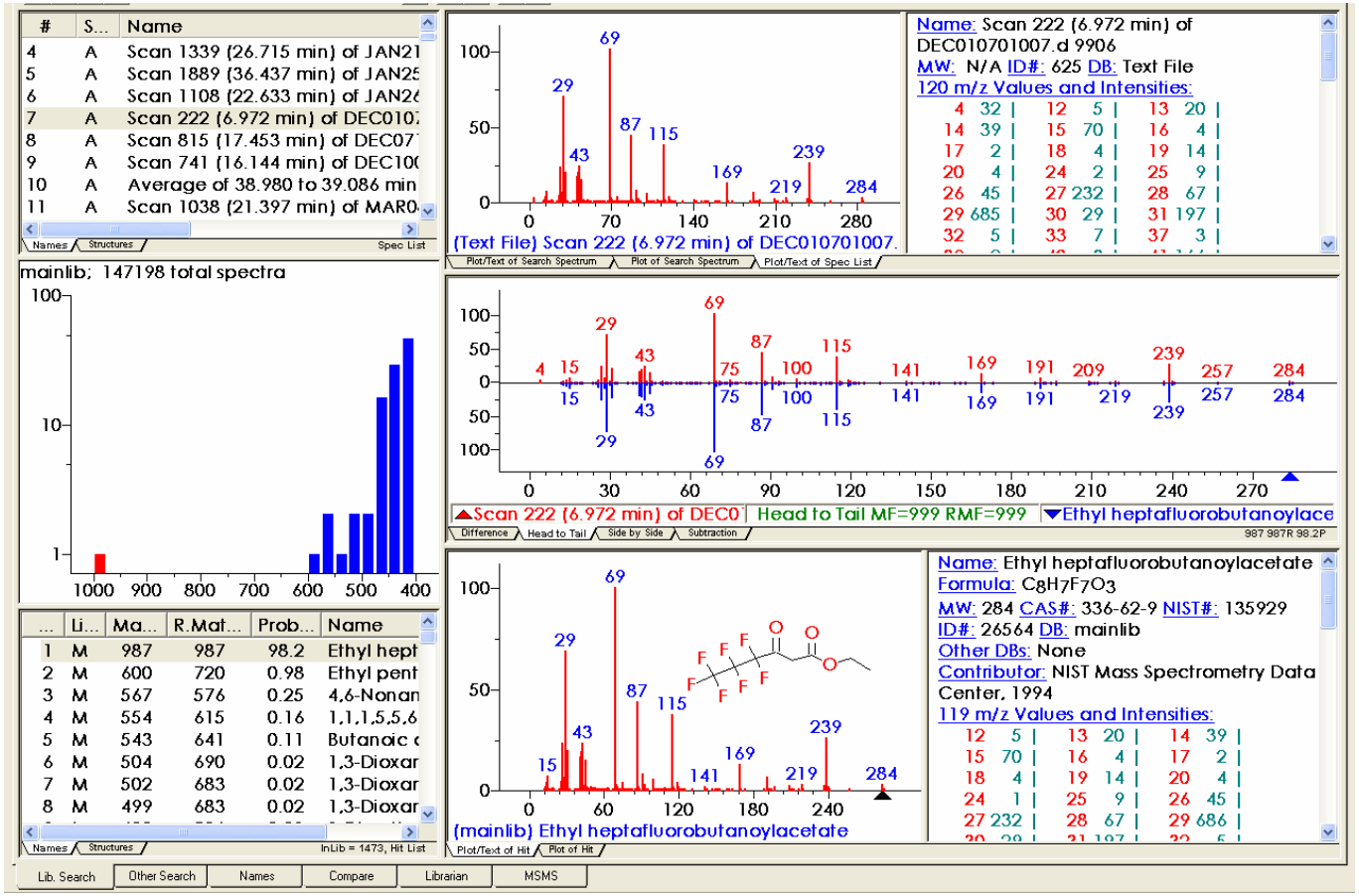
Traditional GC/MS Library Search

Uses a weighted 'dot product' score to quickly evaluate EI spectra – in use for >25 yrs.

$$\frac{\sum \sqrt{MR}}{\sqrt{\sum M \sum R}}$$

(M) Measured
(R) Reference

Cosine of angle between spectra expressed as vectors, weighted to improve performance



NIST/EPA/NIH - 163K Compounds

Compound Identification by GC/MS

But will it work for peptides?

- There are too many biologically relevant peptides! How will you purify and get spectra from them all?
  - Collect spectra from analyses of mixtures (i.e.. ‘naturally-occuring’ sources)
  - Collect spectra from public donations (thank you!)
  - Collect spectra from complex mixtures and synthetic peptides run at NIST
- Spectra are too variable
  - Not with modern instruments
  - Variability is function of S/N
- How do I know the spectra are correct?
  - Identified by up to 4 different algorithms
  - Stringent quality filters, S/N, fraction unexplained abundance
  - Many replicates used to generate consensus, minimizes run-to-run variability
- Aren’t searches limited to the peptides that are in the library (i.e. ones that have been seen before)?
  - Yes.
  - Breadth and depth of library only increased by processing new spectra

Reference Library Building*

- Extract identified spectra from sequence search
  - Multiple search engines
  - Instrument-class specific
  - Use reversed sequence library
- Create ‘consensus’ spectra
  - Two or more matching spectra, also save best
- Assign probability of being correct
  - Refine confidence
- Create searchable spectral library
  - Resolve conflicts, add annotation

* http://chemdata.nist.gov/mass-spc/ftp/mass-spc/PepLib.pdf

‘msp’- formatted library entry

Name: ALTDFERTYMK/2
MW: 923 ID#: 270 DB: yeast_consensus_final_true_lib
Mods: 1/10,M,Oxidation

Protein

Protein: gi|6322847|ref|NP_012920.1| N-terminally acetylated protein component of the large (60S) ribosomal subunit, nearly identical to Rpl14Bp and has similarity to rat L14 ribosomal protein; rpl14a csh5 double null mutant exhibits synthetic slow growth [Saccharomyces cerevisiae]; gi|300233|gb|AAC60550.1| orf YKL153 [Saccharomyces cerevisiae]; gi|320756|pir||S30133 ribosomal protein L14.e.A, cytosolic - yeast (Saccharomyces cerevisiae); gi|485979|emb|CAA81839.1| RPL14A [Saccharomyces cerevisiae]; gi|549656|sp|P36105|R14A_YEAST 60S ribosomal protein L14-A

SE scores

Comment: Spec=Consensus Pep=Tryptic Fullname=R.ALTDFERTYMK.F/2 Mods=0 Parent=461.736 Inst=it Mz_diff=0.184 Mz_exact=461.7356 Mz_av=462.011 Se=4^M13:sc=61.88/4.489,td=58.46/8.338,sr=16.68/3.719,sd=32.8/3.959,bs=23.8,b2=22.79,bd=69.63^X21:ex=0.0021/0.004377,td=6900/1.467e+004,sd=0/0,hs=30.9/0.1857,bs=0.00031,b2=0.00036,bd=45200^O21:ex=0.000222/0.02367,td=225000/2.262e+005,pr=4.48e-008/4.357e-006,bs=4.84e-005,b2=6.41e-005,bd=1.03e+006^P3:sc=24/0.5667,dc=15.1/0.4,ps=3.01/0.04667,bs=0

Sources

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Consensus statistics and measures

63 m/z Values and Intensities:

