Quantitation with XPRESS

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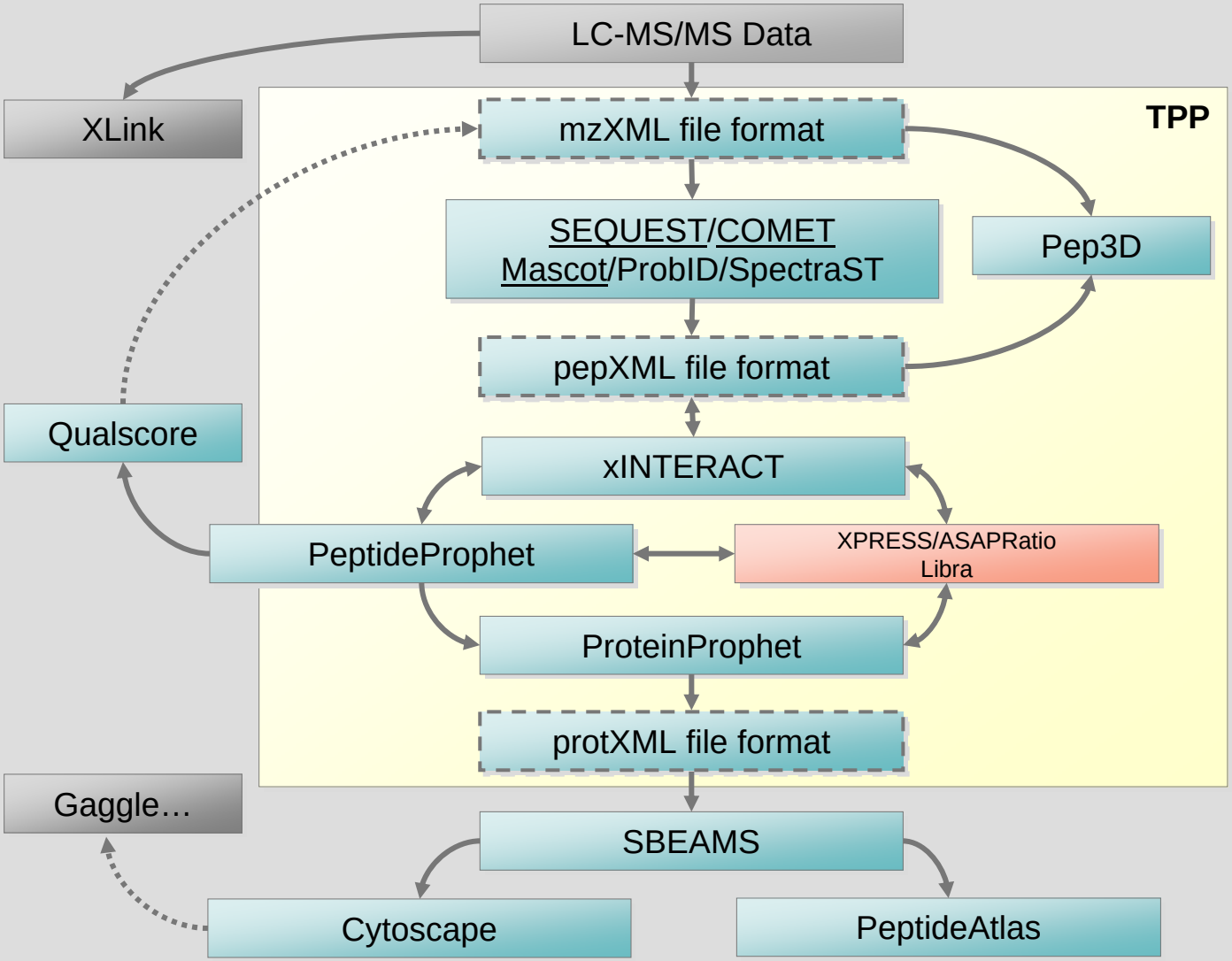
Peptide and Protein Quantitation



XPRESS Publication

Quantitative profiling of differentiation-induced microsomal proteins using isotope-coded affinity tags and mass spectrometry

