Isotopic-Labeling and Mass Spectrometry-Based Quantitative Proteomics

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Day 4

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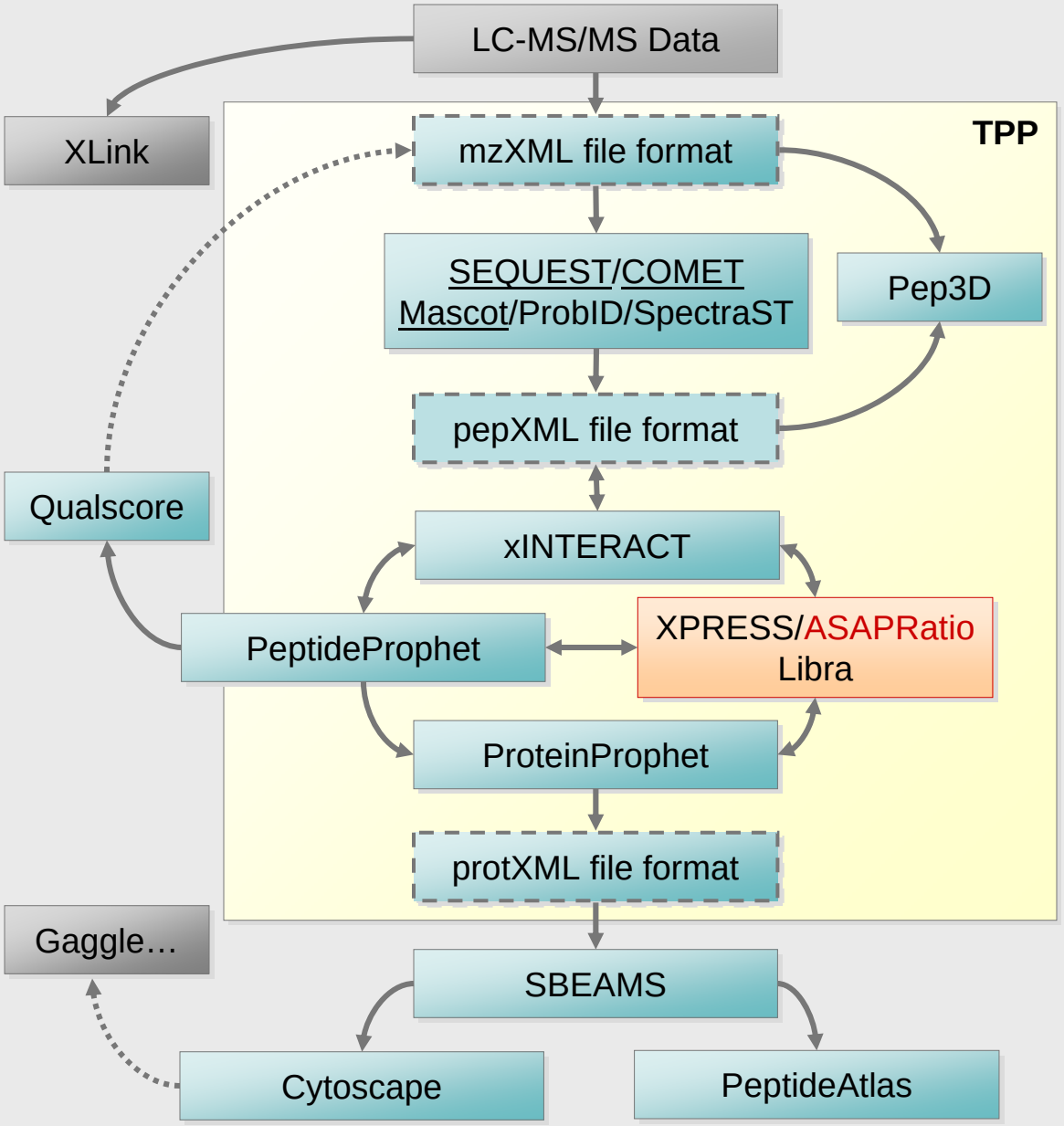
INSTITUTE FOR Systems Biology

Protein Quantification



Outline

- Principles of quantitative proteomics using LC-ESI-MS/MS
- Introduction to ASAPRatio: software tool for quantitative proteomics using isotopic labeling

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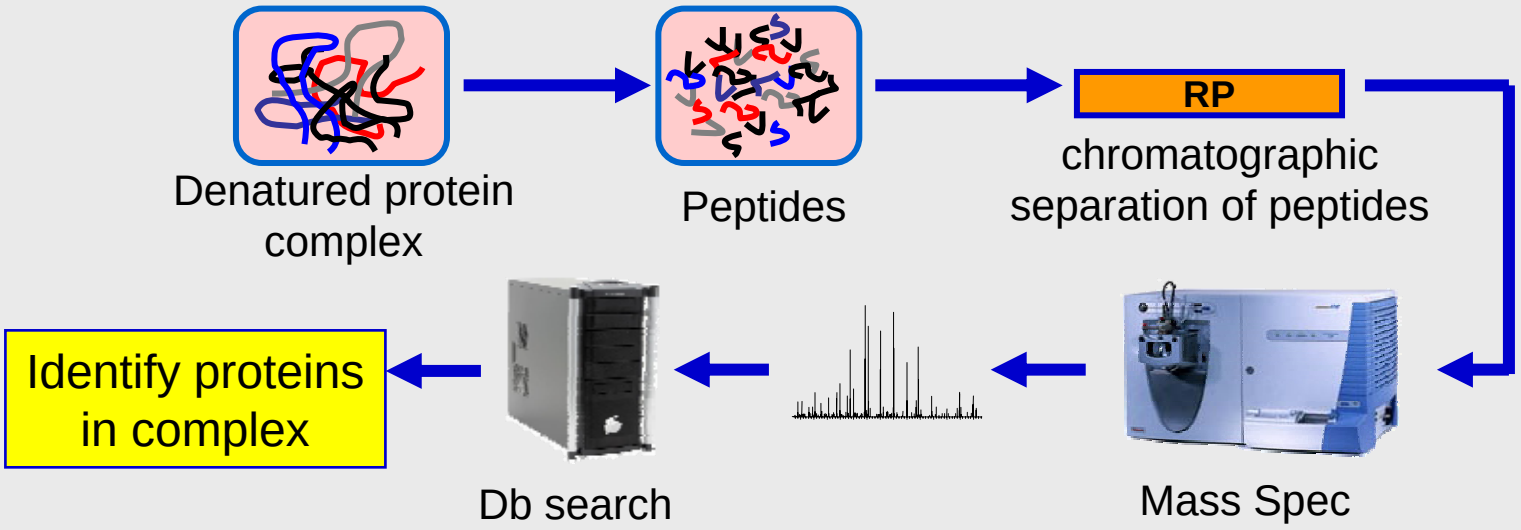
Summary of LC-ESI-MS/MS

- Protein mixtures are digested into peptides
- Peptides are concentrated and fractionated by separation technologies such as SCX, IEF, RP, etc.
- While eluting from RP column, peptides are ionized by ESI and analyzed by MS/MS
- Peptides are identified from CID spectra
- Peptides are usually quantified from MS signatures
  - Except in the case of iTRAQ



Complications

Shotgun MS detects peptides not proteins

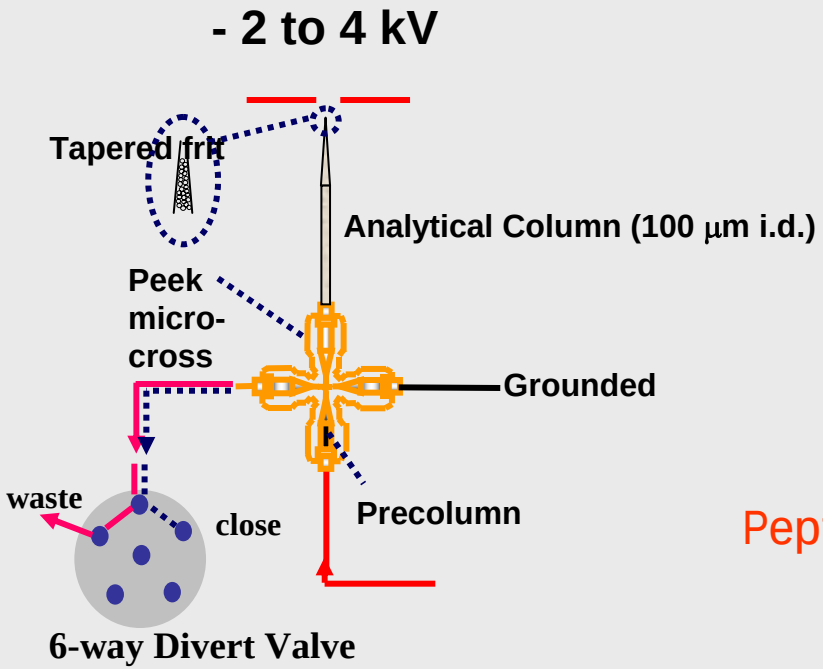
- Multiple peptides per protein
- Multiple proteins per peptide

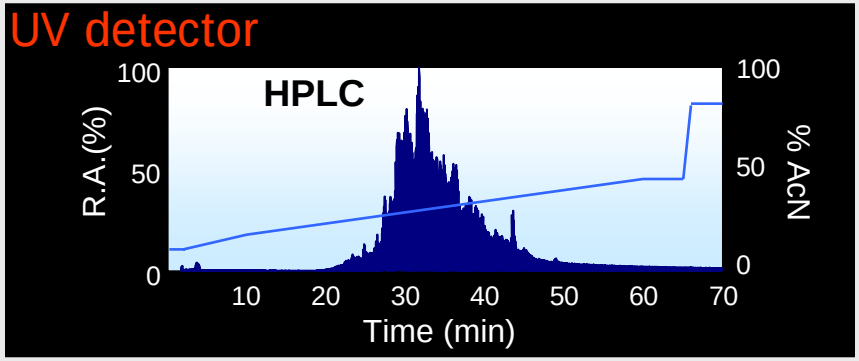
Strong Cation-Exchange Chromatograph

- Fair but not great separation power
- Same peptide separated into several fractions