142.2	2.19	? 13/20 0.2
143.0	20.88	b2/-0.07 21/21 0.8
175.1	17.38	y1/-0.02 21/21 0.3
183.2	6.79	? 20/21 0.1
187.2	3.79	? 21/20 0.2
197.2	1.79	? 12/20 0.1
199.1	1.49	? 12/19 0.0
211.1	61.73	? 21/21 1.1
215.1	14.18	b5-44^2/0.48 21/21 0.7
227.6	2.29	b5-18^2/-0.02 17/20 0.3
244.1	2.89	? 19/20 0.2
256.1	46.75	b3/-0.06 21/21 1.2

[…]

Annotated spectrum
m/z, normalized intensities

steve.stein@nist.gov

NCBI Entrez Protein

My NCBI [Sign In] [Register]

PubMed | Nucleotide | Protein | Genome | Structure | PMC | Taxonomy | OMIM | Books

Search Protein for Go Clear

Limits Preview/Index History Clipboard Details

Display GenPept Show 5 Send to

Range: from begin to end Features: CDD + Refresh

1: AAA39883. Reports p53...[gi:200203]

BLink, Conserved Domains, Links

Features Sequence

```
LOCUS       AAA39883                 381 aa            linear   ROD 27-APR-1993
DEFINITION  p53.
ACCESSION   AAA39883
VERSION     AAA39883.1  GI:200203
DBSOURCE    locus MUSP53C accession M13874.1
KEYWORDS    .
SOURCE      Mus musculus (house mouse)
  ORGANISM  Mus musculus
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia;
            Sciurognathi; Muroidea; Muridae; Murinae; Mus.
REFERENCE   1  (residues 1 to 381)
  AUTHORS   Arai,N., Nomura,D., Yokota,K., Wolf,D., Brill,E., Shohat,O. and
            Rotter,V.