David K. Han[1], Jimmy Eng[2], Huilin Zhou[2], and Ruedi Aebersold[2*]

An approach to the systematic identification and quantification of the proteins contained in the microsomal fraction of cells is described. It consists of three steps: (1) preparation of microsomal fractions from cells or tissues representing different states; (2) covalent tagging of the proteins with isotope-coded affinity tag (ICAT) reagents followed by proteolysis of the combined labeled protein samples; and (3) isolation, identification, and quantification of the tagged peptides by multidimensional chromatography, automated tandem mass spectrometry, and computational analysis of the obtained data. The method was used to identify and determine the ratios of abundance of each of 491 proteins contained in the microsomal fractions of naïve and *in vitro*–differentiated human myeloid leukemia (HL-60) cells. The method and the new software tools to support it are well suited to the large-scale, quantitative analysis of membrane proteins and other classes of proteins that have been refractory to standard proteomics technology.

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Proteins that are integral to or associated with lipid membranes perform a wide range of essential cellular functions. Pores, channels, pumps, and transporters facilitate the exchange of membrane-impermeable molecules between cellular compartments and between the cell and its extracellular environment. Transmembrane receptors sense changes in the cellular environment and, typically via associated proteins, initiate specific intracellular responses. Cell-adhesion proteins mediate cell-specific changes in the membrane protein profiles of different cell populations or tissues would be of considerable importance for biology and for applied biomedical research.

A main objective of proteomics research is the systematic identification and quantification of the proteins expressed in a cell, tissue, or body fluid. The standard approach to proteomics has been the combination of high-resolution two-dimensional gel electrophoresis (2D-E) and mass spectrometry. Proteins in complex

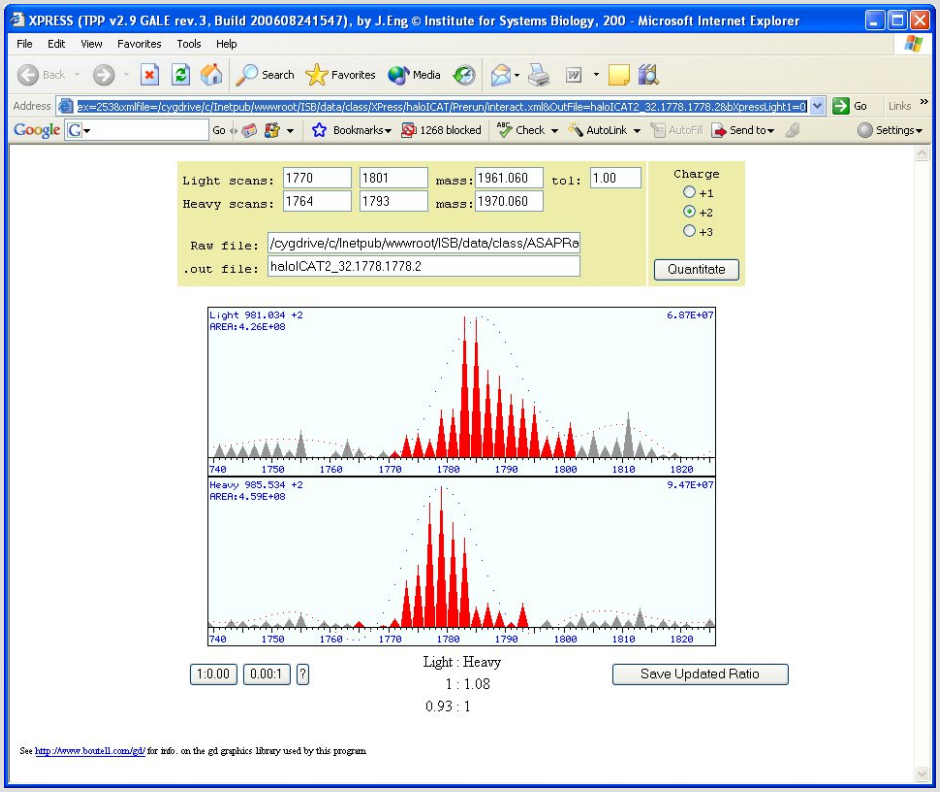
Han DK, Eng J, Zhou H, and Aebersold R. (2001) *Nature Biotechnology* 19:946-51.

