Enhanced information extraction by photo-fragmenting peptide and protein ions

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Conventional protein ID by MS

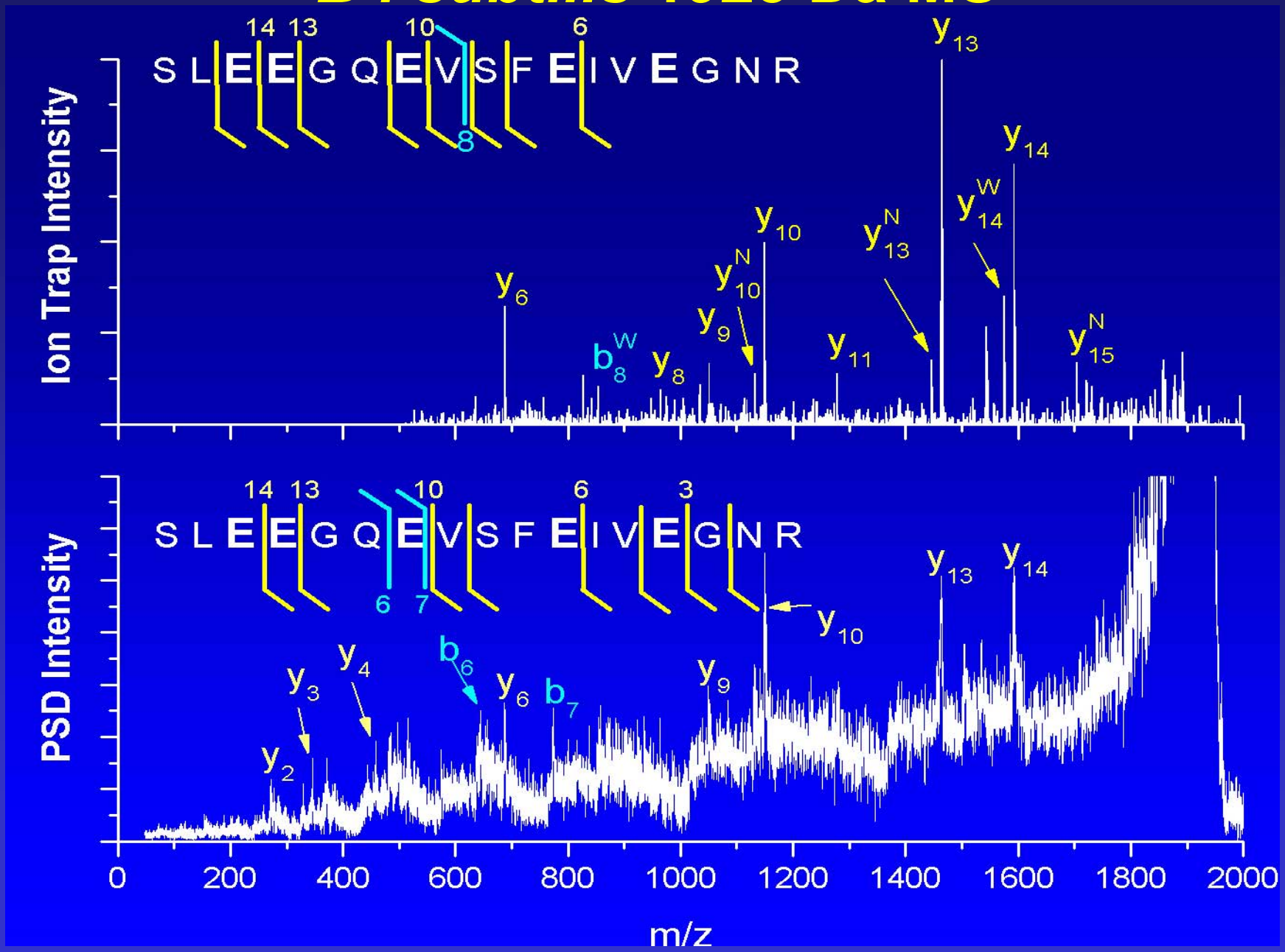
- Protein molecular weight

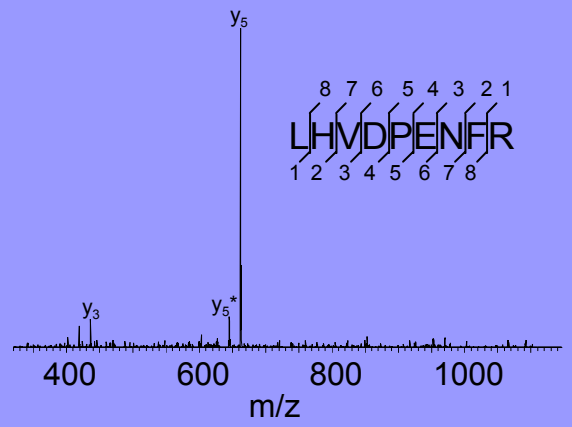
- Proteolytic fragment analysis
  - peptide mass mapping
  - peptide ion fragmentation