  TITLE     Immunologically distinct p53 molecules generated by alternative
            splicing
  JOURNAL   Mol. Cell. Biol. 6 (9), 3232-3239 (1986)
   PUBMED   3023970
REFERENCE   2  (sites)
  AUTHORS   Han,K.A. and Kulesz-Martin,M.F.
  TITLE     Alternatively spliced p53 RNA in transformed and normal cells of
            different tissue types
  JOURNAL   Nucleic Acids Res. 20 (8), 1979-1981 (1992)
   PUBMED   1579500
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FEATURES             Location/Qualifiers
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                     /db_xref="taxon:10090"
                     /map="11"
                     /cell_line="normal T-cell line L12"
                     /cell_type="Ab-MuLV transformed Meth A fibroblast"
     Protein         1..381
                     /name="p53"
     Region          8..31
                     /region_name="P53_TAD"
                     /note="P53 transactivation domain (TAD). The binding of
                     the p53 transactivation domain by regulatory proteins
                     regulates p53 transcription activation. This domain is
                     comprised of a single amphipathic alpha helix and contains
                     a highly conserved motif; pfam08563"
                     /db_xref="CDD:71989"
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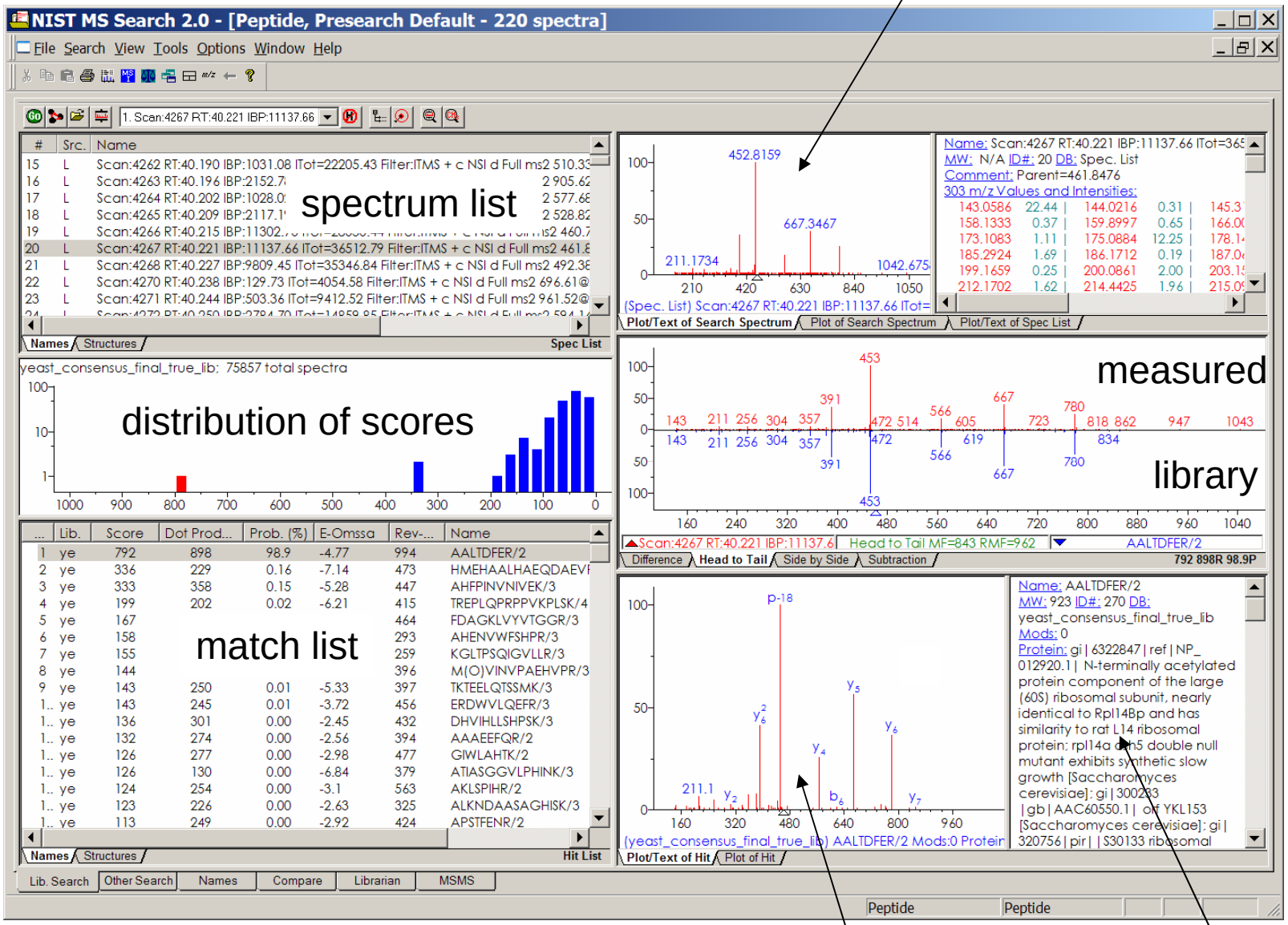
Not that different than other types of molecular biology data records

NIST MS Search 2.0 – Peptide Version (free demo)

measured spectrum



library spectrum

annotation

http://www.peptideatlas.org/speclib/

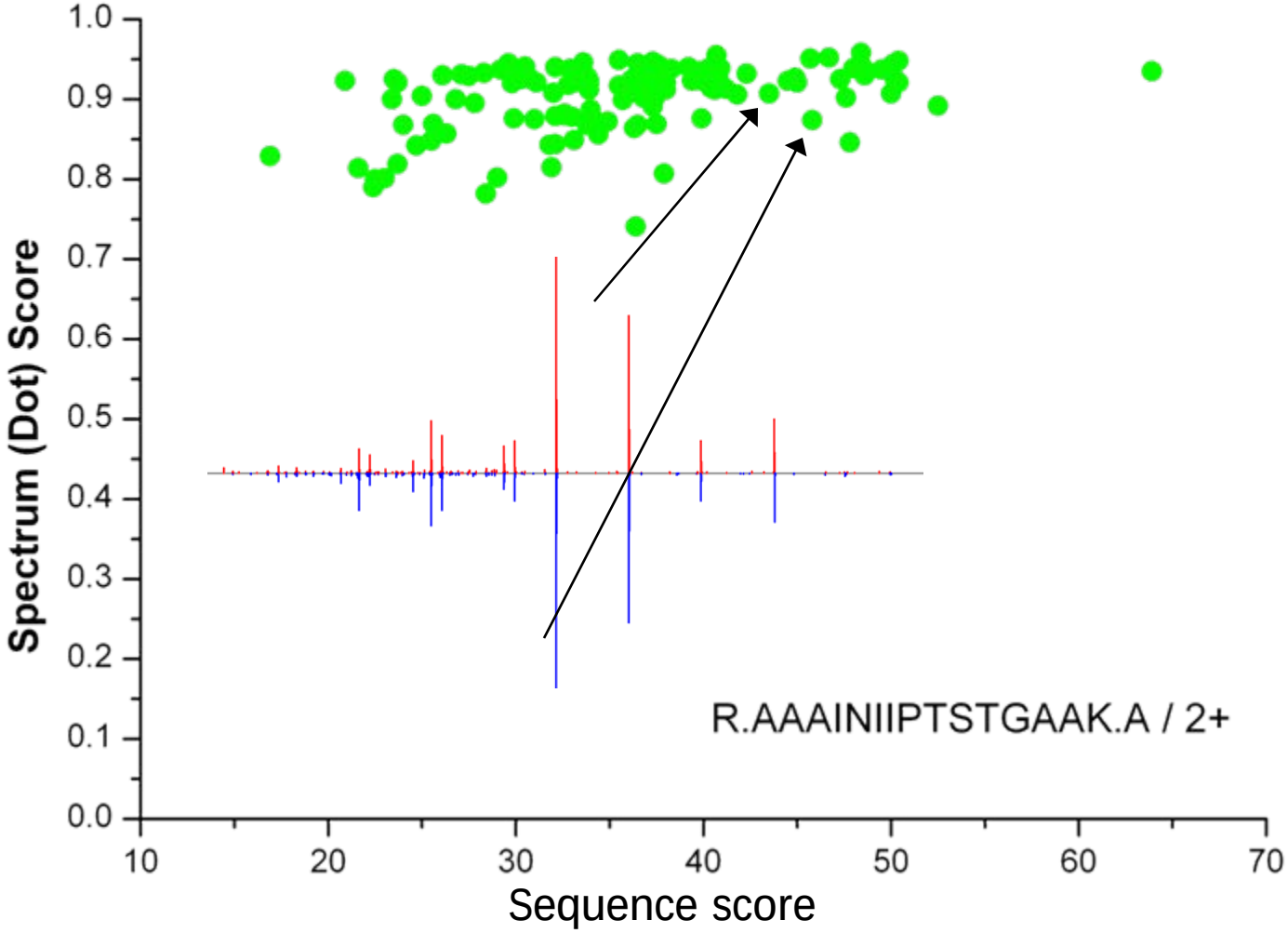
To download software & libraries

Library Searching for Peptides

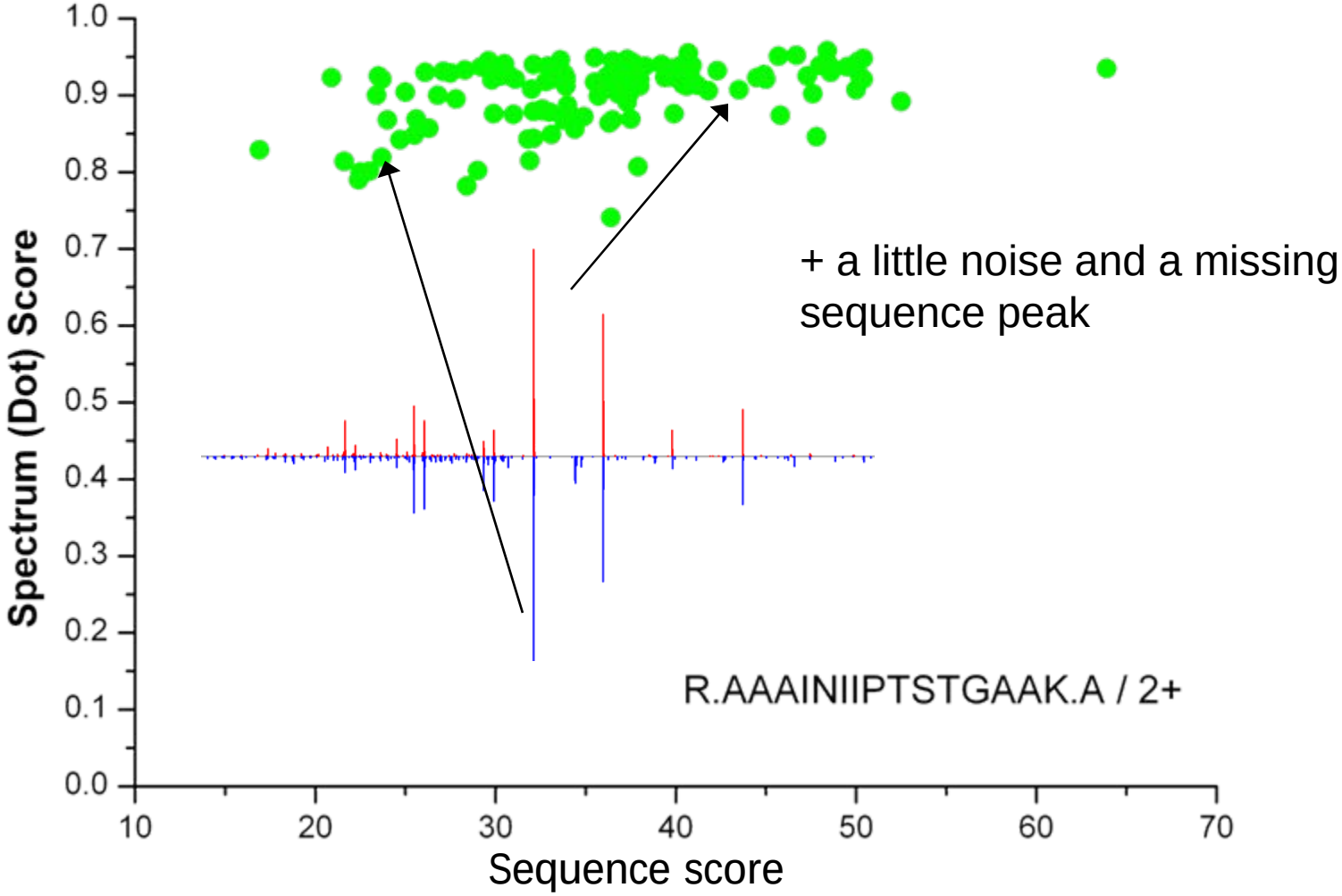
- LIBQUEST (Yates)
  - Yates et al, Anal. Chem., 1998, 70, 3557
- X!Hunter (Beavis)
  - Craig et al, J. Proteome Res., 2006, 5, 1843
- BiblioSpec (MacCoss)
  - Frewen et al., Anal. Chem. 2006, 78, 5678
- Spectral Comparison (Kearney)
  - Liu et al, Proteome Science 2007, 5:3
- SpectraST (Aebersold)
  - Lam et al., *Proteomics* 2007 6, 655-667
  - Lam et al., *in press* (SpectraST tool for building custom libs.)

- NIST Peptide Ion Fragmentation Library
  - June 2006 1st release (US-HUPO – March 2004)

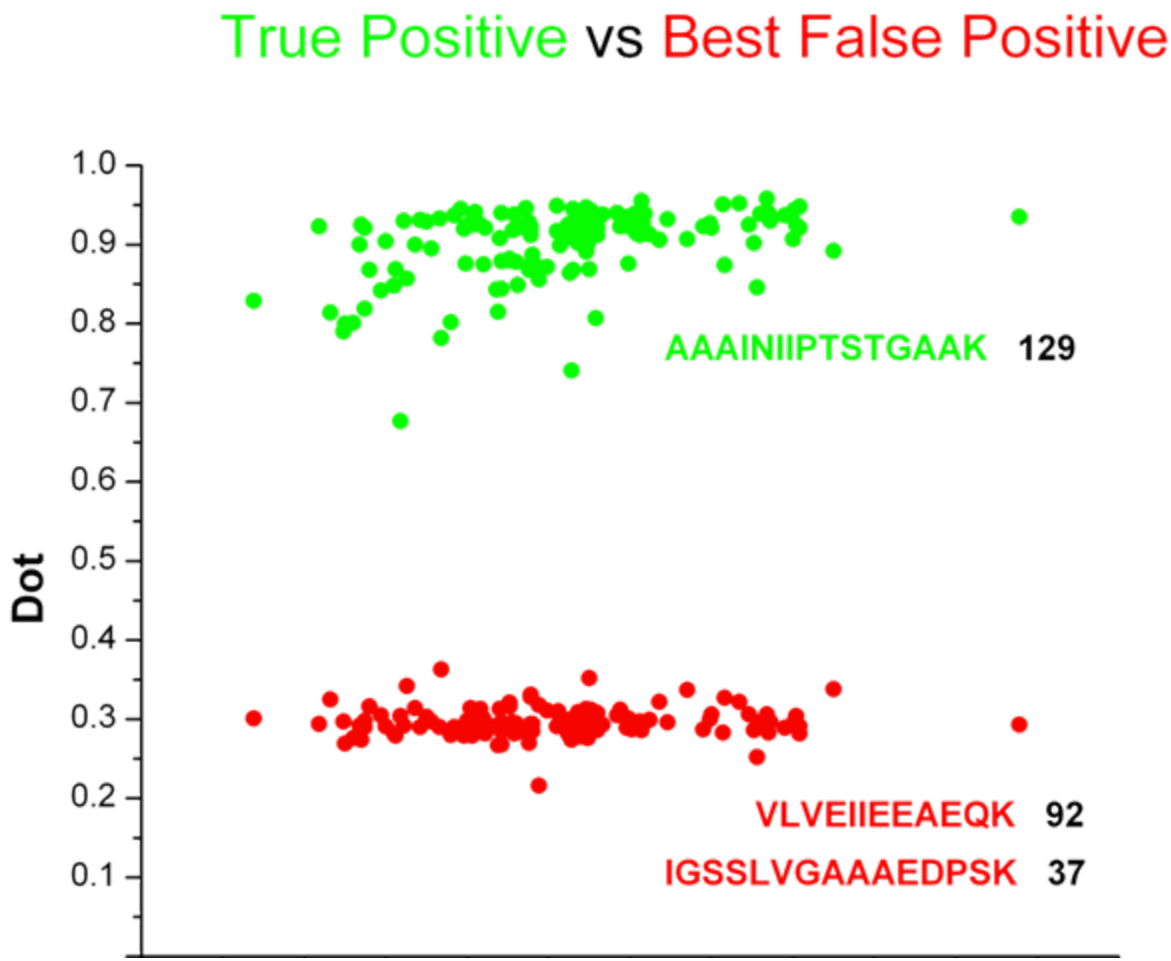
Spectrum/Sequence Scores Vary More than Spectrum/Spectrum Scores



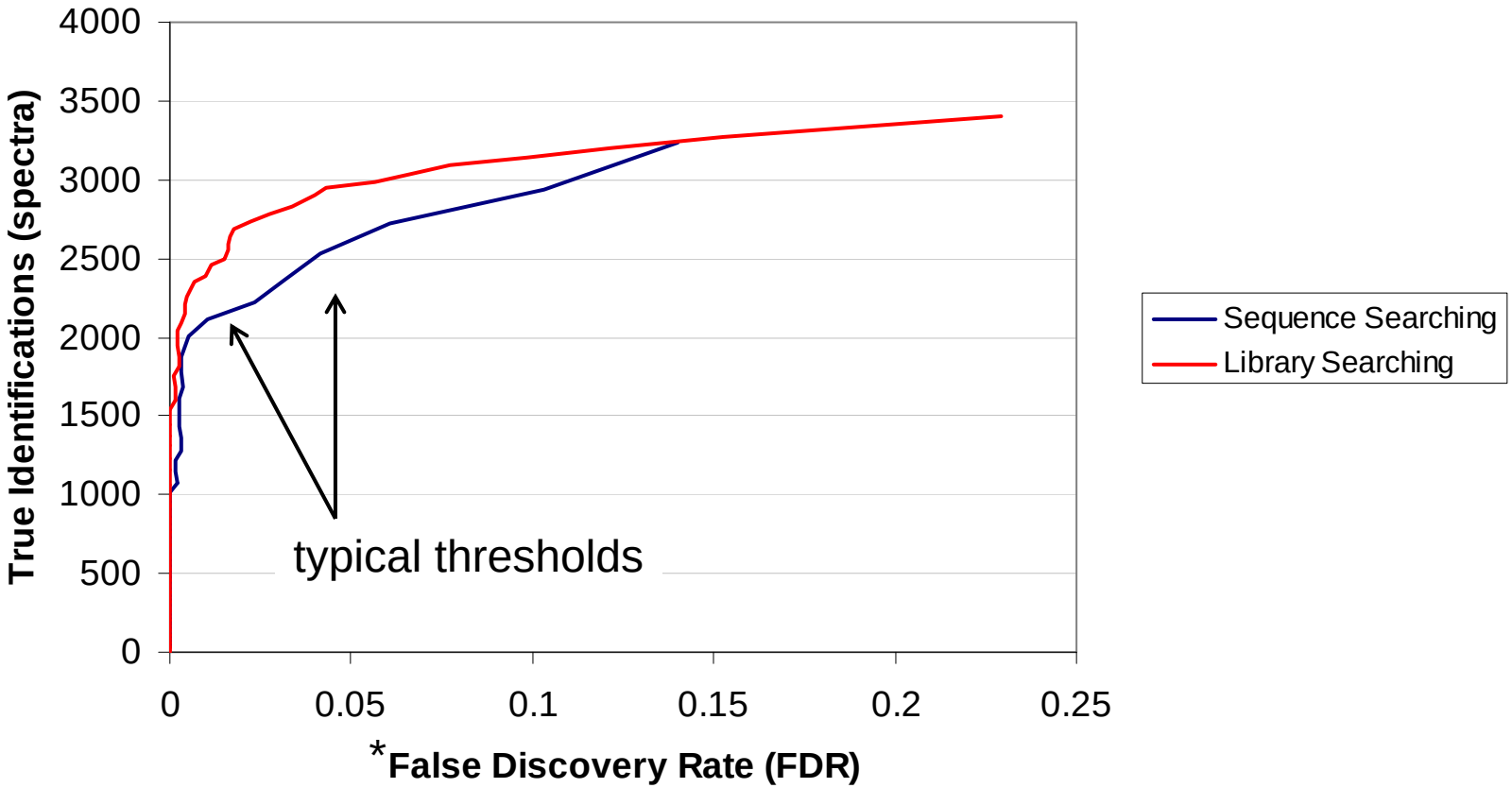
Spectrum/Sequence Scores Vary More than Spectrum/Spectrum Scores



Spectrum/Spectrum Score Separates True and False IDs



A Real Example: Spectral Library Searching Identifies More Peptides



Biological Source: yeast lysate
Separation: 1D RPLC
Instrument: Thermo LTQ (VUMC)