XPRESS Overview

- XPRESS MS-level Quantitation
  - An alternative to ASAPRatio
- Peptide Quantitation
- Protein Quantitation
- Running XPRESS
- Looking at results

XPRESS Peptide Ratio

- Calculated from SIC of charge state in which peptide was identified
- Smoothing done with a Butterworth low-pass filter
- No background estimation
- Works with different labeling methods
  - ICAT, SILAC



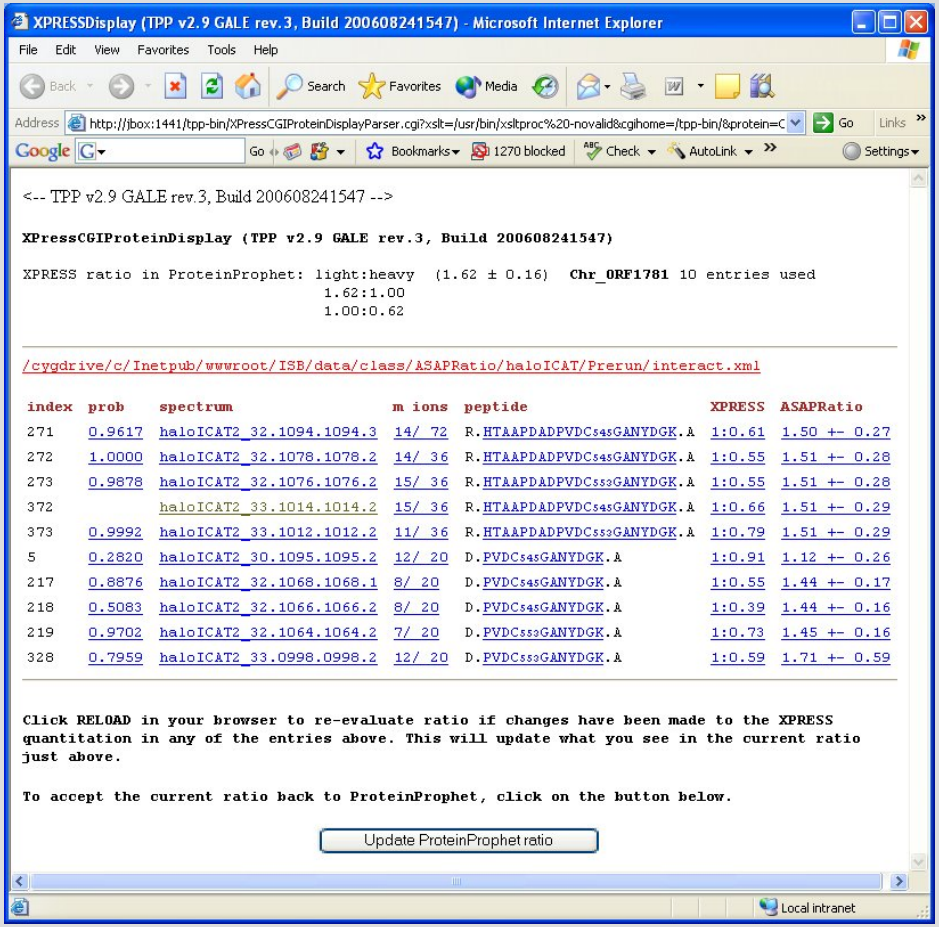
XPRESS Protein Ratio

- Calculated as the Geometric Mean of the constituent peptide ratios

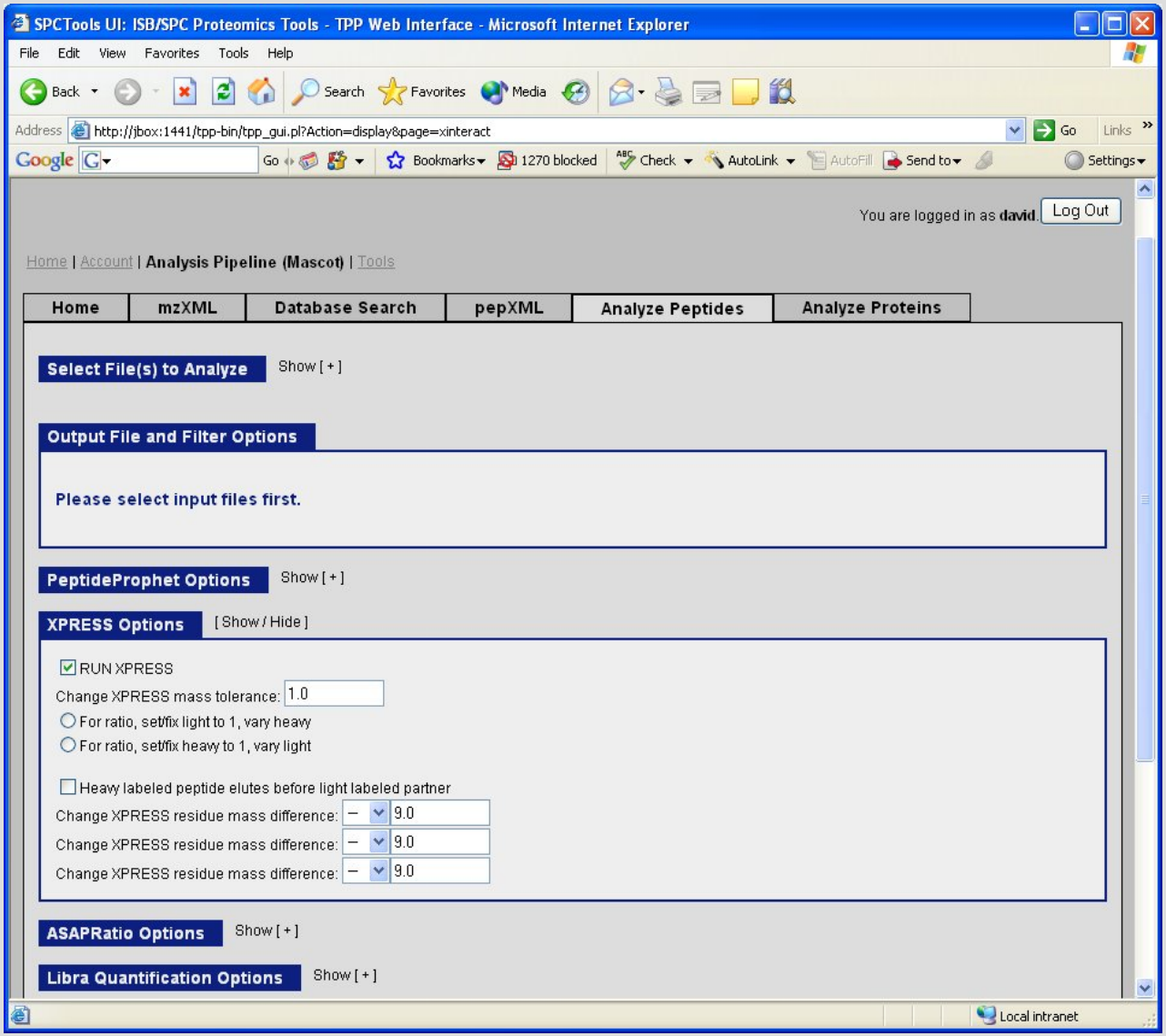
$$R = 10^{\frac{\sum \log r_i}{n}}$$

- Uncertainty is also calculated

$$\varepsilon = R \cdot \sqrt{\frac{\sum (\log r_i)^2}{n} - \left(\frac{\sum \log r_i}{n}\right)^2}$$