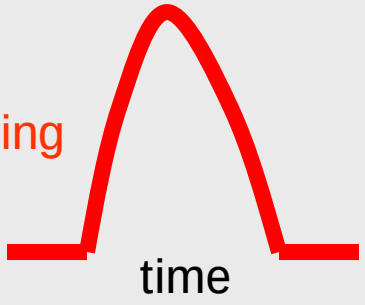
Reversed-Phase Chromatography

Reproducible: but a few erratic data points may exist





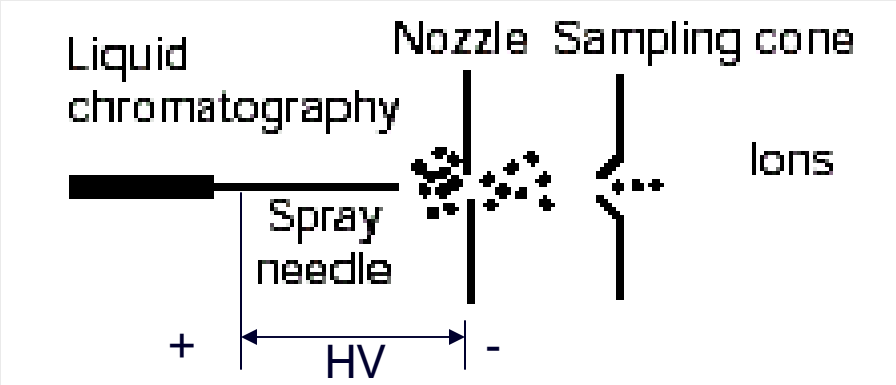
Peptide eluting profiling

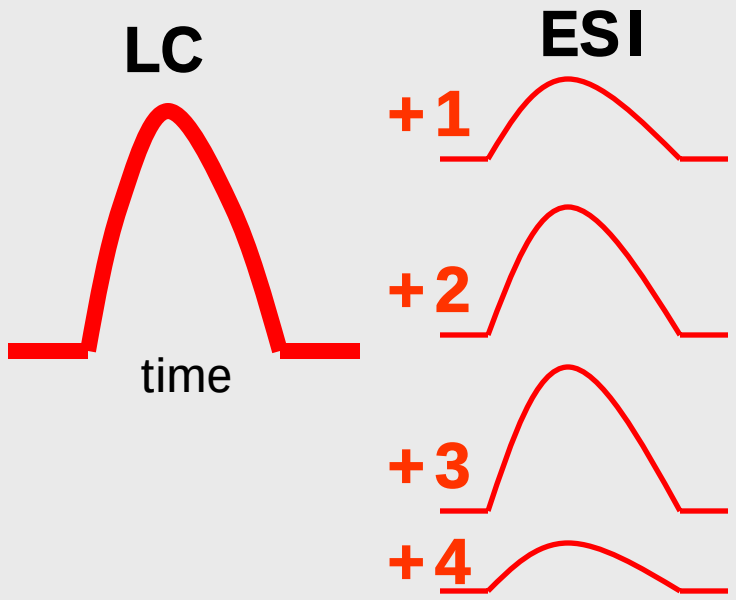


Electrospray Ionization

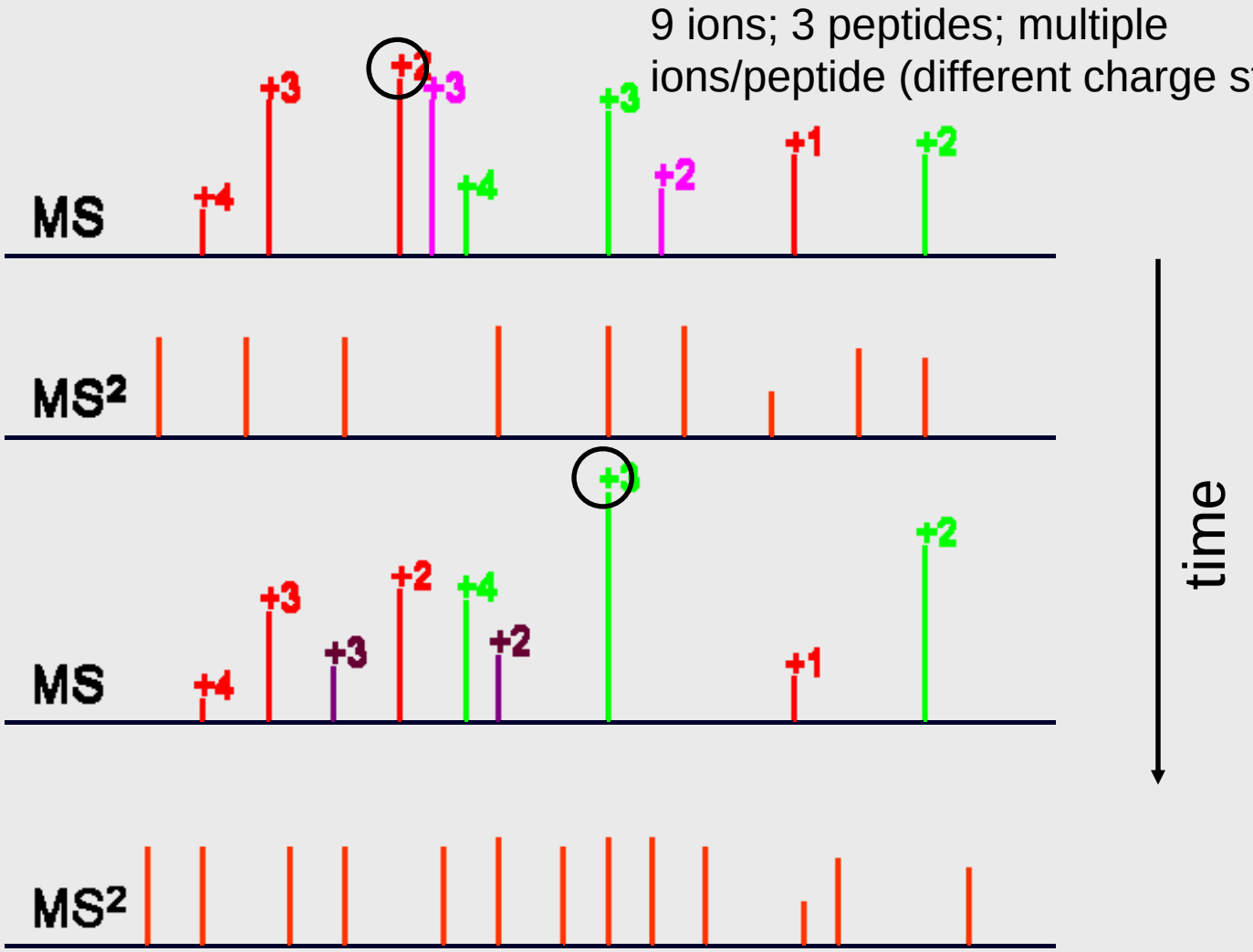
- Multiple charge states: from +1 to +4

$M + z\ H^+ = M(H^+)_z$ $\qquad$ $m/z = (M+z*H)/z$



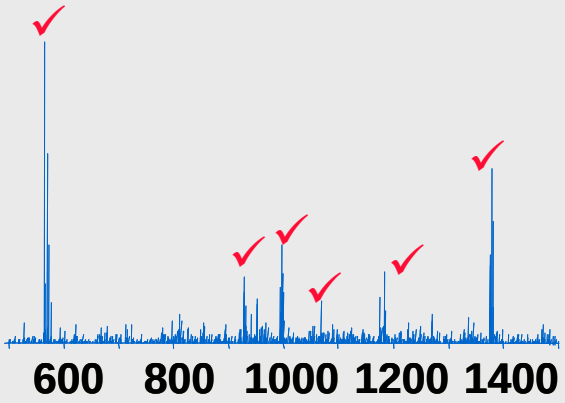


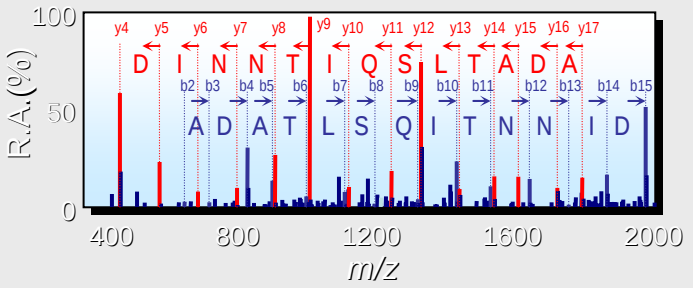
ESI-Tandem Mass Spectrometry



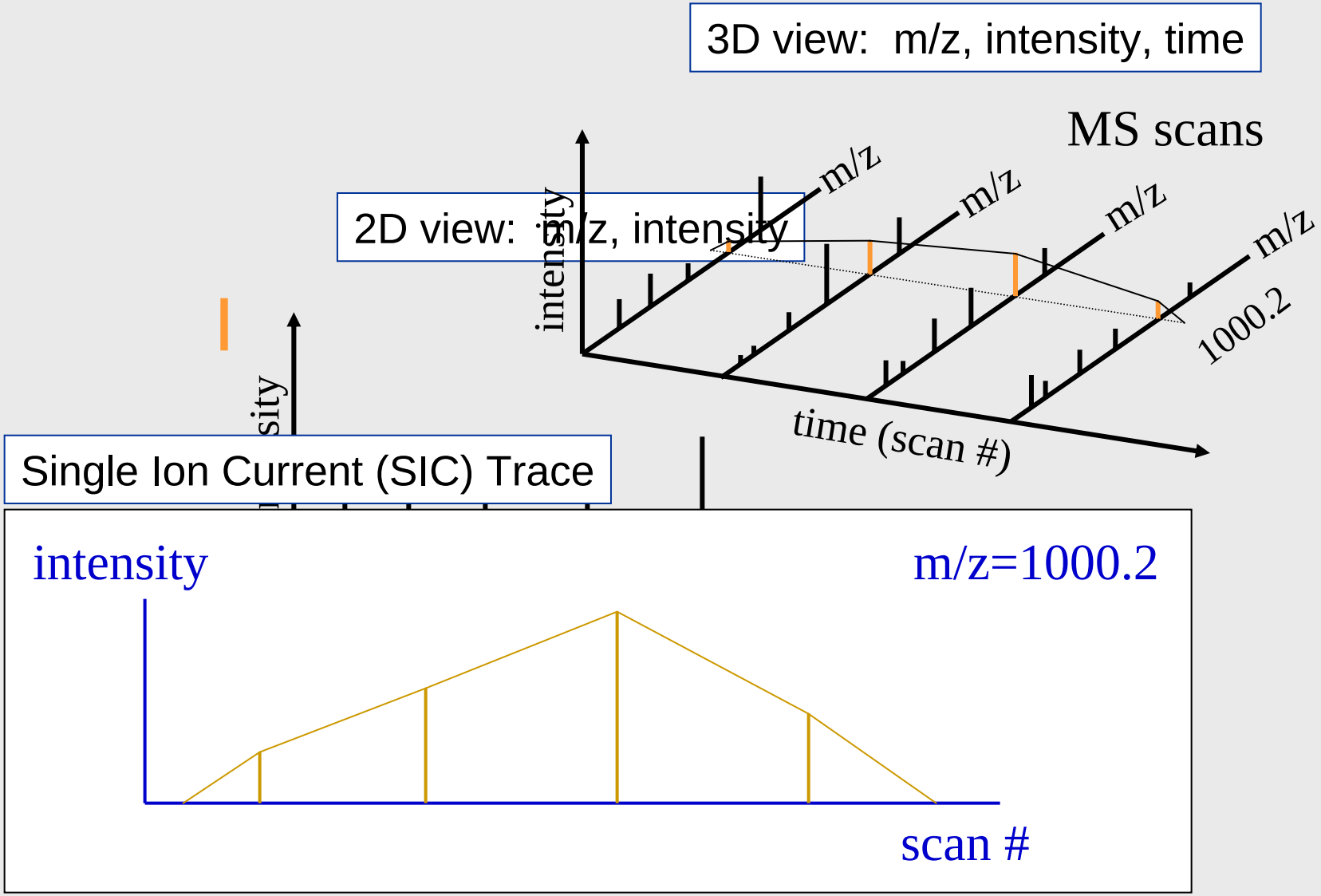
Peptide Identification

- Match CID (MS/MS) spectra with database
  - SEQUEST, MASCOT, X!Tandem, …
- Multiple IDs for the same peptide
  - different isotopes: light and heavy
  - different charge states: +1, +2, +3
  - repeating IDs: same isotope and same charge state