Spectra: 10,227 (not used in library construction)
Sequence Library: (Yeast SGD + reversed sequences)
Spectral Library: NIST Yeast Lib 2007 (78,450 entries)
Decoy Library: *Drosophila melanogaster* (65,503 entries)

*FDR=2F/(F+T) [integral]
'F' does **not** include 'homologous' or identical forward/decoy matches

Comparison of search space: NIST human lib vs. Human IPI sequence database (~50,000 proteins)

Criterion		Search space	
Sequence characteristics	Precursor m/z range	Sequence Search	Spectral Search
Tryptic, with no missed internal cleavage, +2/+3	300 – 2000	**3.5×10^5**	**1.1×10^4**
	800 – 803	**7.2×10^2**	**52**
At least semi-tryptic, with at most 1 missed internal cleavage, +2/+3	300 – 2000	**1.5×10^7**	**1.7×10^4**
	800 – 803	**3.6×10^4**	**77**

leads to a ~500X reduction in CPU time

(Henry Lam, ISB)

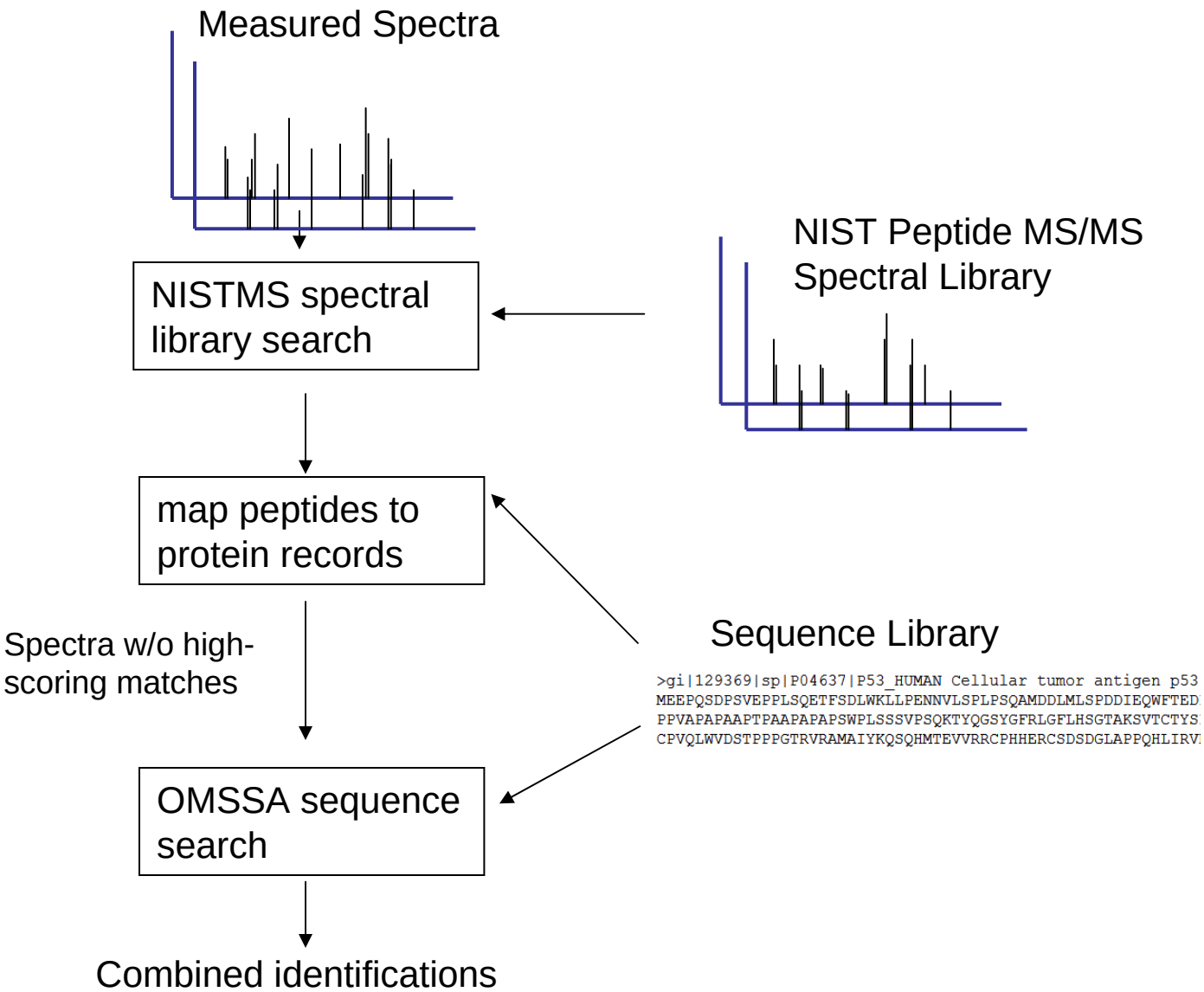
Advantages of Spectral Library Searching

1. *Higher confidence matches*
   - Each entry has been identified by up to 4 algorithms
   - Quality measures ‘built-in’
2. *Scoring is more precise*
   - Natural use of peak intensities
   - Makes full use all annotated peaks (e.g. recurring, non-sequence peaks)
3. *Scoring is more robust*
   - Small changes in spectra give small changes in score
   - Better separation of true and false matches
