novatia

Innovative Solutions For Science

ProMass Deconvolution User Training

ProMass for Xcalibur

Novatia LLC

2015

Overview

- General info about ProMass
- Features
- Basics of how ProMass Deconvolution works
- Example Spectra
- Manual Deconvolution with ProMass
- Deconvolution Parameters
- Automated Deconvolution with ProMass
- Questions and User Examples?

ProMass General Info

- OPTON-20885 for ProMass Deconvolution 2.8
- OPTON-20876 for ProMass Deconvolution Upgrade from BIOMASS Deconvolution.
- Those who purchased ProMass 2.0 can upgrade to ProMass 2.8 at no charge, download from http://www.enovatia.com/products/promass/promass-support/. Your existing license key is valid.
- ProMass 2.8.0 is compatible with Xcalibur 1.2 - 2.0.7
- ProMass 2.8.2 is compatible with Xcalibur 2.0.7 – 2.2
- ProMass license model: 1 license per CPU

ProMass Features

- Xcalibur layered application
- Uses Novatia's proprietary ZNova algorithm for "*artifact-free*" charge deconvolution of biomolecule ESI mass spectra
- Allows for automated deconvolution of spectra in LC/MS data from the Xcalibur sample list
- Produces web-based results format including chromatograms, spectra, color-coded summary, and tabular results
- Works with proteins, peptides, and oligonucleotides, etc.
- Applications include:
  - *Detailed intact protein characterization – impurities, degs, PTM's*
  - *HT oligonucleotide synthesis QC*
  - *HT intact protein expression and bioprocess monitoring*
  - *Peptide mapping – works with data-dependent files*

Why we need charge deconvolution

Unfortunately mass spectrometers measure m/z NOT Mass:

$$\text{Mass } (M) \neq m/z$$

But:

$$m/z = (M + zA)/ z$$

Where:

A = mass of adduct providing charge

z = number of charges

Given 2 adjacent m/z peaks (mz_1 and mz_2) in a charge series of unknown charges (z_1 and z_2), you can use Algebra:

$$z_1 = z_2 + 1$$

$$z_2 = (mz_1 – A)/(mz_2 – mz_1)$$

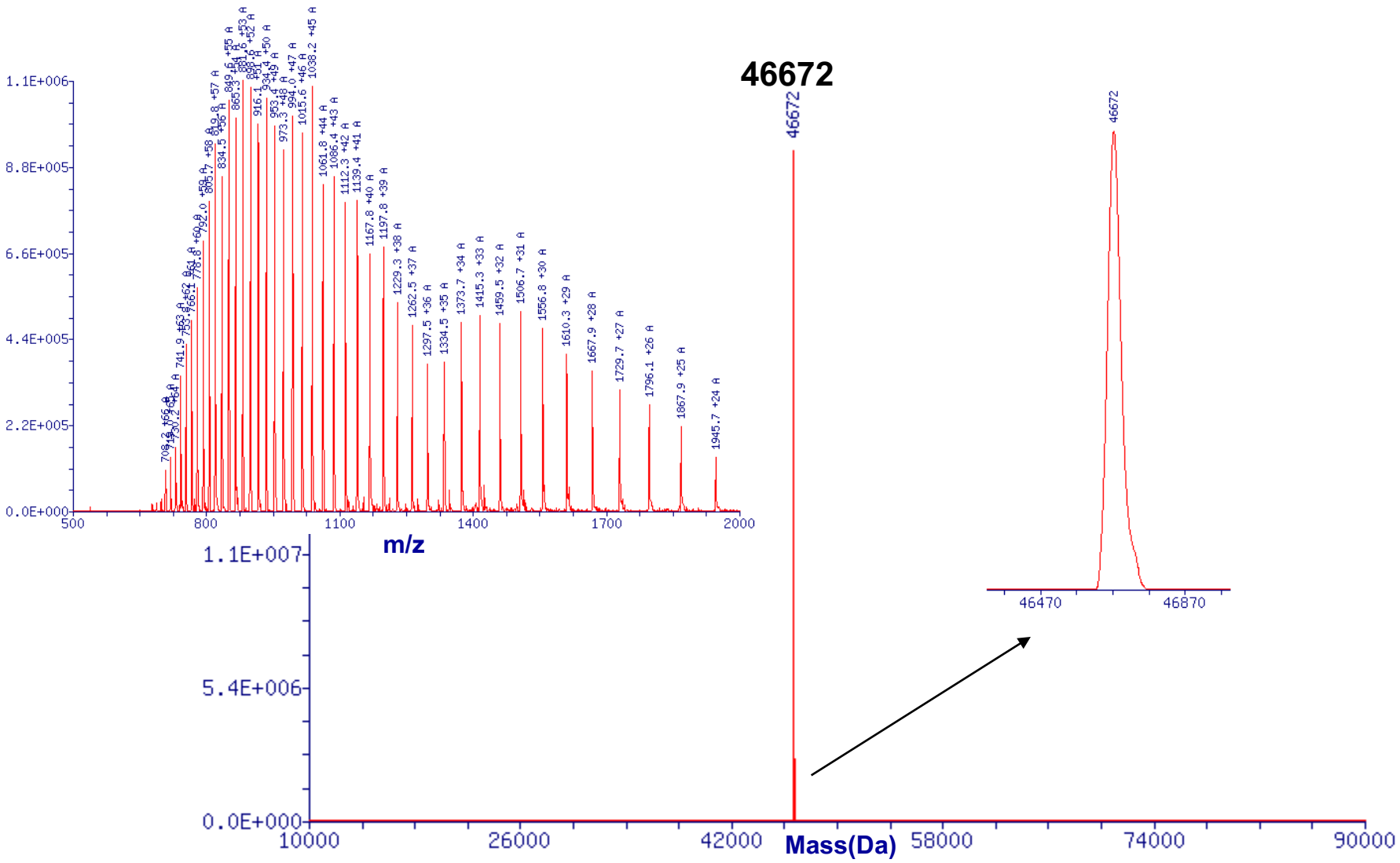
You would go insane if you had to do this for every spectrum!