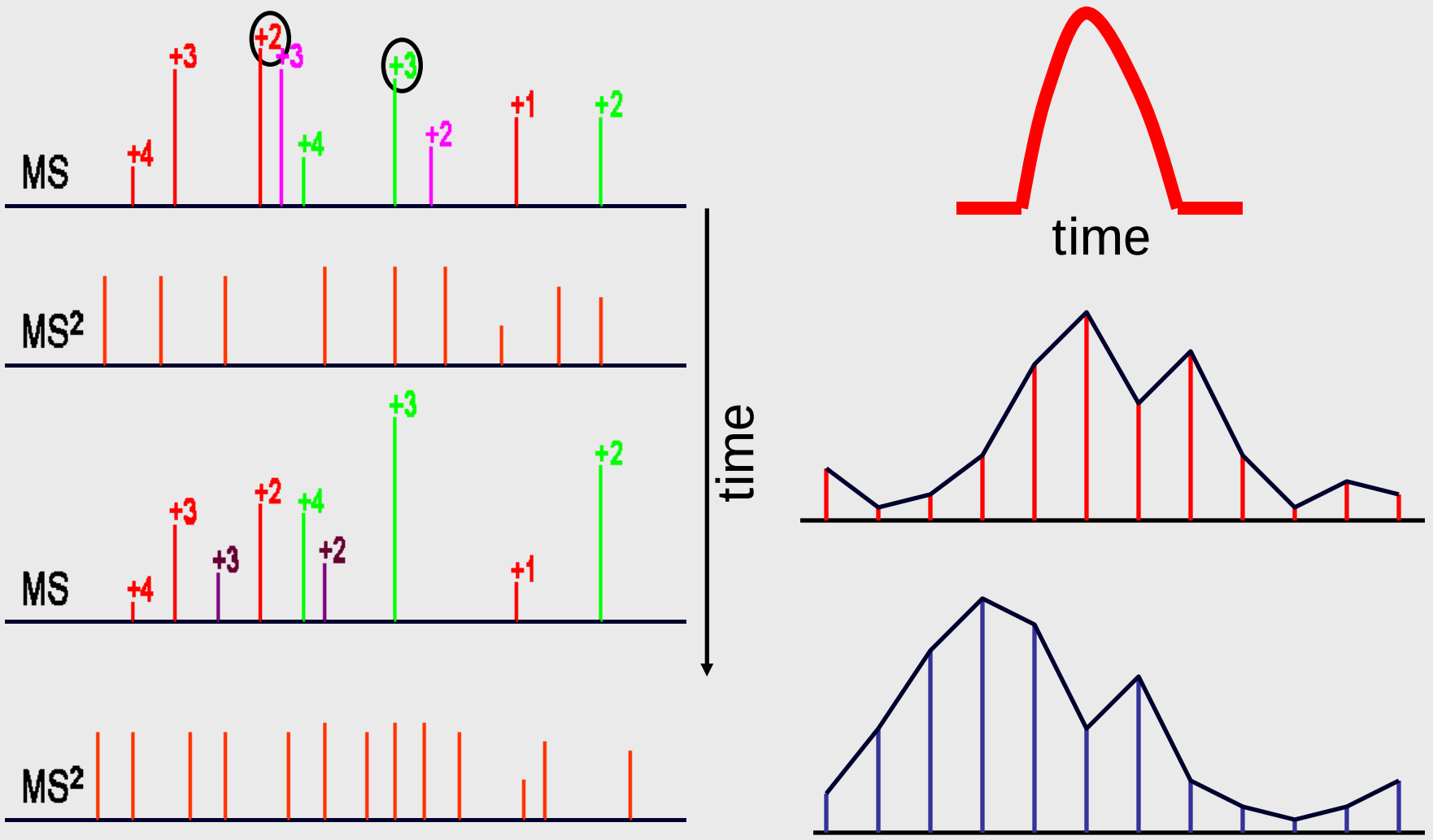




Single Ion Chromatogram



Single-Ion Chromatogram



Peptide Quantitation

- Area under SIC is proportional to peptide abundance
- **PROBLEM**

  Ionization efficiency of each peptide is different

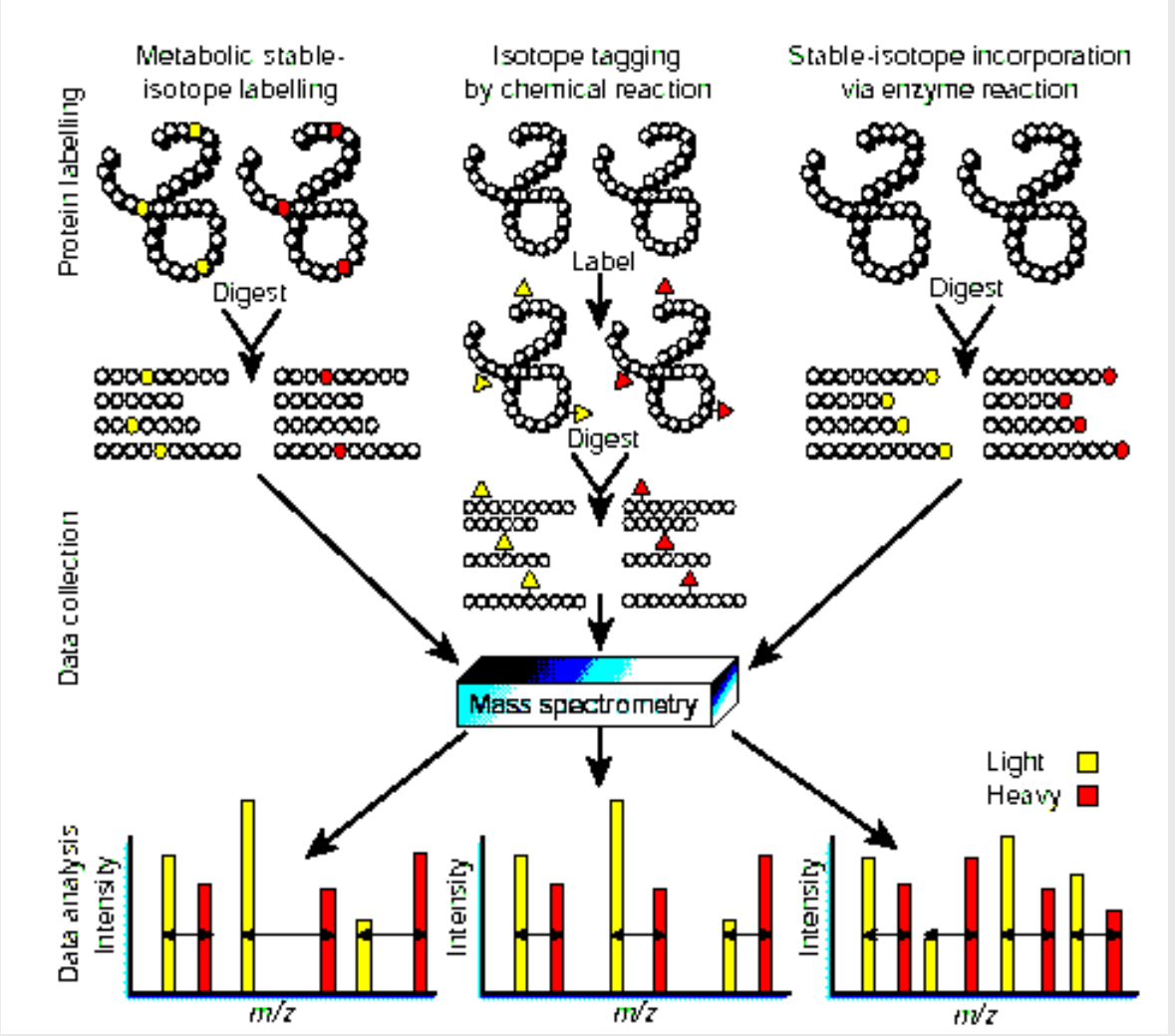
  – Depends on the peptide molecular properties (e.g. number of basic residues)
- **ONE SOLUTION**

  Samples labeled with different stable isotopes

  - Chemically identical
  - Peptides are identified before quantification
  - Distinguishable by MS in mass shift
  - Peptide abundance ratio measured by ratio of SIC areas

Different Labeling Methods

- Metabolic labeling ^{13}C, ^{15}N, SILAC
- Chemical reaction ICAT, cleavable ICAT iTRAQ
- Enzyme reaction ^{18}O



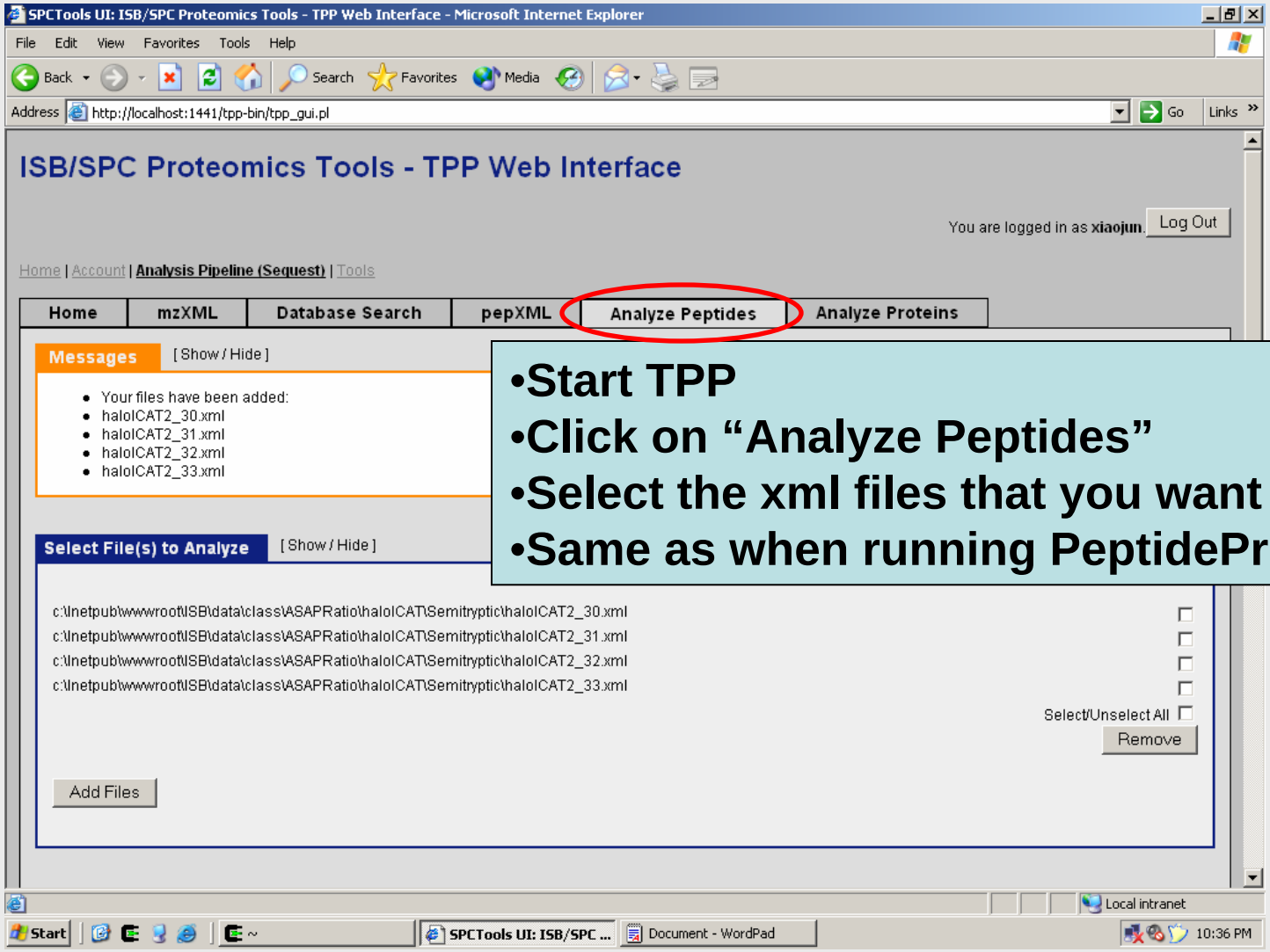
Summary of Quantitative LC-MS/MS Approach

- Samples are isotopically labeled
- Simultaneously identify & quantify thousands of proteins in complex samples
- Accuracy: ±20-30%
- Dynamic range: ~100 fold

Outline

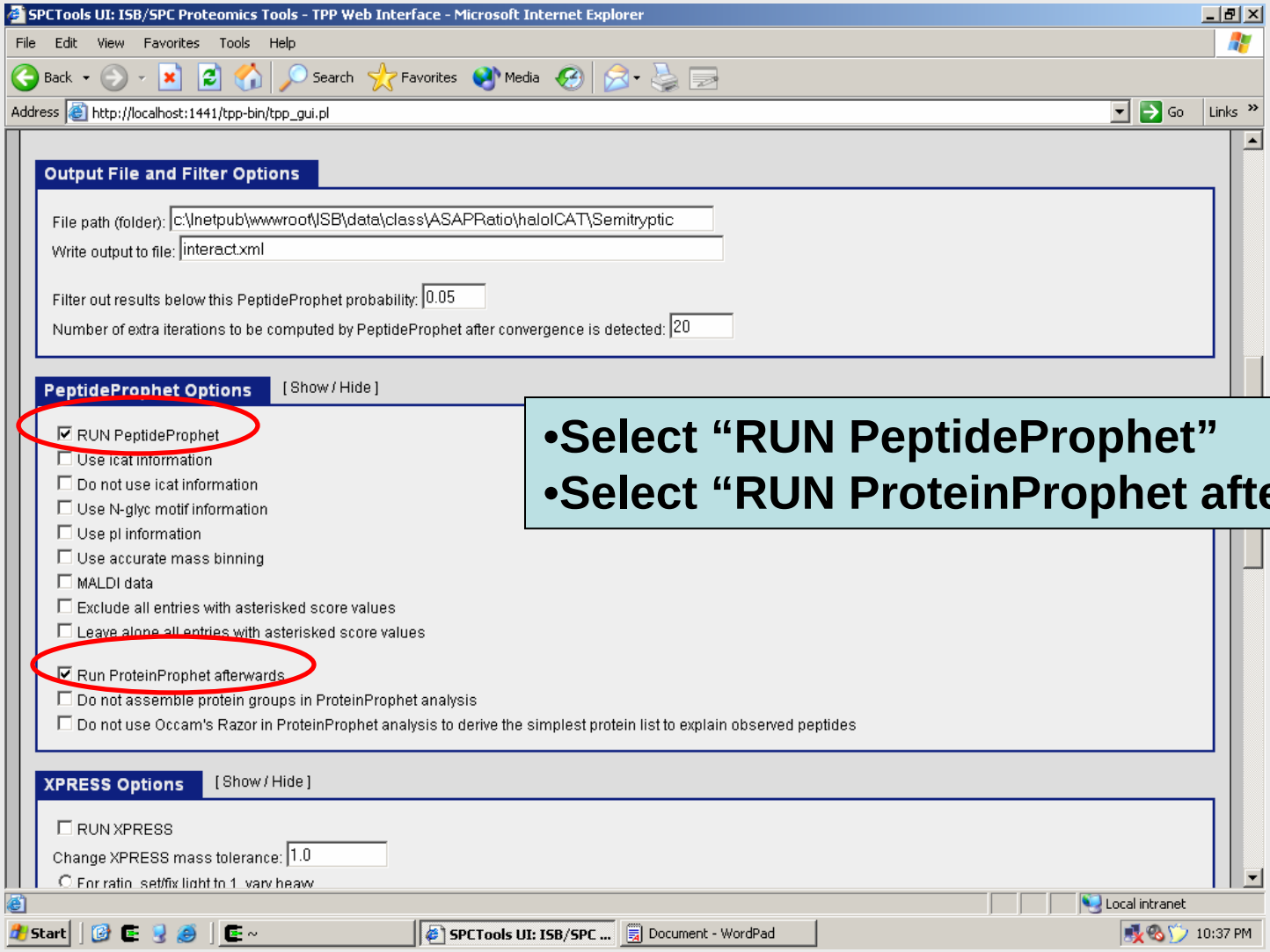
- Principles of quantitative proteomics using LC-ESI-MS/MS
- Introduction to ASAPRatio: software tool for quantitative proteomics using isotopic labeling