   - Reduces false positives and false negatives
4. *Faster*
   - Up to 500X faster, search space is limited to technologically-identifiable peptides (i.e.. *m/z* 300-2,000)
5. Bonus: Spectral library searching can also match ‘unidentified’ consensus spectra for later interpretation (e.g. SNPs, extensive known/unknown PTMs)

Integrating Sequence Searching with Spectral Library Searching

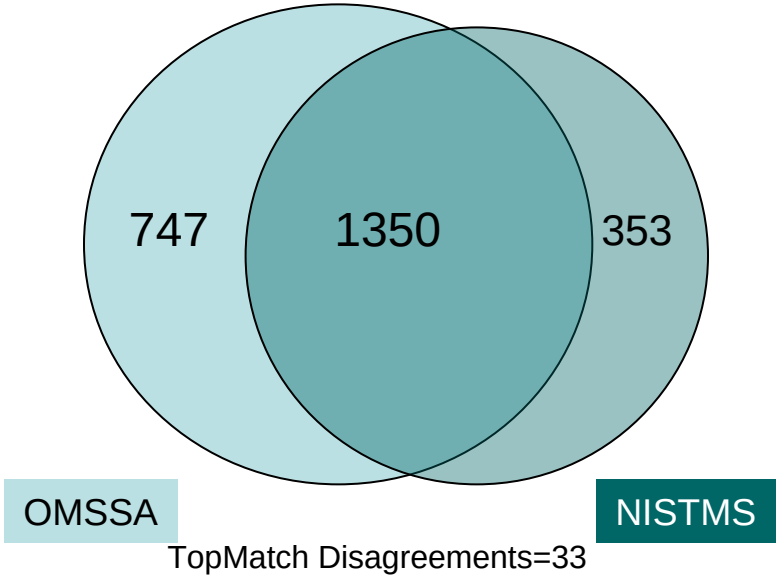
- Problem: Most missed IDs are because the peptide ions are not represented in the library
- Solution: Create a combined search program for matching sequences when no spec lib match is found
- Components
  - NISTMS, 'NIST MS Search 2.0' handling and scoring routines
  - OMSSA (Lewis Geer/NCBI), probability-based, sequence search engine
  - 'Pep2Prot' matches spec lib peptides to proteins for reporting
  - Wrapper script
  - Tools for handling/parsing output
  - Methods for calculating false discovery rate
  - Browser

OMSSA/NISTMS Workflow

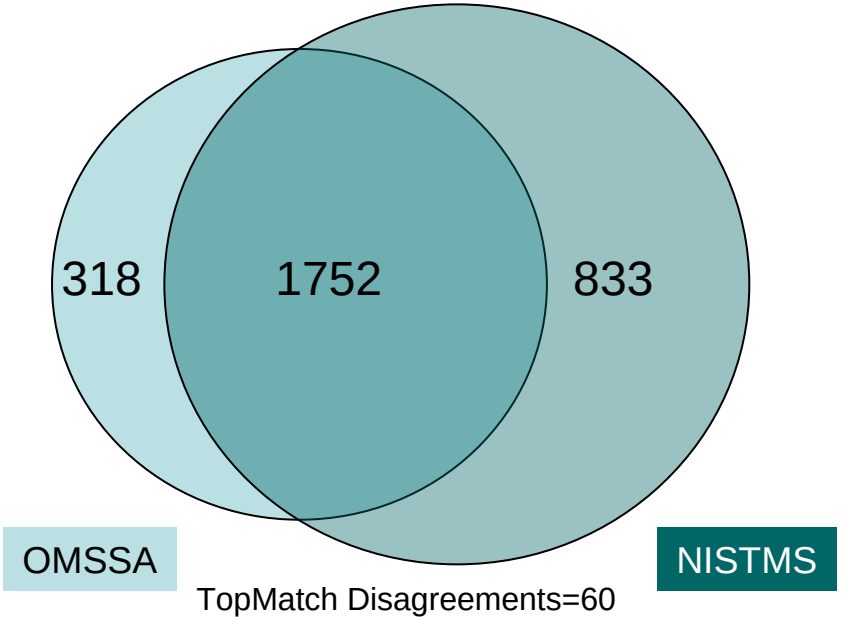


Separate & Combined Performance

NIST Yeast Lib. (06/06)



NIST Yeast Lib. (05/07)



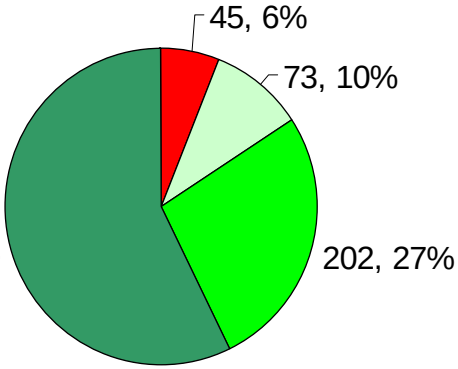
10,227 LTQ Spectra (1% FDR)	Spectra in Library	Spectral Counts				Unique Peptides	
		OMSSA	NISTMS	Union	Gain over OMSSA alone	OMSSA	NISTMS
NIST Yeast Peptide Fragmentation Lib. 06/06	34,426	2,130	1,736	2,450	+15%	1,677	1,375