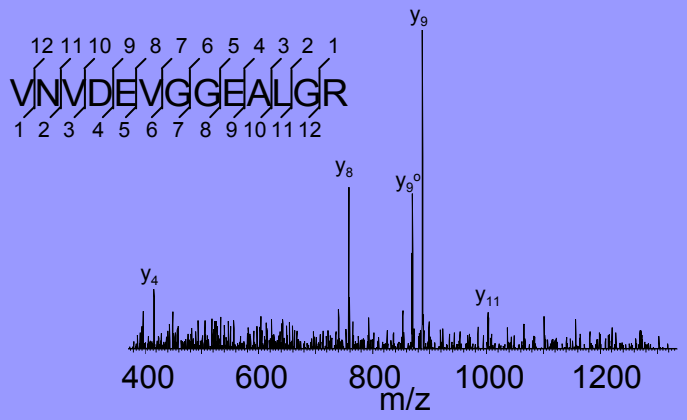
Ion fragmentation shortcomings

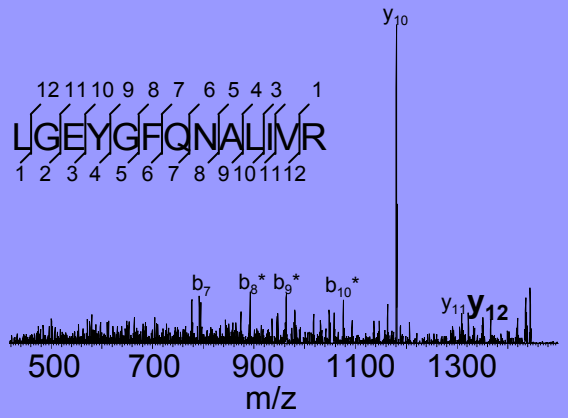
- Errors in proteome sequencing
- Mutations in proteome
- Post-translational modifications
- Sample handling modifications
- Spectra often hard to interpret
  or contain limited information

B . subtilis 1923 Da MS^2




y5
8 7 6 5 4 3 2 1
LHVDPENFR
1 2 3 4 5 6 7 8
y3
y5*
400
600
800
1000
m/z


y9
12 11 10 9 8 7 6 5 4 3 2 1
VNVDEVGGEALGR
1 2 3 4 5 6 7 8 9 10 11 12
y8
y9o
y4
y11
400
600
800
1000
1200
m/z


y10
12 11 10 9 8 7 6 5 4 3 1
LGEYGFQNALIVR
1 2 3 4 5 6 7 8 9 10 11 12
b7
b8*
b9*
b10*
y11
y12
500
700
900
1100
1300
m/z

Typical Scenario

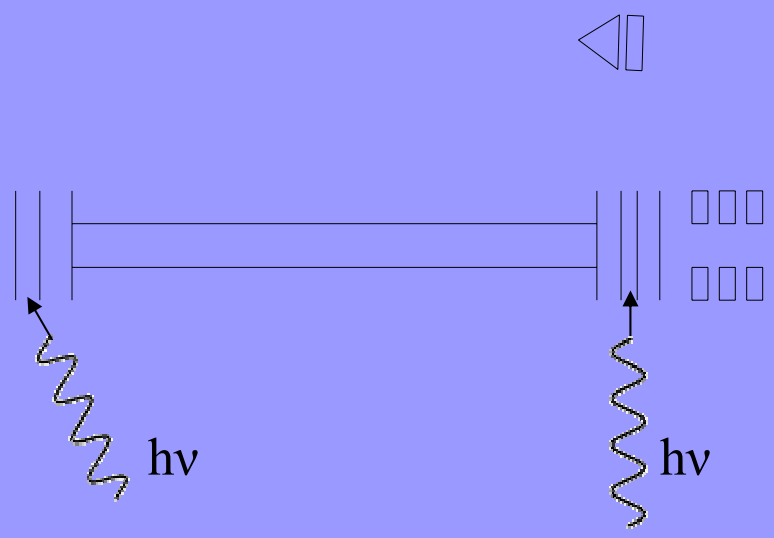
"More than 162,000 MS/MS spectra were collected with 26,815 matched to yeast peptides."