How to Use TPP for Data Analysis in Quantitative Proteomics



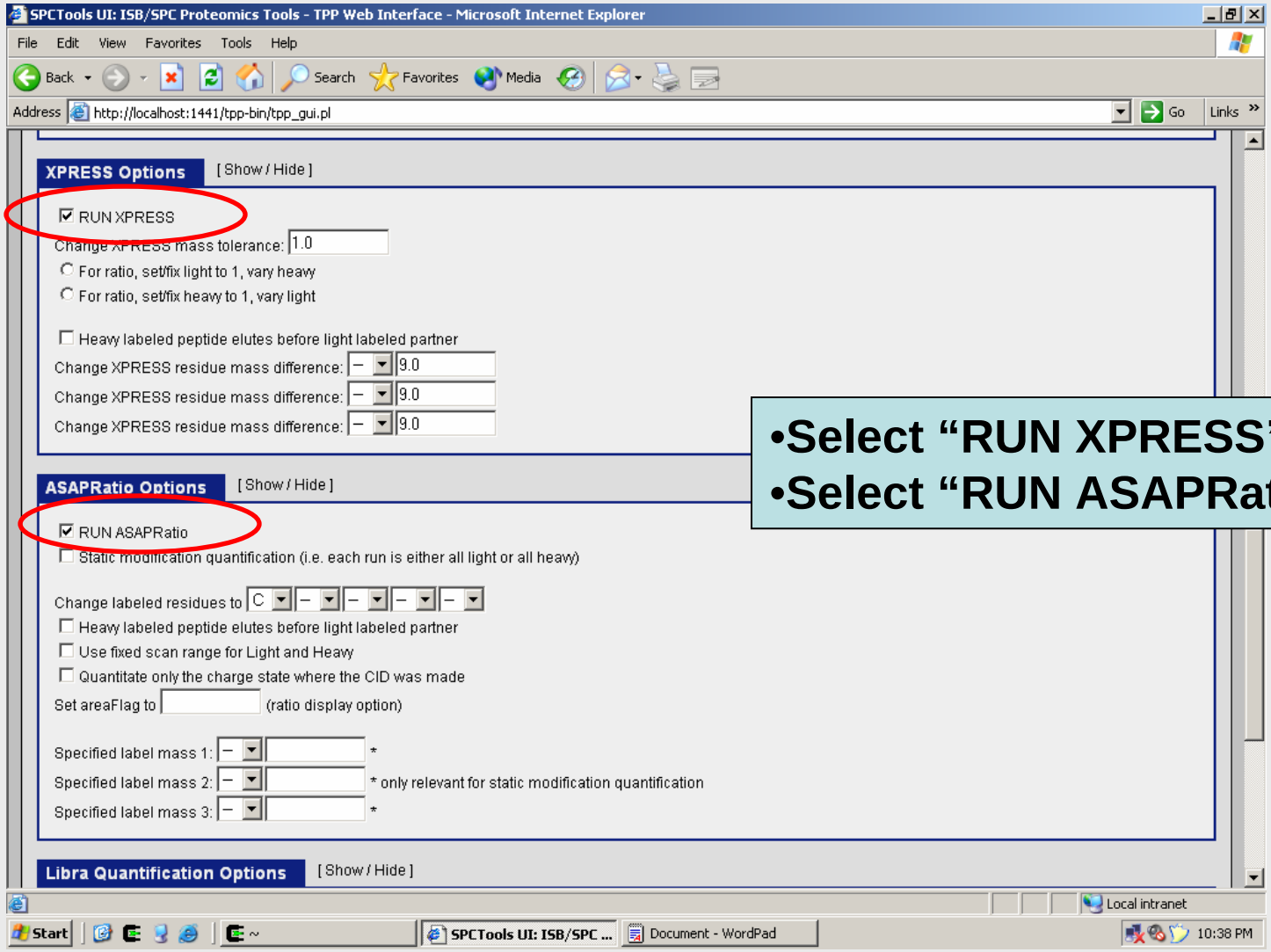
- **Start TPP**
- **Click on "Analyze Peptides"**
- **Select the xml files that you want to analyze**
- **Same as when running PeptideProphet**

How to Use TPP for Data Analysis in Quantitative Proteomics



- **Select "RUN PeptideProphet"**
- **Select "RUN ProteinProphet afterwards"**

How to Use TPP for Data Analysis in Quantitative Proteomics



- **Select "RUN XPRESS"**
- **Select "RUN ASAPRatio"**

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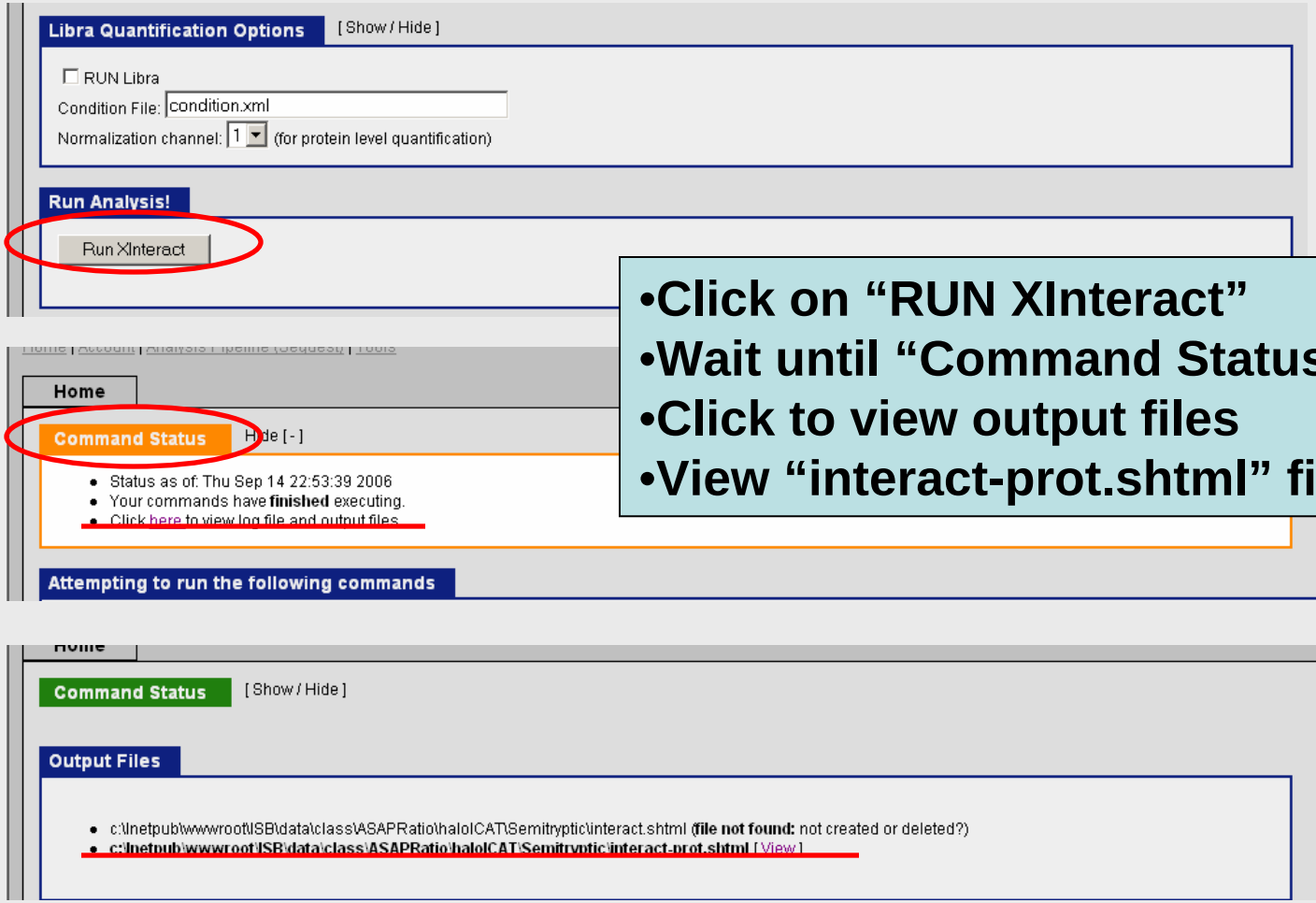
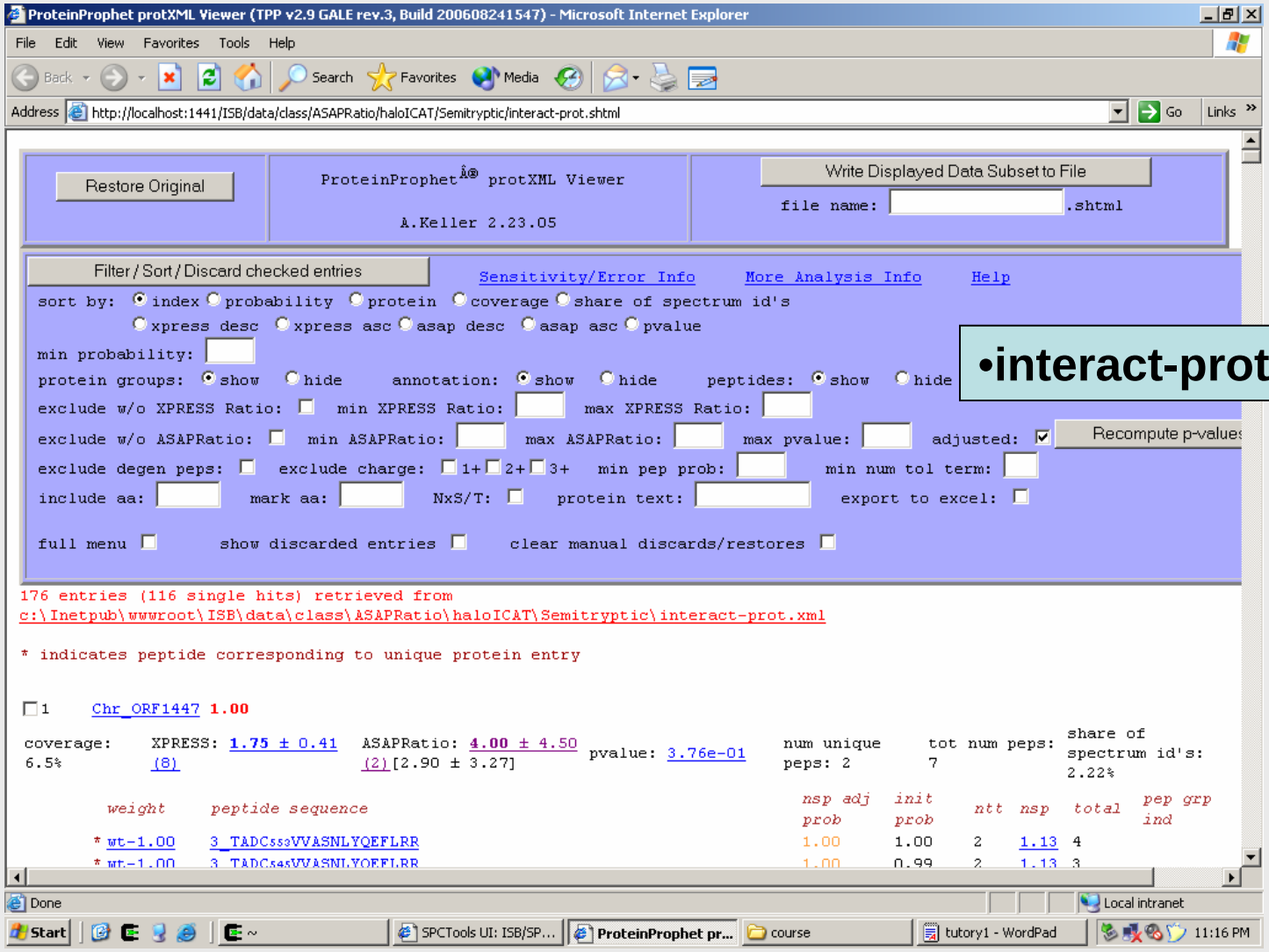