NIST Yeast Peptide Fragmentation Lib. 07/07	78,450	2,130	2,645	2,903	+36%	1,677	1,974

Bonus OMSSA Matches

Green = ‘not in spec lib’

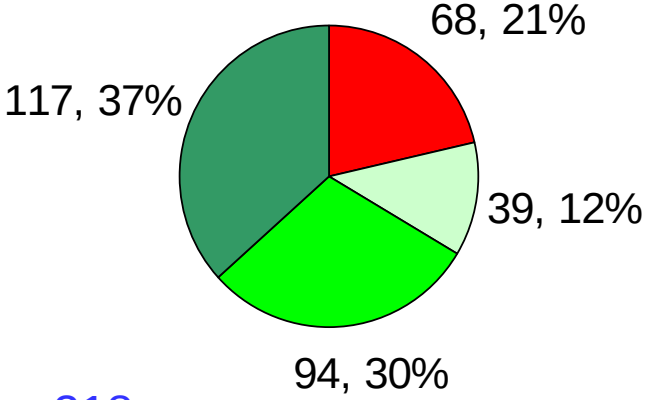
Red = below NISTMS threshold or no match

2006



747

2007



318

- In spec. lib. same charge/mods
- In spec. lib. same charge, diff. mods
- In spec. lib. same sequence, diff. charge
- Not in spec. lib.

OMSSA/NISTMS – ‘pipeline’

- Accepts common input formats (dta, mgf, pkl, etc.)
- NISTMS will run as a pre-search against any ‘.msp’- formatted NIST reference library
- All NISTMS scores used by MS Search 2.0 included in output
- OMSSA will run as an iteration with optional ‘E-value’ cutoffs for spectra and/or protein sequences to include
- Either an NISTMS or an OMSSA match is returned
- Peptide matches from NISTMS mapped to proteins
- Output is one OMSSA search result file (XML or ASN.1)
- Output will be viewable in the OMSSA Browser
- OMSSA available now- http://pubchem.ncbi.nlm.nih.gov/omssa/
- When finished (2007), will be freely available for testing (paul.rudnick@nist.gov).

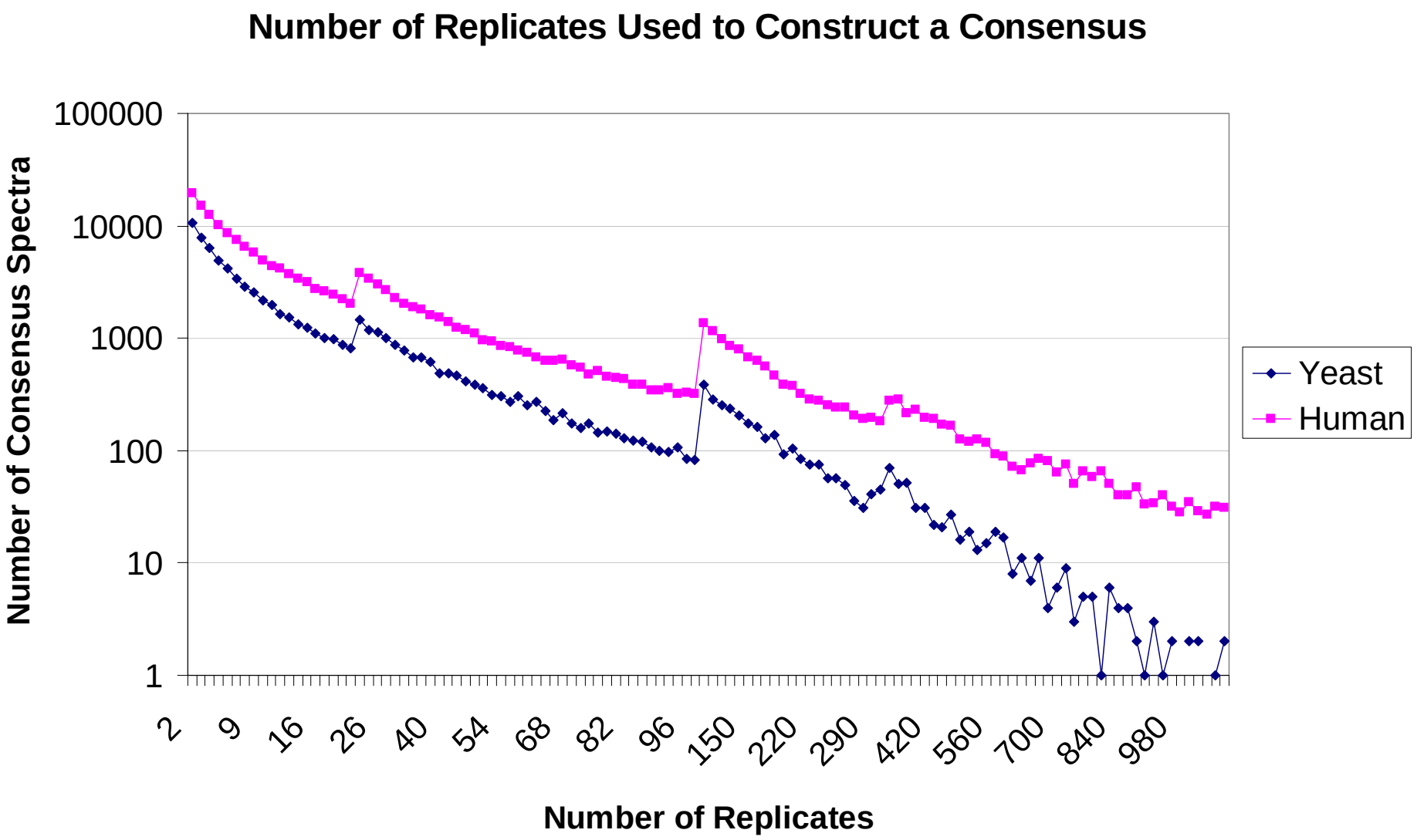
NIST Peptide Fragmentation Libraries: Status Update

Ion trap

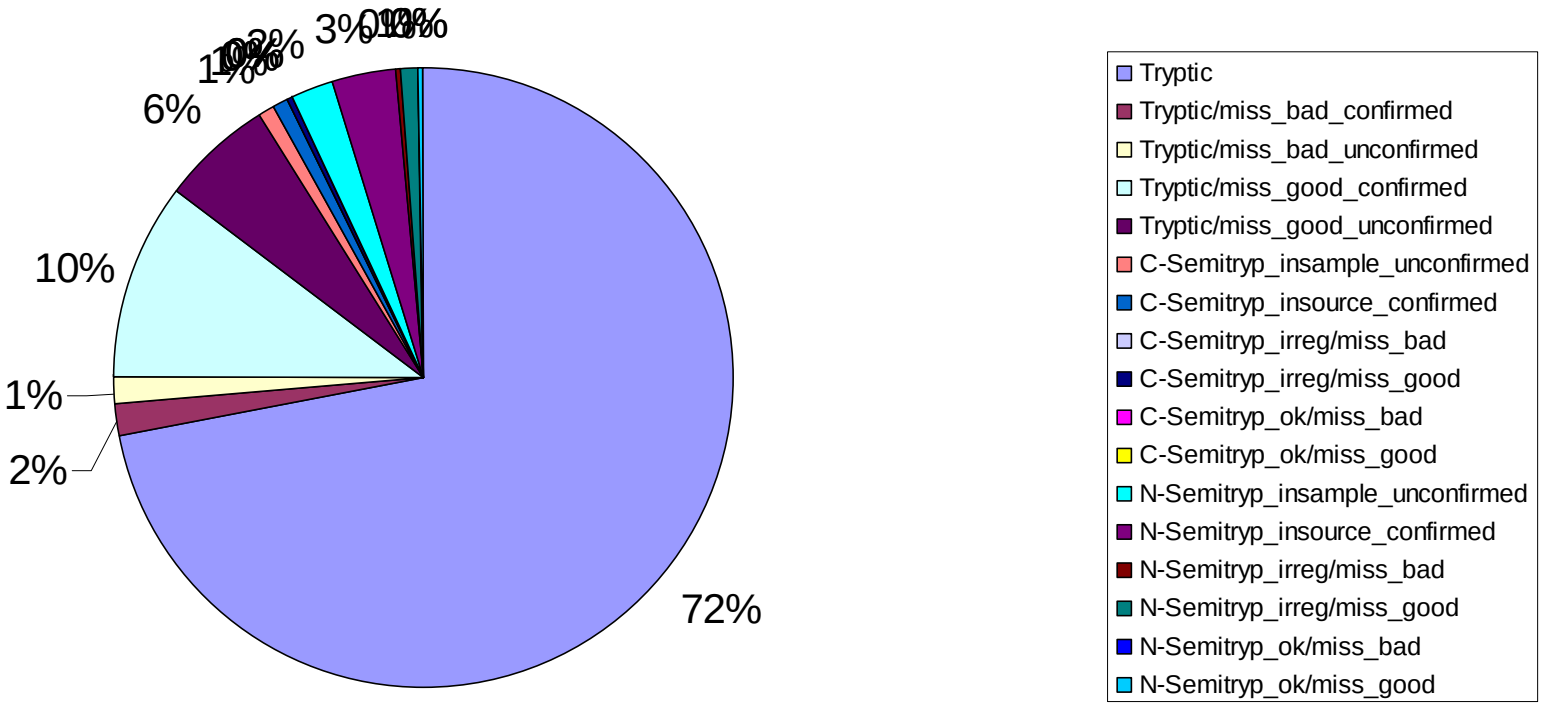
2007 Library	*Increase over '06*	*Approx. Coverage (simple inference)*	*Datasets used*	*Peptide Identifications*	*Consensus spectra*	*Different sequences*	*+1*	*+2*	*+3*	*+4*