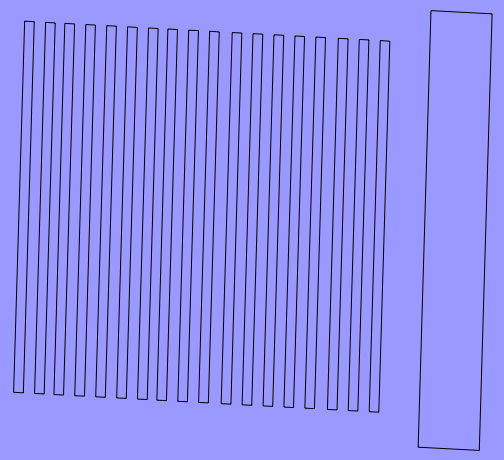
J. Proteome Research **2**, 43 (2003)

Alternative strategy:

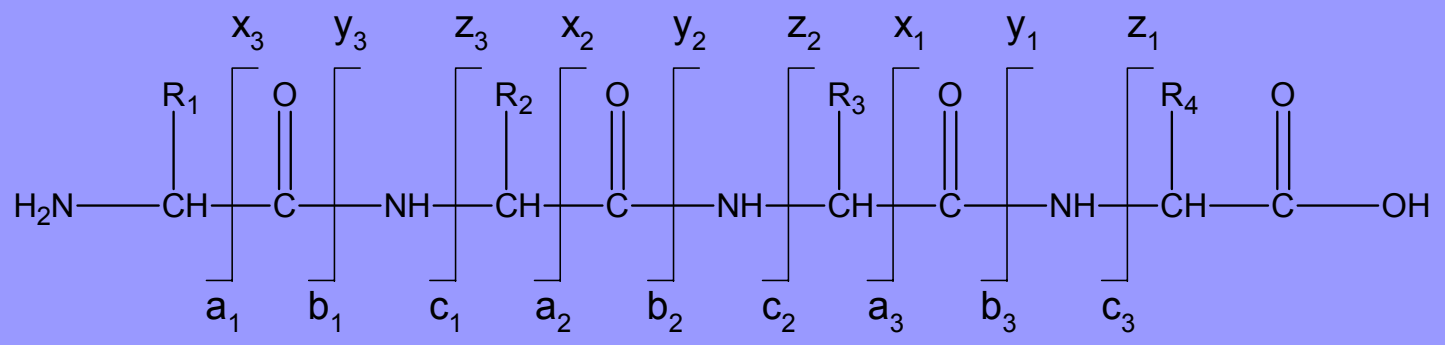
VUV Photofragmentation

Peptide photodissociation by MALDI-TOF/TOF





Peptide fragment nomenclature



Experimental Conclusions

- Unique, non-thermal fragmentation
- Relatively uniform fragment intensity
- Much improved sequence coverage

  => better mass matching

  => *de novo* sequencing

De novo sequencing

- Interpret MS
- Accelerate database searches
- Avoid confusion caused by PTMs, sample handling modifications, genome sequencing errors and mutations

M Q P S G G L F R

M\Q\P\S\G\G\L\F\R → QPSGGLF

M Q P X G G L F R

M\Q\P\X\G\G\L\F\R → QP?GGLF

Conclusions…

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- Dr. Noah Christian
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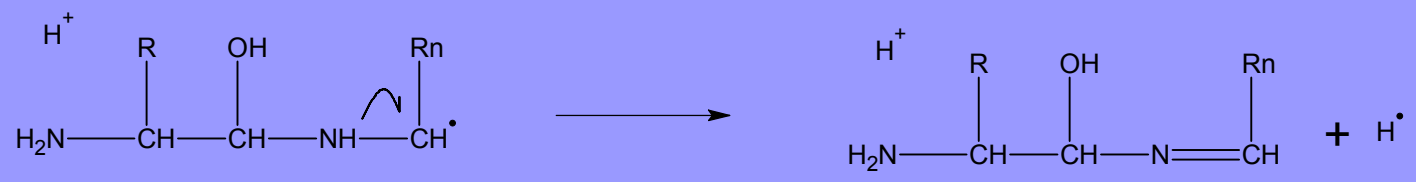
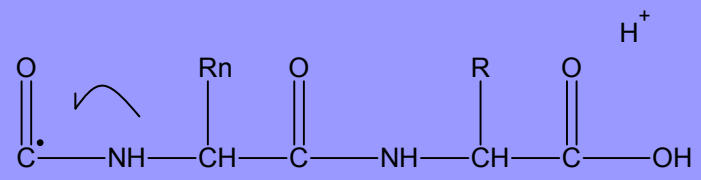
a_n ion formation



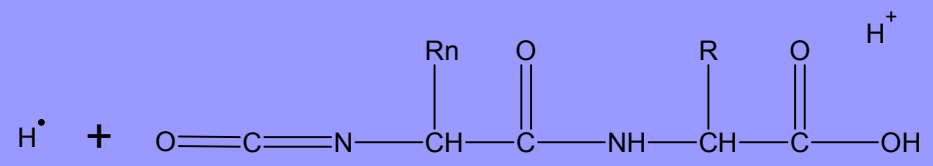
a_n+1 radical precursor

standard a_n ion

x_n ion formation



x_n radical ion precursor



Standard x_n ion