Running XPRESS: Petunia Interface



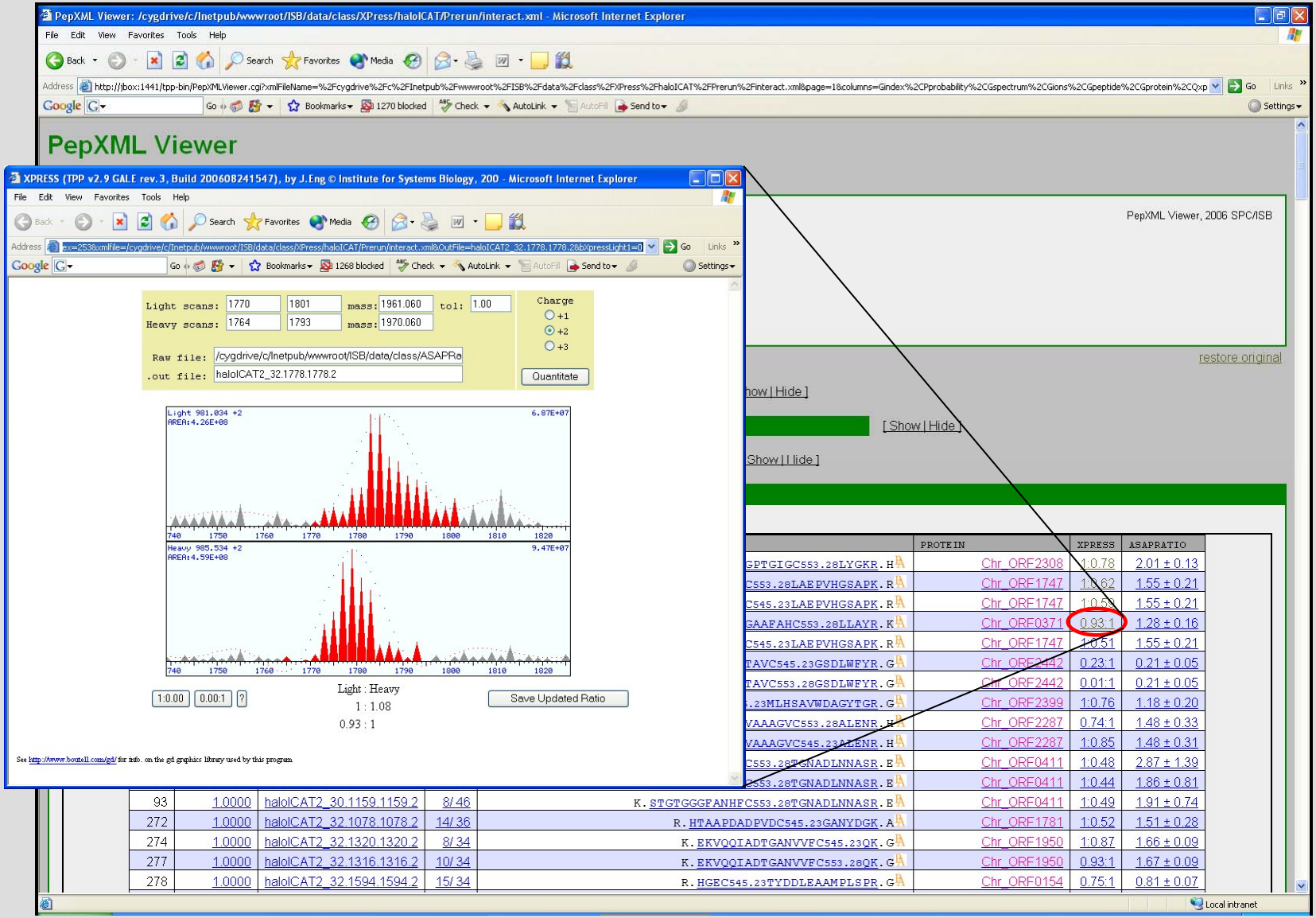
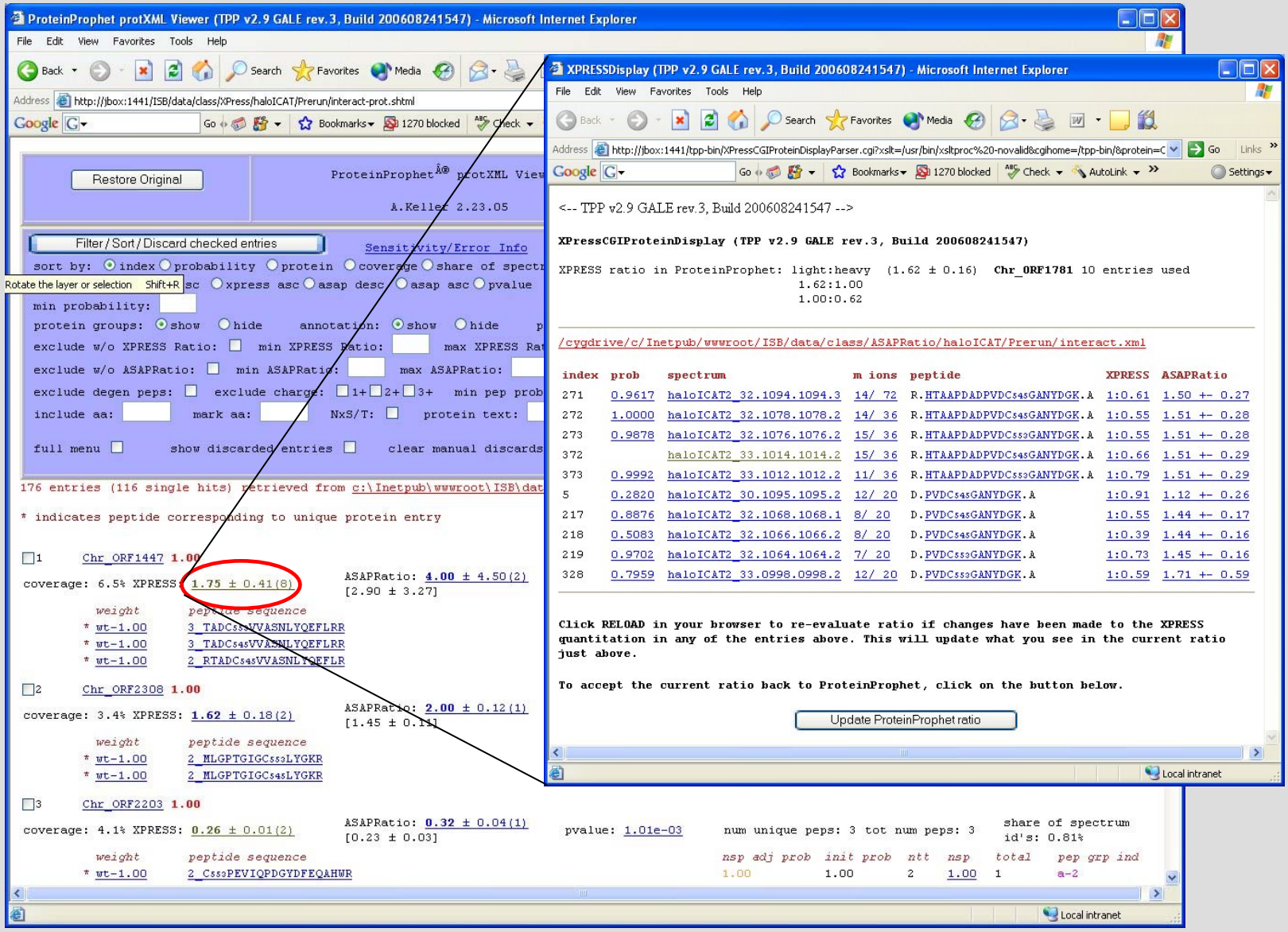
Running XPRESS: Commandline

- Use the –X flag for xinteract

xpressoptions [will run XPRESS analysis with any specified options that follow the 'X']:

-m<num>	change XPRESS mass tolerance (default=1.0)
-l<str>	change labeled residues (default='C')
-n<str>,<num>	change XPRESS residue mass difference for <str> to <num> (default=9.0)
-b	heavy labeled peptide elutes before light labeled partner
-F<num>	fix elution peak area as +-<num> scans (<num> optional, default=5) from peak apex
-L	for ratio, set/fix light to 1, vary heavy
-H	for ratio, set/fix heavy to 1, vary light
-M	for metabolic labeling; ignore all other parameters, assume IDs are normal and quantify w/corresponding 15N heavy pair
-N	for metabolic labeling; ignore all other parameters, assume IDs are 15N heavy and quantify corresponding 14N light pair

XPRESS PeptideProphet Results



XPRESS ProteinProphet Results