Human	4.1x	13%	274	17,779,962	179,857	112,945	15,802	107,700	50,891	5,464
Yeast	2.2x	20%	59	3,535,532	76,044	46,819	5,569	46,125	23,206	1,144

Both new libraries set for release (09/07).

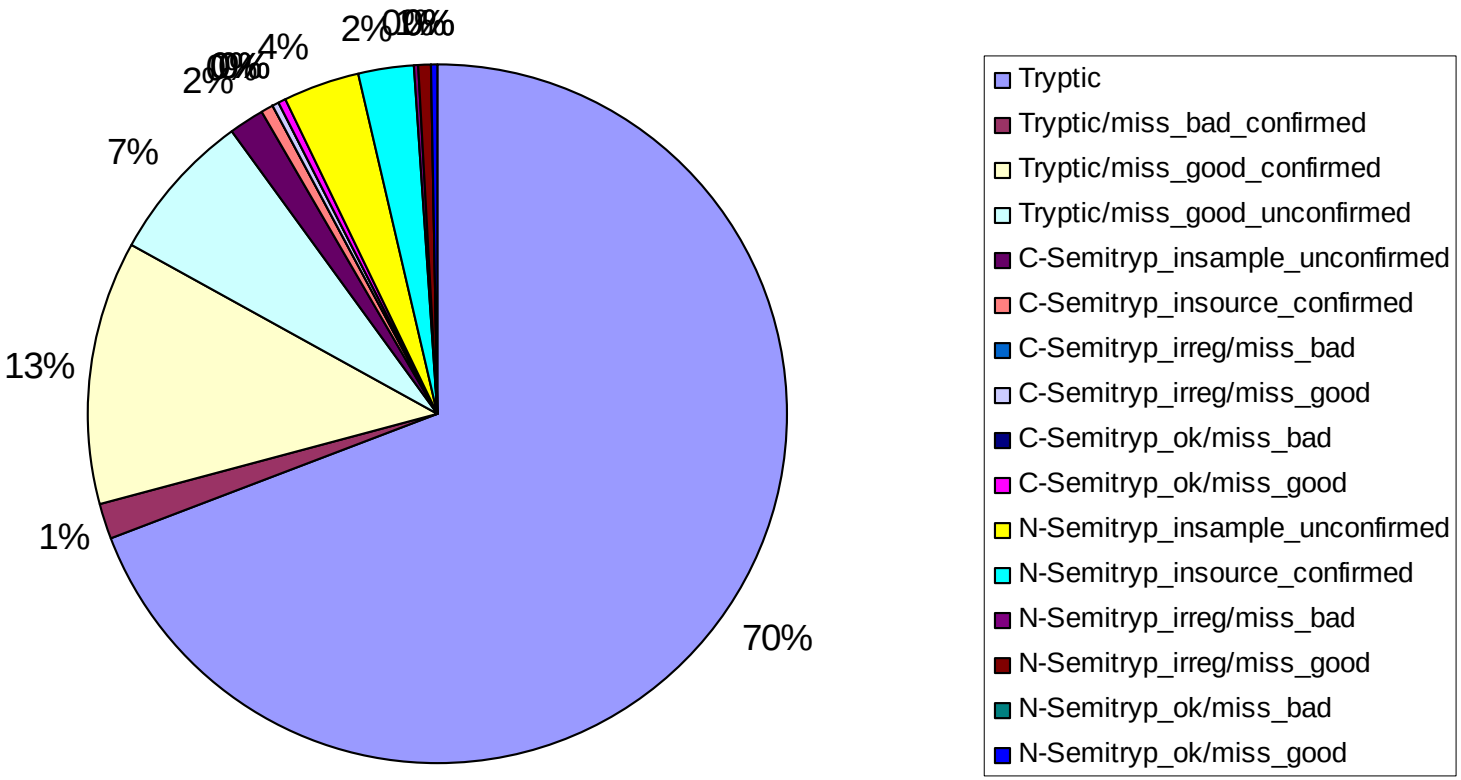
http://www.peptideatlas.org/speclib/


Number of Replicates Used to Construct a Consensus
Number of Consensus Spectra
100000
10000
1000
100
10
1
2
9
16
26
40
54
68
82
96
150
220
290
420
560
700
840
980
Number of Replicates
Yeast
Human

Human Peptides



Yeast Peptides



Next

- More data to improve releases (human > mouse > rat > yeast > fly > microbes / viruses – all instruments)
- Custom library building available by agreement
- More ‘batch’ software and integration with existing systems
- NIST’s focus is on library construction and maintenance
- Development of specialized applications (e.g. ‘real-time’ exclusion lists)

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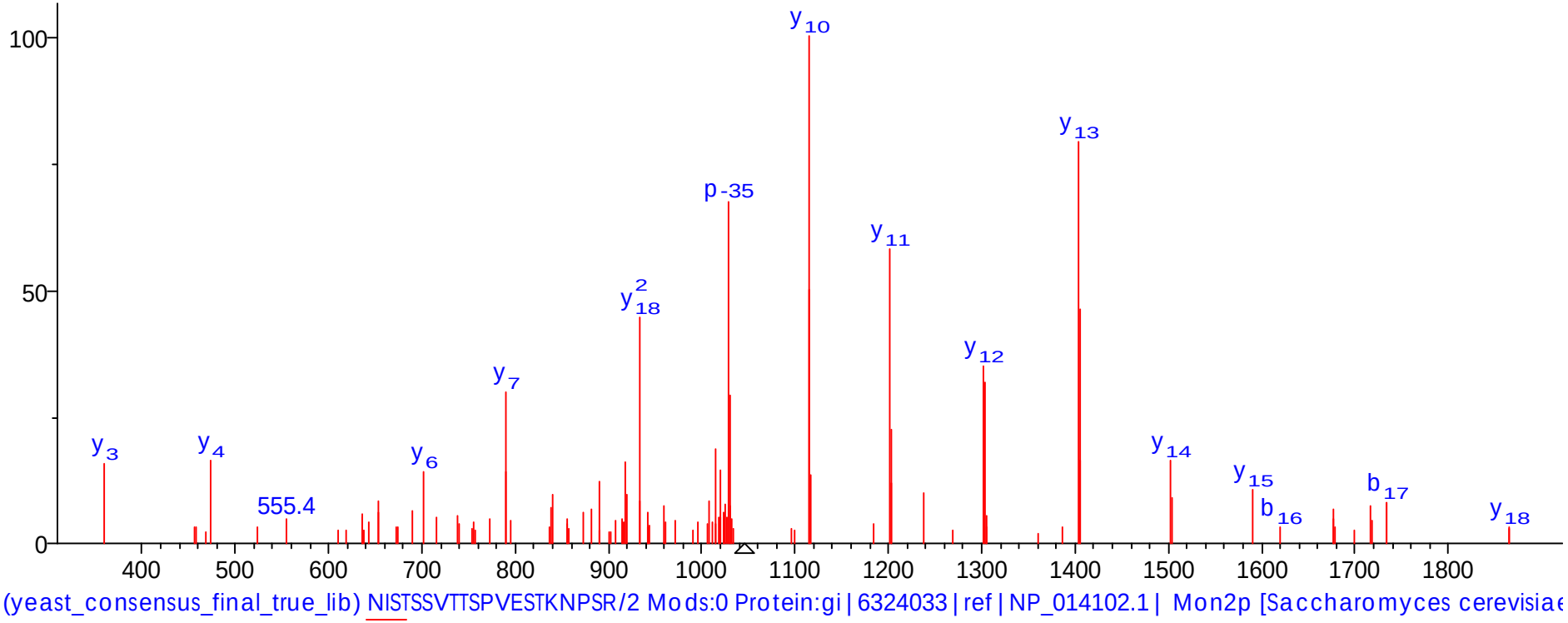
Thank YOU to everyone making public and private data donations!

End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p-35
y11
y2 18
y12
y7
y3
y4
y6
555.4
y14
y15
b17
b16
y18
(yeast_consensus_final_true_lib) NISTSSVTTSPVESTKNPSR/2 Mods:0 Protein:gi|6324033|ref|NP_014102.1| Mon2p [Saccharomyces cerevisiae