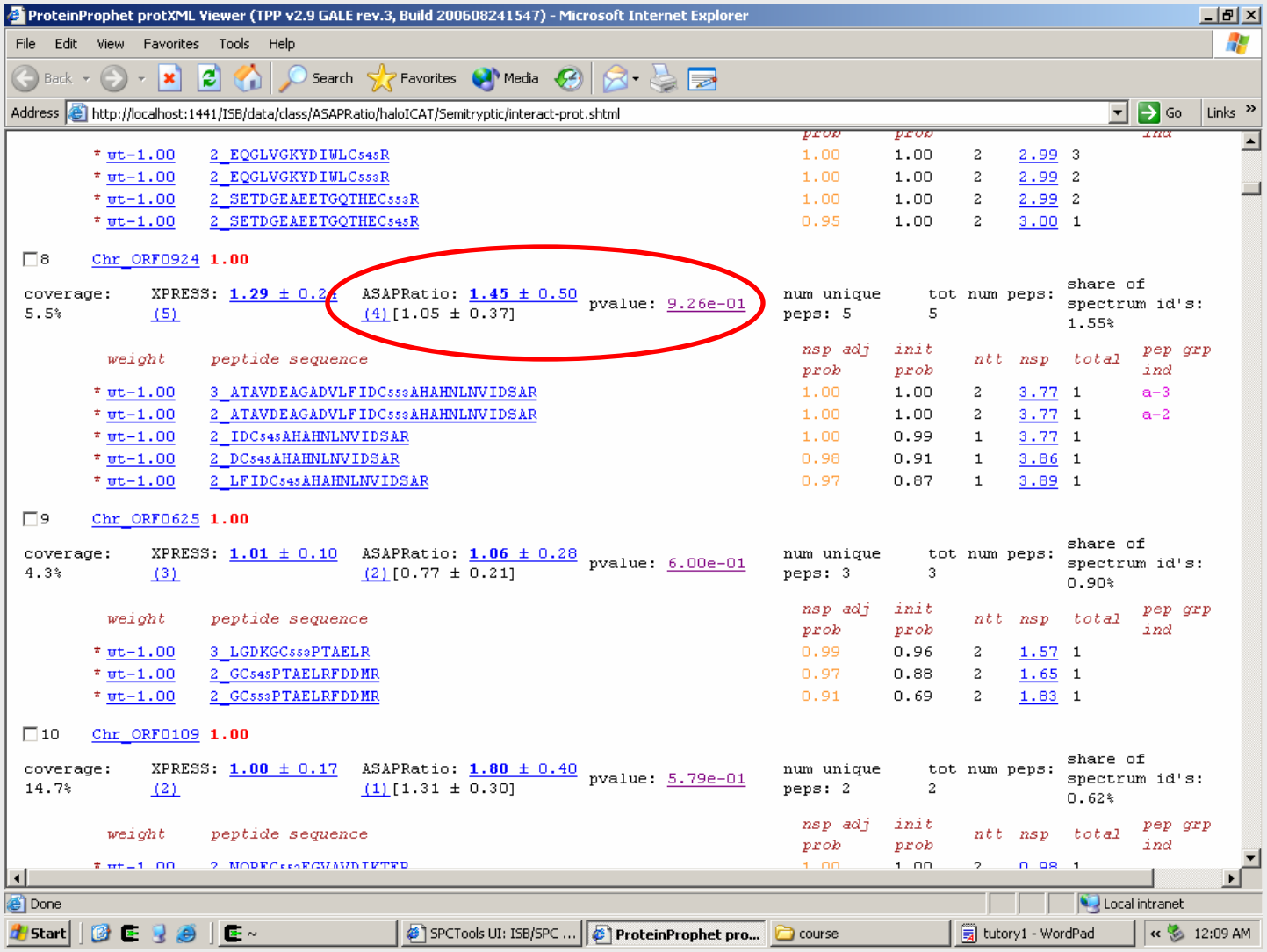
- **Click on "RUN XInteract"**
- **Wait until "Command Status" turns orange**
- **Click to view output files**
- **View "interact-prot.shtml" file**

How to Use TPP for Data Analysis in Quantitative Proteomics



- interact-prot.shtml

How to Interpret ASAPRatio Results



How to Interpret ASAPRatio Results

Protein ratio and its standard deviation

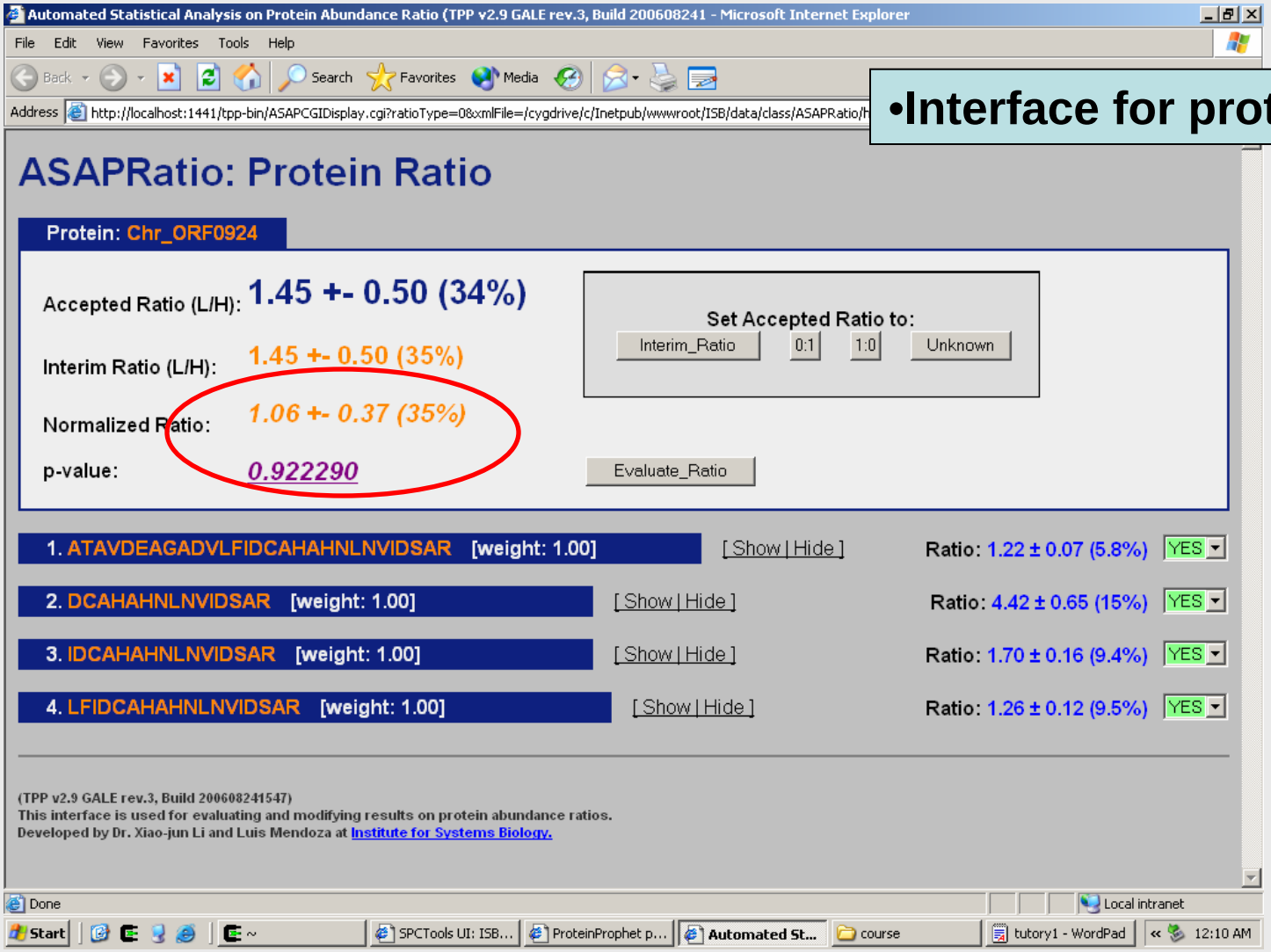
Protein p-value for differential expression

```
ASAPRatio: 1.45 ± 0.50                pvalue: 9.26e-01
[4][1.05 ± 0.37]
```

Number of unique peptides

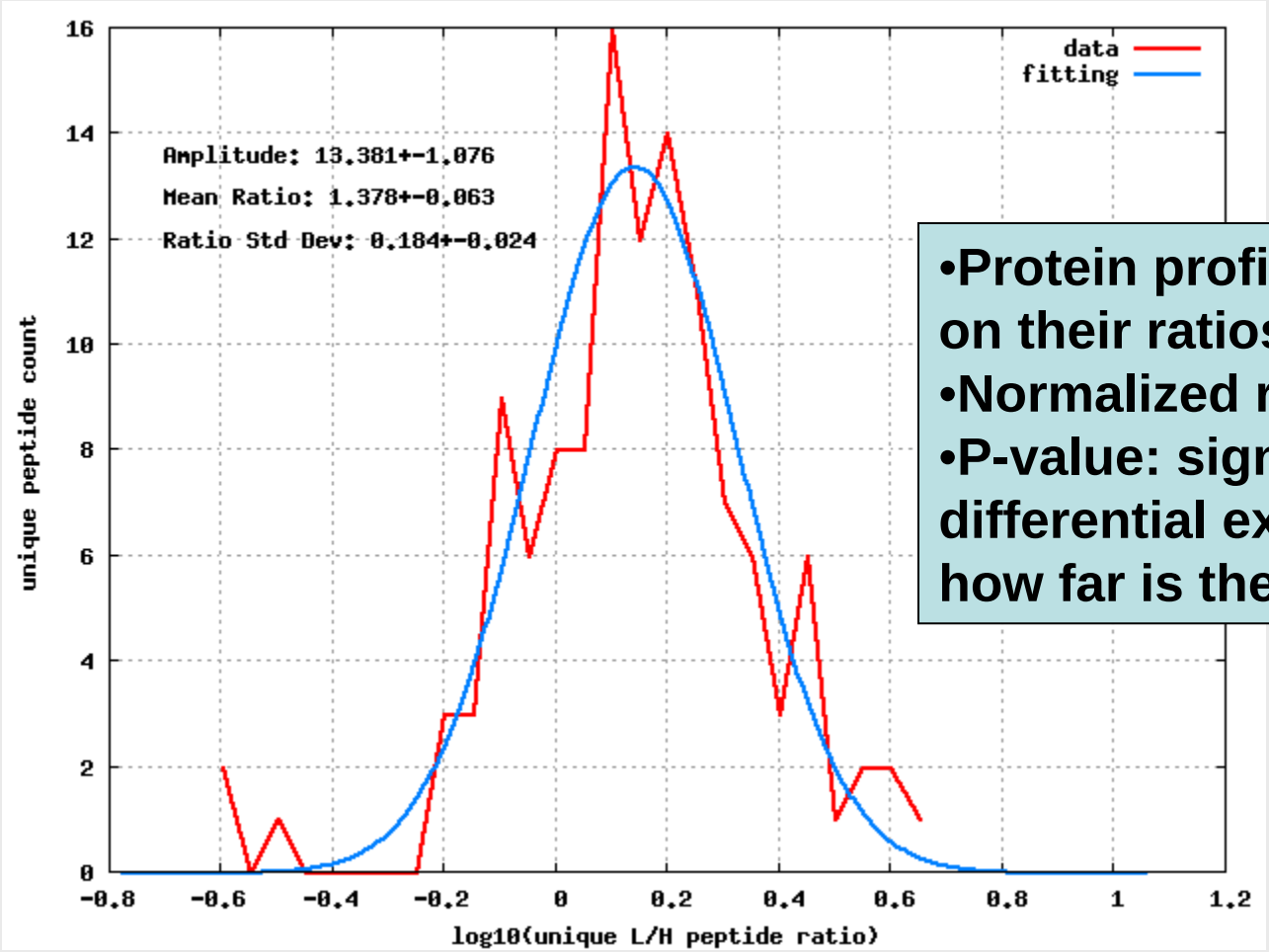
Normalized protein ratio and its standard deviation

How to Interpret ASAPRatio Results



- **Interface for protein ratio**

How to Interpret ASAPRatio Results



- **Protein profiling based on their ratios**
- **Normalized ratio: $r^* = r/r_0$**
- **P-value: significance in differential expression; how far is the data from r_0**

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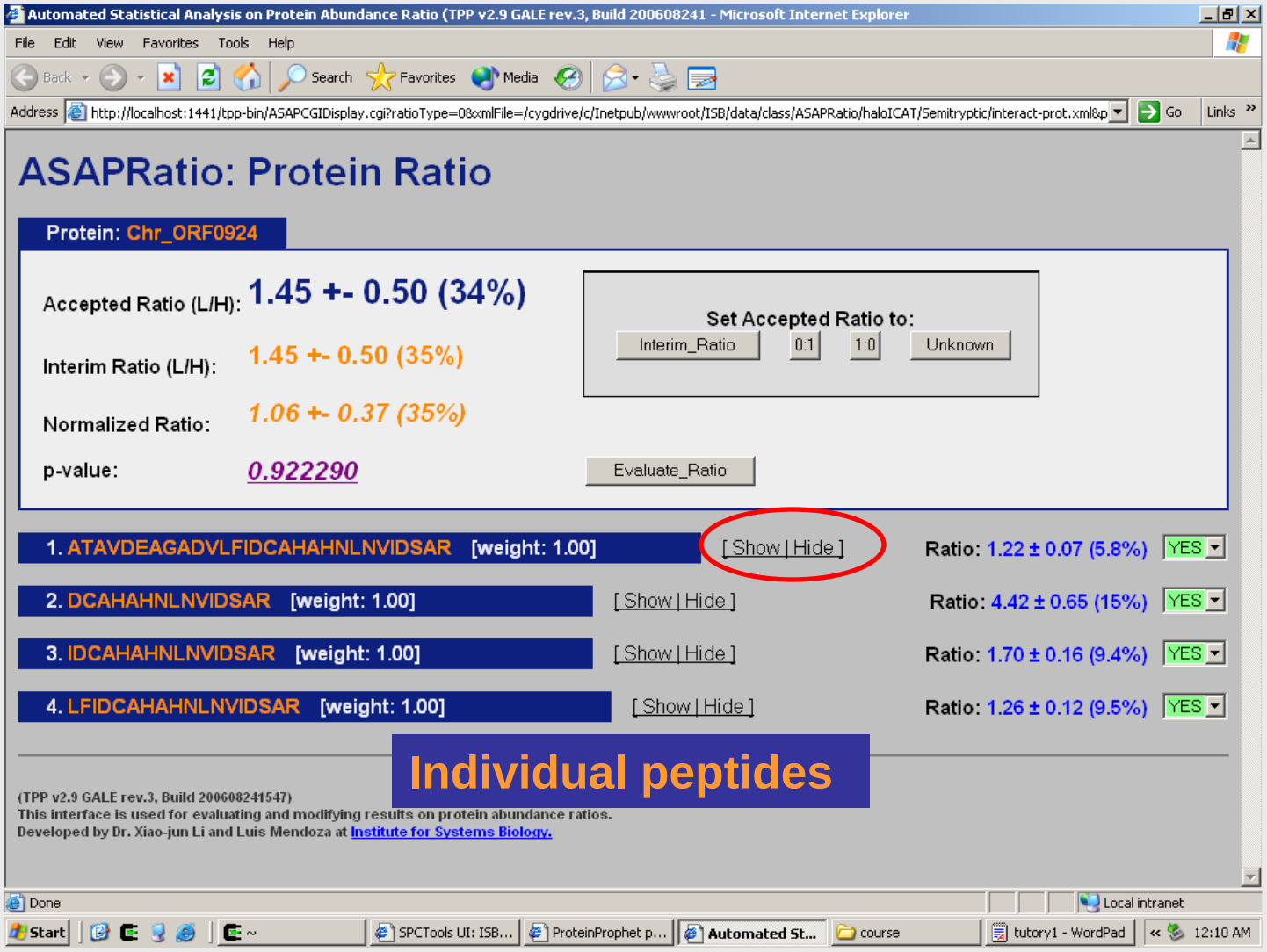

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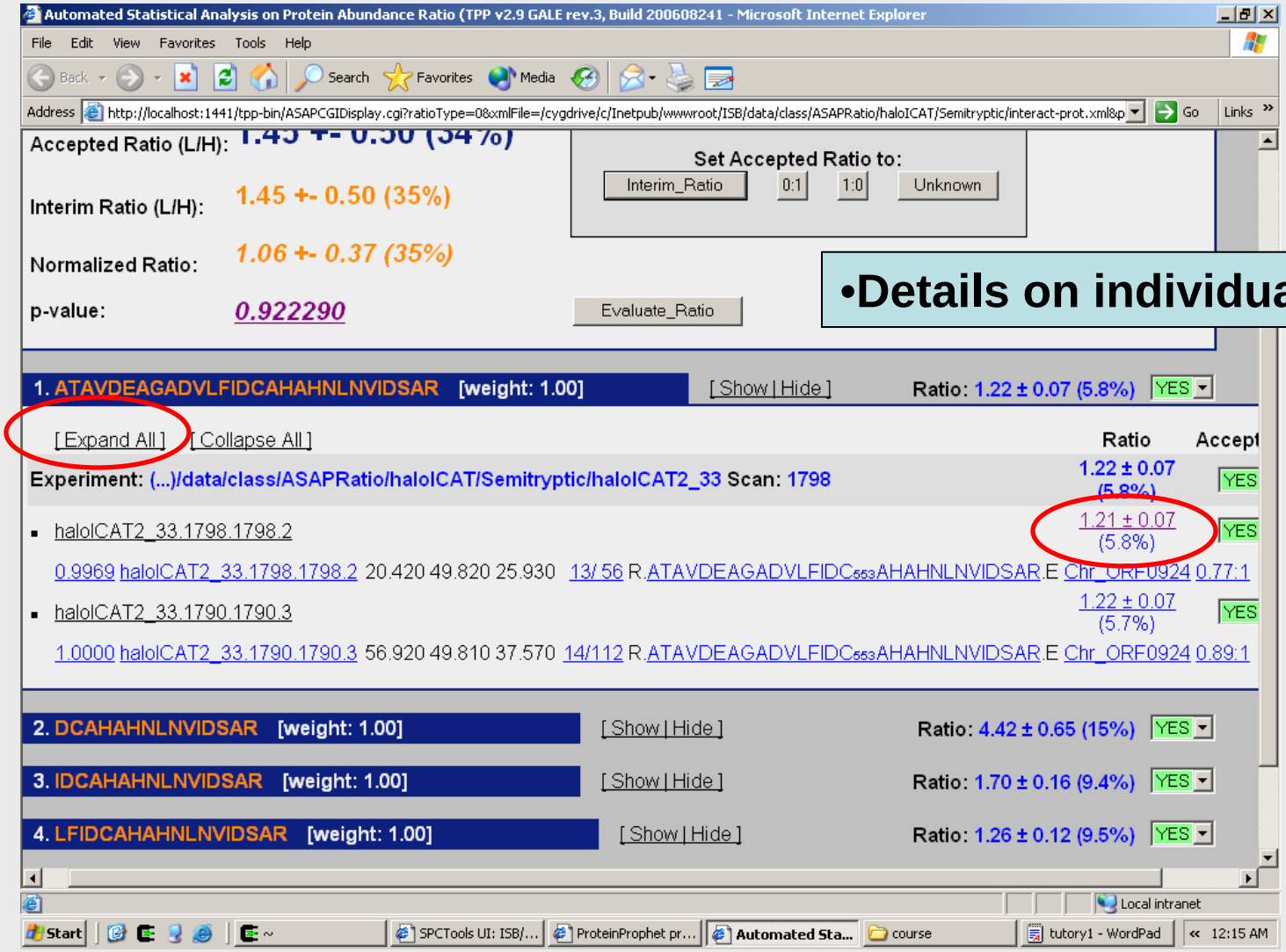
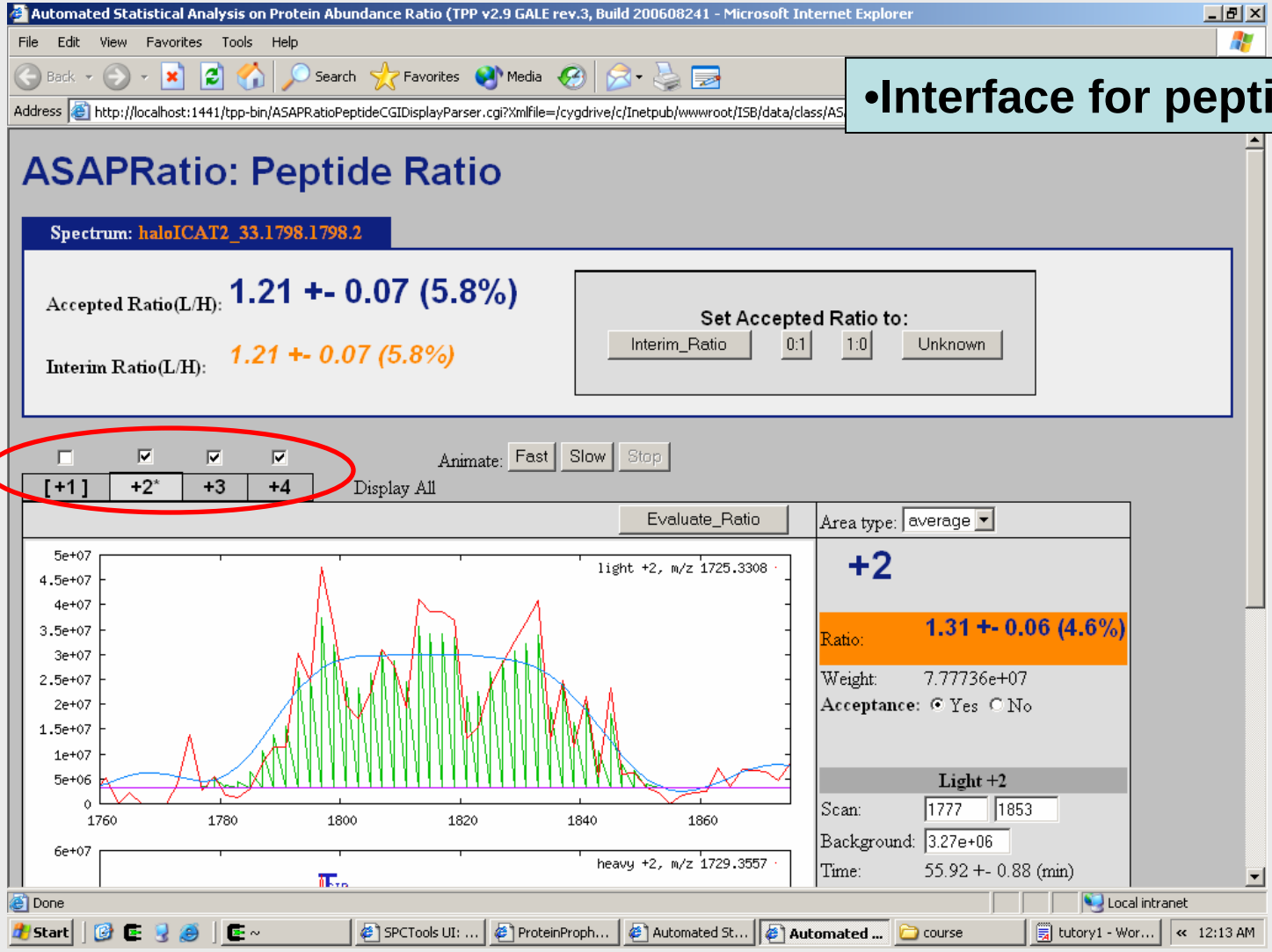


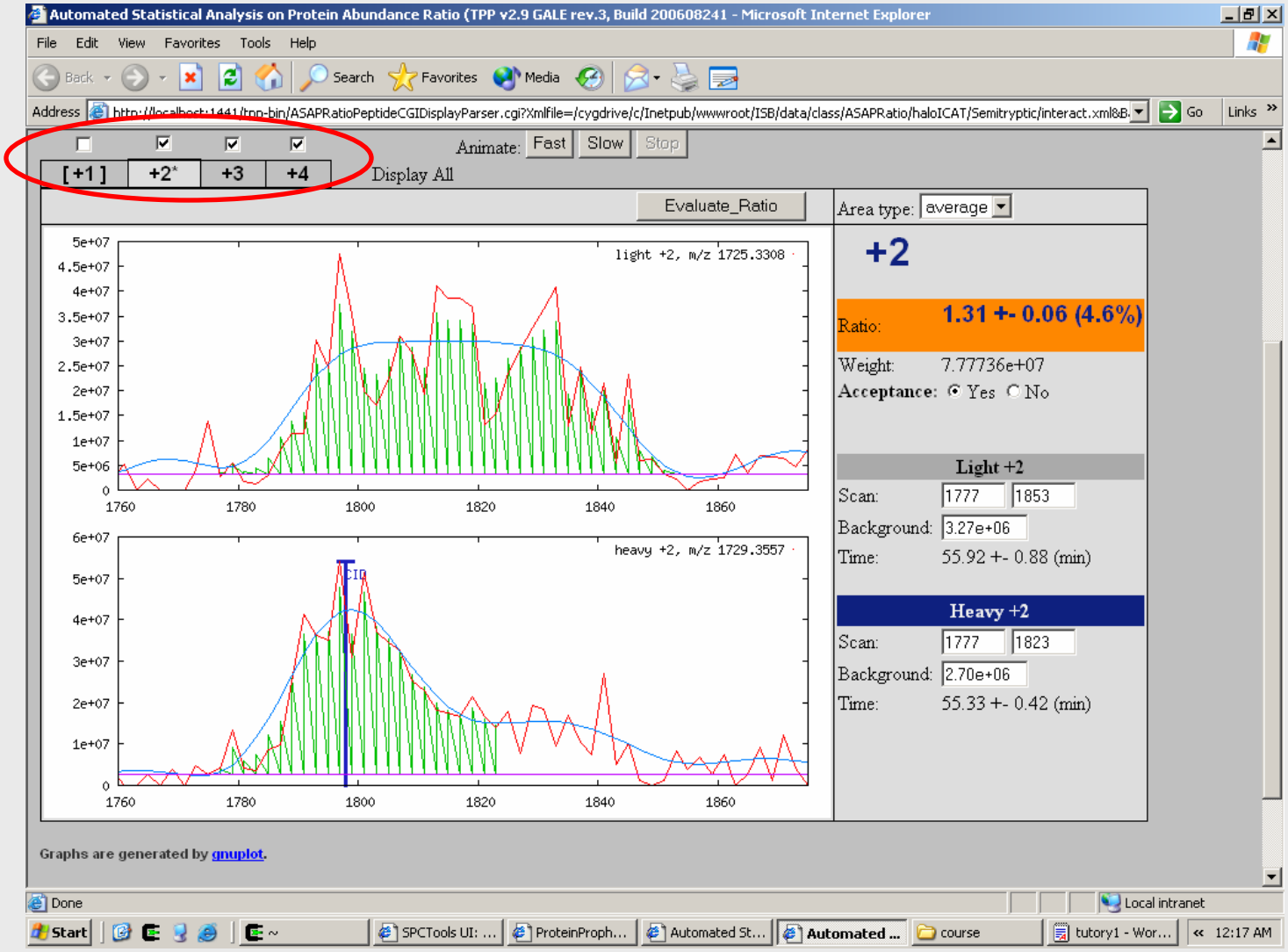
- **Details on individual peptides**

How to Interpret ASAPRatio Results



- **Interface for peptide ratio**

How to Interpret ASAPRatio Results



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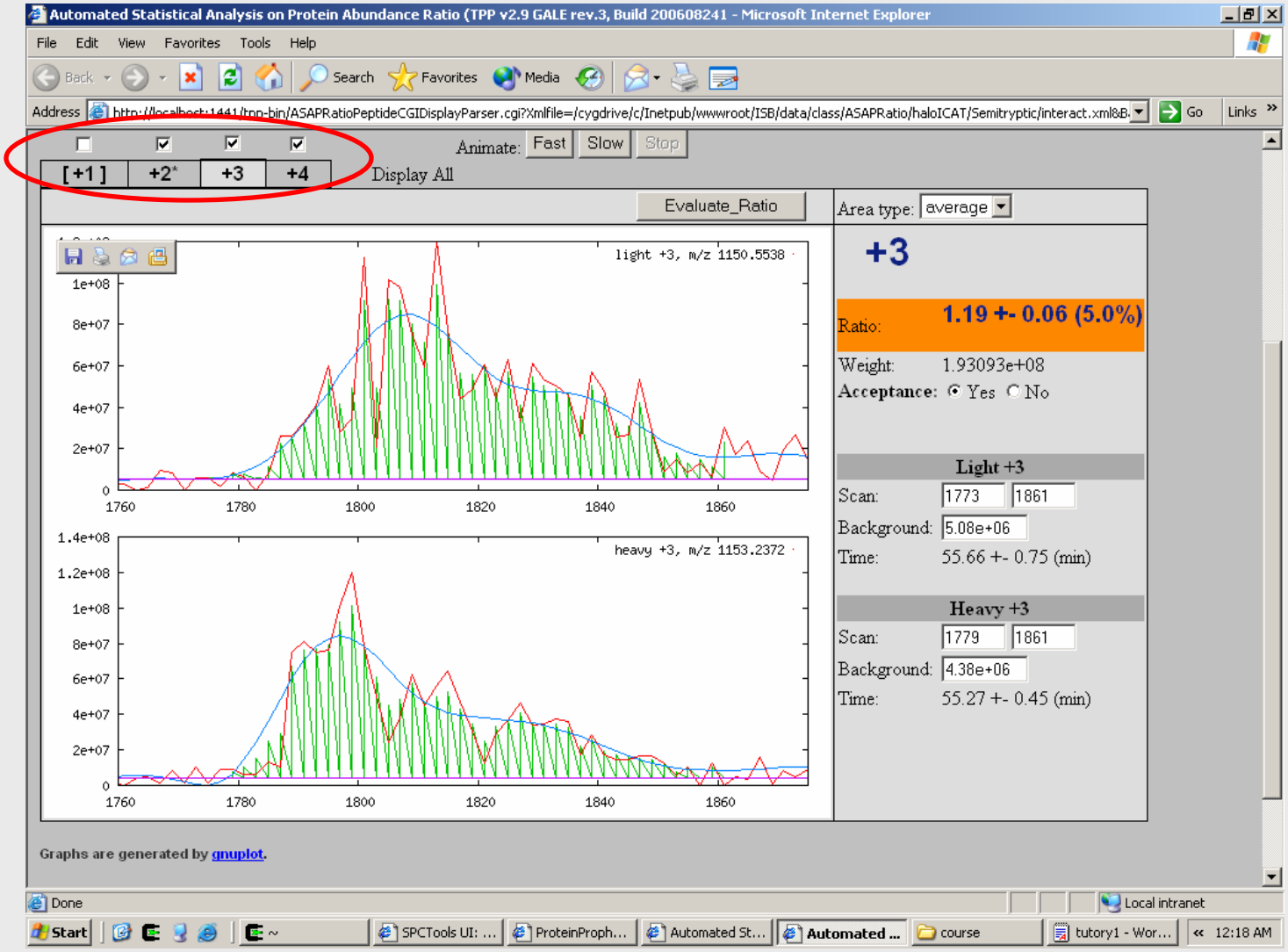

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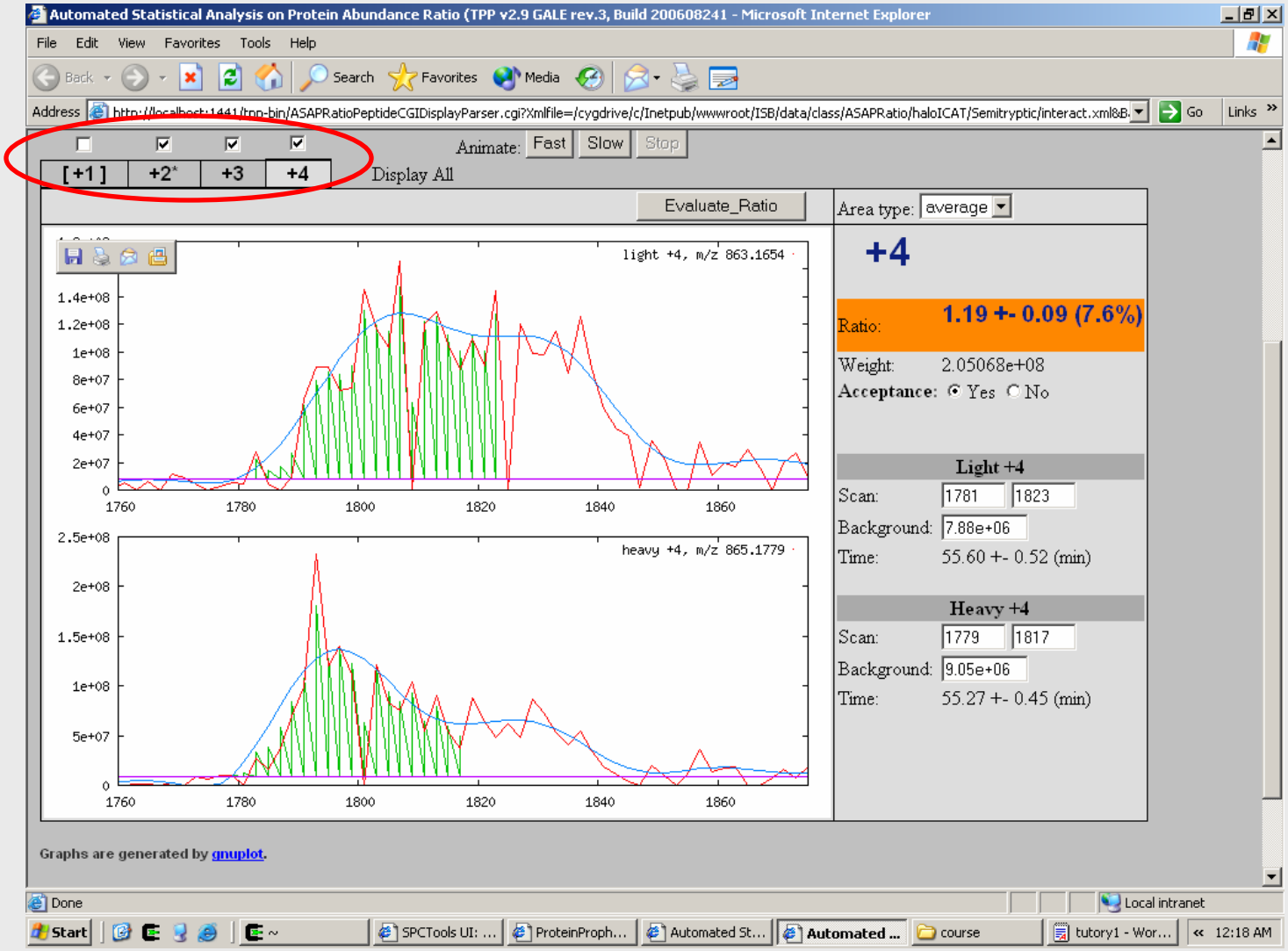

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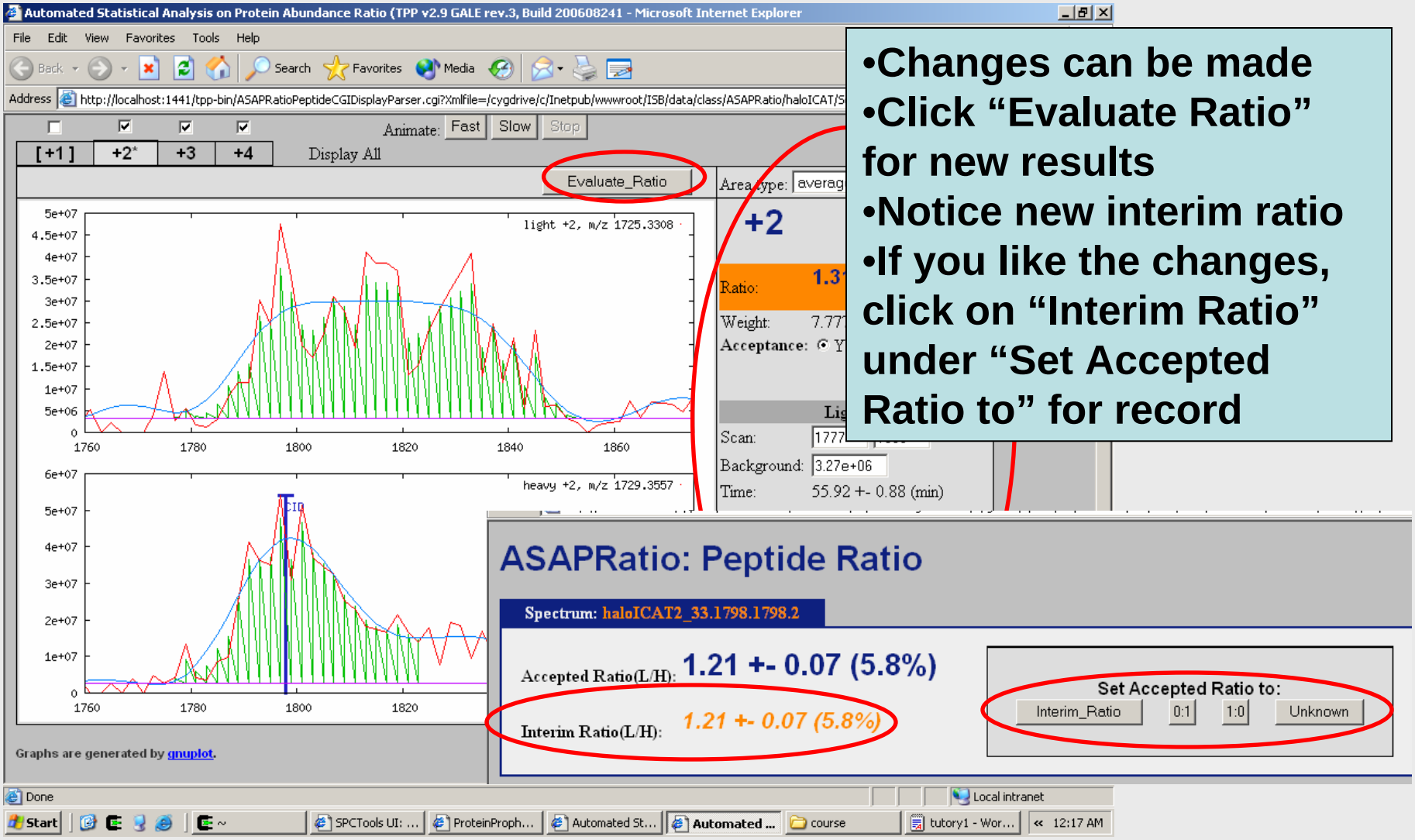
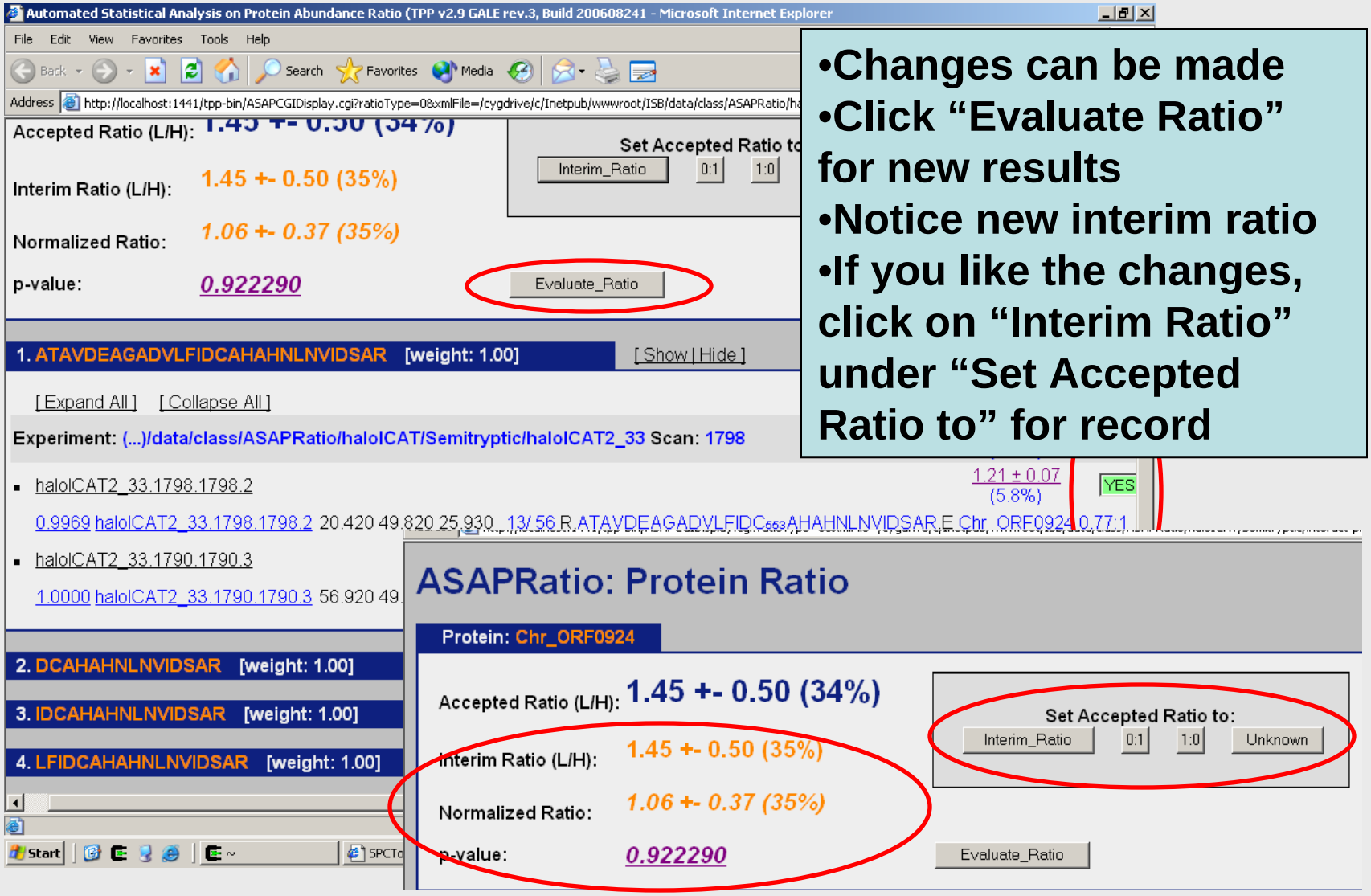


- Changes can be made
- Click "Evaluate Ratio" for new results
- Notice new interim ratio
- If you like the changes, click on "Interim Ratio" under "Set Accepted Ratio to" for record

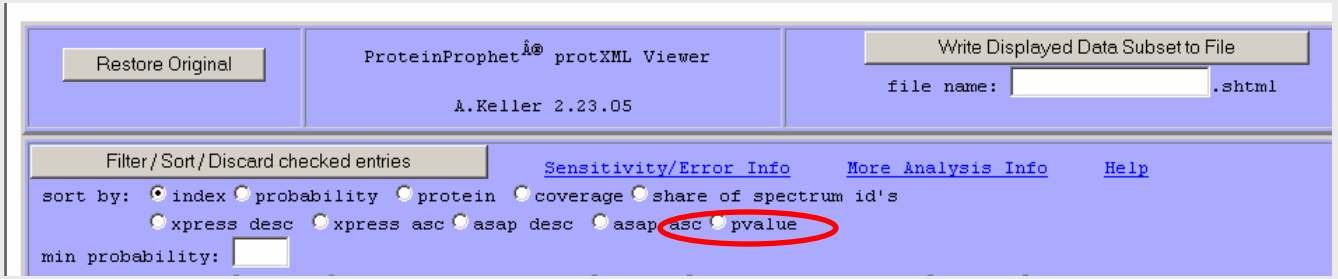
How to Interpret ASAPRatio Results



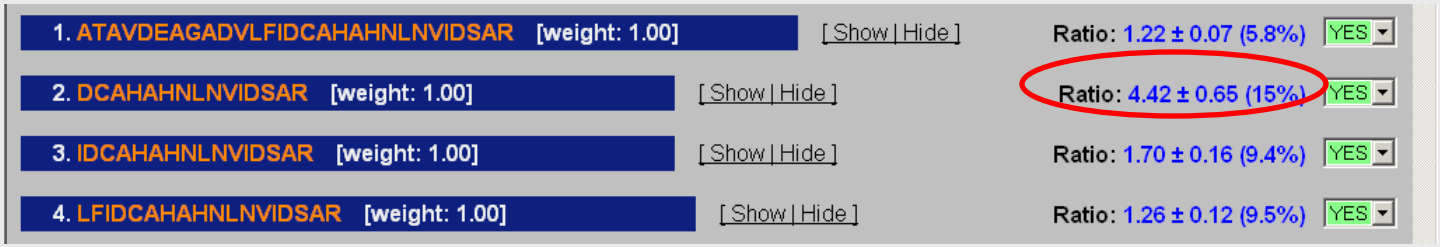
- **Changes can be made**
- **Click "Evaluate Ratio" for new results**
- **Notice new interim ratio**
- **If you like the changes, click on "Interim Ratio" under "Set Accepted Ratio to" for record**

How to Interpret ASAPRatio Results

- **Sort by p values first and verify potentially interesting data**

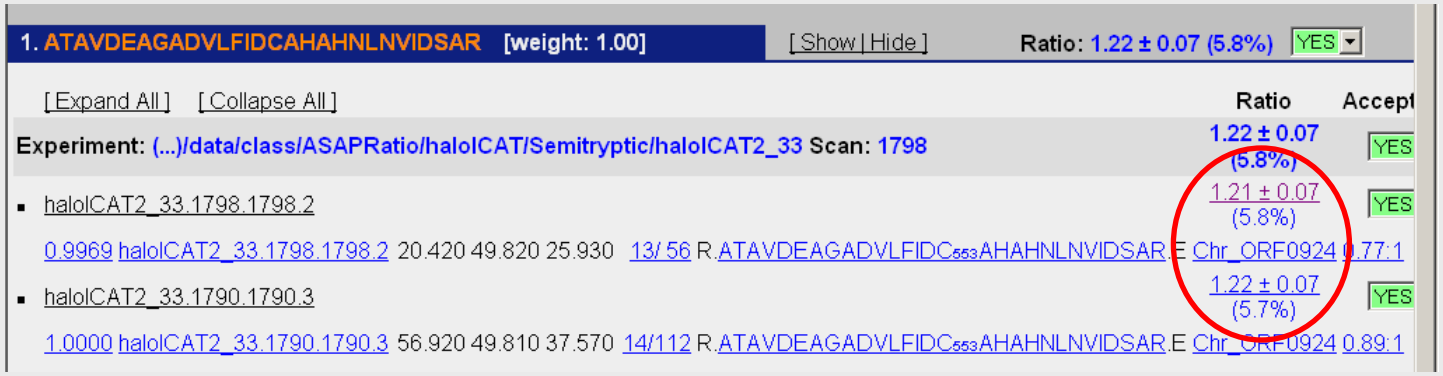


- **Identify and verify troublesome unique peptide ratios**

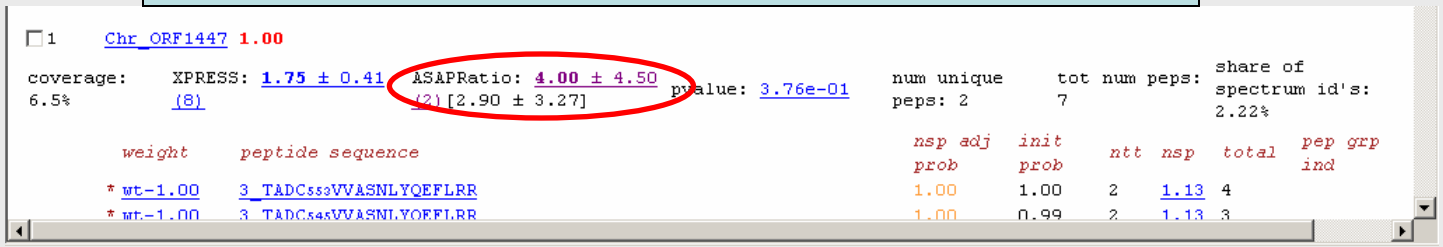


How to Interpret ASAPRatio Results

- **For peptides of same experiment, verify one peptide ratio and reject others**



- **Pay attention to unusual data: large error, 1:0, 0:1, or "unknown"**



ASAPRatio Main Features

- Able to handle various labeling methods (except iTRAQ)
- Estimate error on peptide and protein ratios
- Calculate peptide ratios from multiple charge states
  - Not just from charge state in which the CID was matched
- Chromatogram signal background subtraction to increase the dynamic range
- Calculate protein ratios by using connection of peptides to proteins computed by ProteinProphet
- Evaluate p-value for protein profiling
- Detect outliers: Dixon's test
- Easy to use user interface for manual validation of ratios

Summary

- Quantitative LC-ESI-MS/MS is a powerful method for identifying and quantifying proteins in complex samples
- ASAPRatio provides a user-freindly platform for analyzing LC-ESI-MS/MS data