ZNova Deconvolution Algorithm

- *ZNova* is the algorithm used by the ProMass software
- *ZNova* uses a "component deconvolution" approach which tests and determines the charge of every peak in the raw mass spectrum (Zhang & Marshall, JASMS 1998)
- *ZNova* uses a simple intensity-based scoring algorithm
- *ZNova* has built-in signal processing techniques to improve reliability of deconvolution even on noisy data
  - *Automatic baseline removal*
  - *Decentroiding of centroid input data*
  - *Smoothing prior to decon*
  - *Normalization of scoring based on observed/predicted peaks*
- *ZNova* exhibits low incidence of deconvolution artifacts - unlike Mann algorithm
- Clean deconvolutions allow reliable confirmation of target components

ProMass Deconvolution of Yeast Enolase (MW 46670.9)

Example of Artifact-free Deconvolution

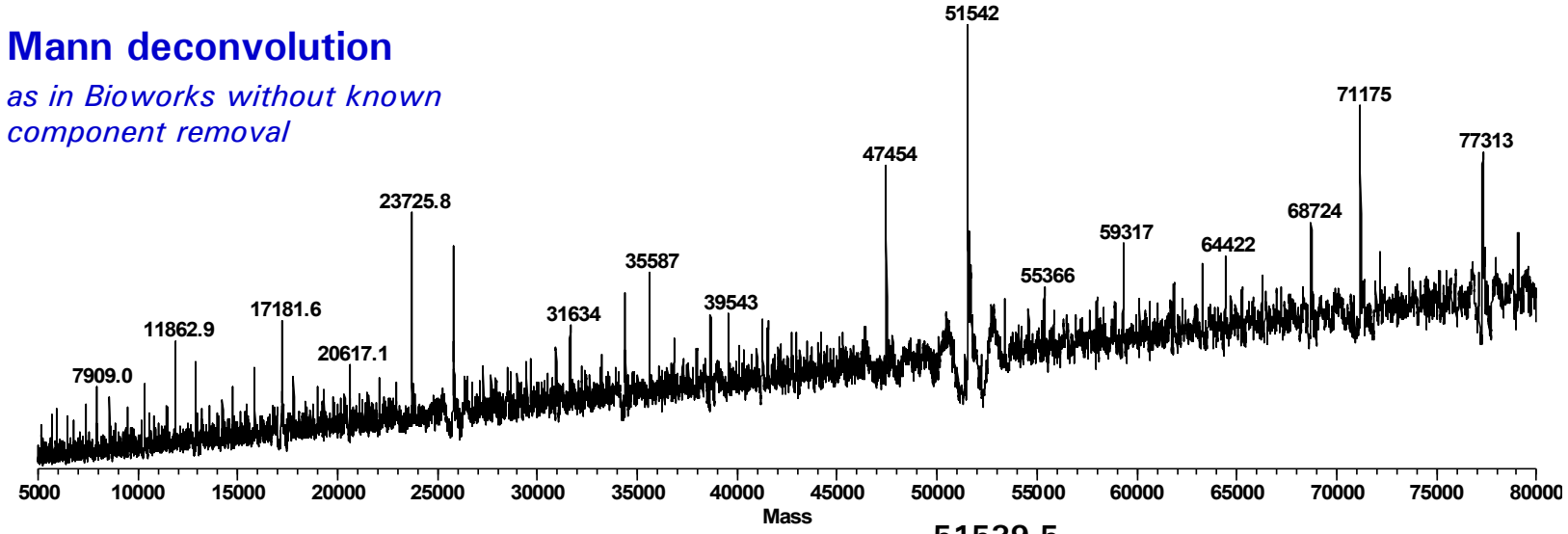


Comparison of Deconvolution Algorithms

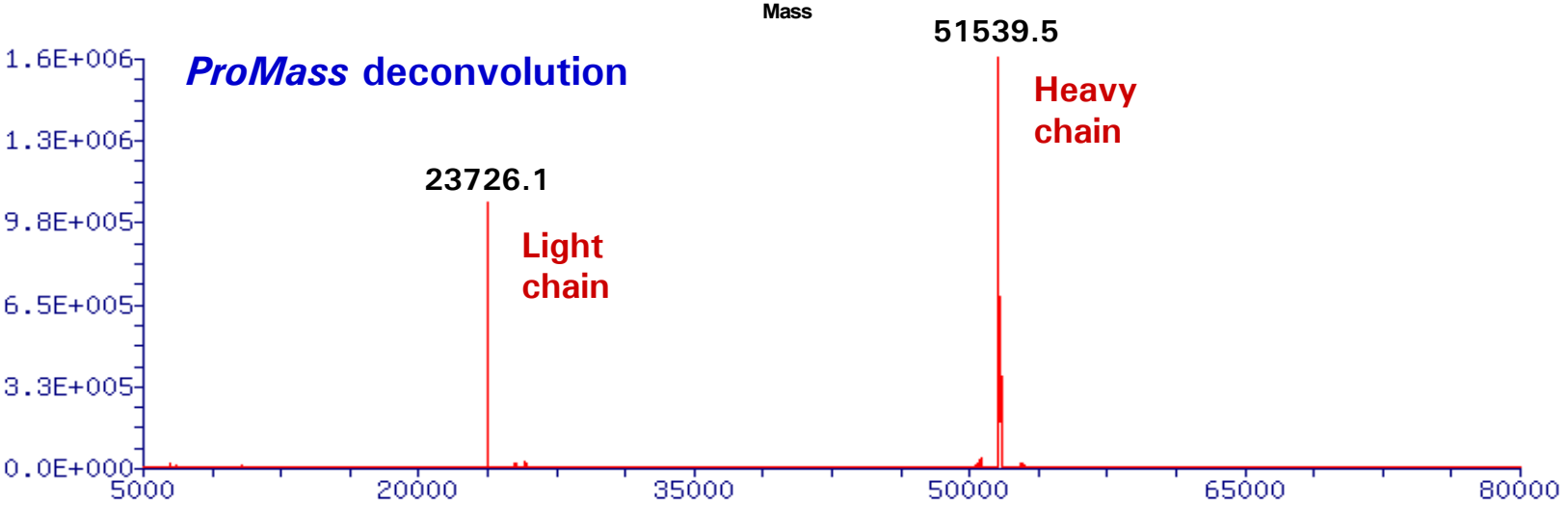
heavy / light chain IgG mixture

Mann deconvolution

as in Bioworks without known component removal



ProMass deconvolution



How *ZNova* Deconvolution Works

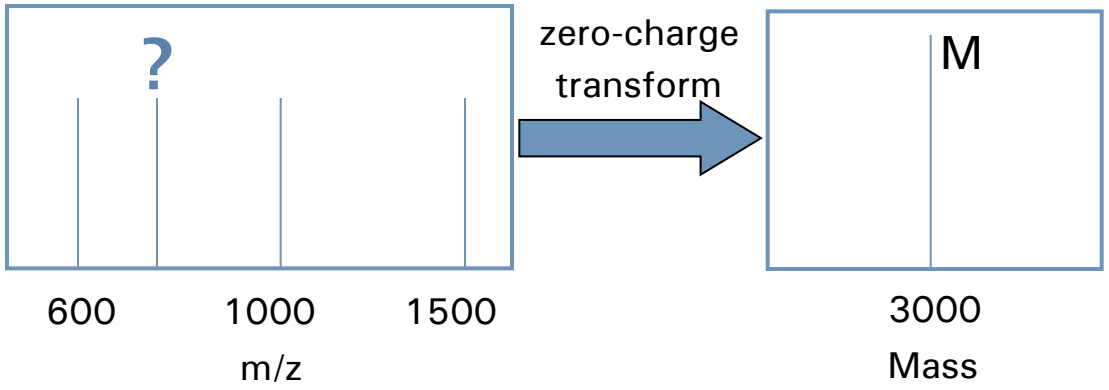
Example: Test all z values for m/z 751 in the spectrum below

Assume +2 (MW 1500) : +1 = 1501, score = 2

Assume +3 (MW 2250) : +4 = 563.5, +2 = 1126, +1 = 2251, score = 1

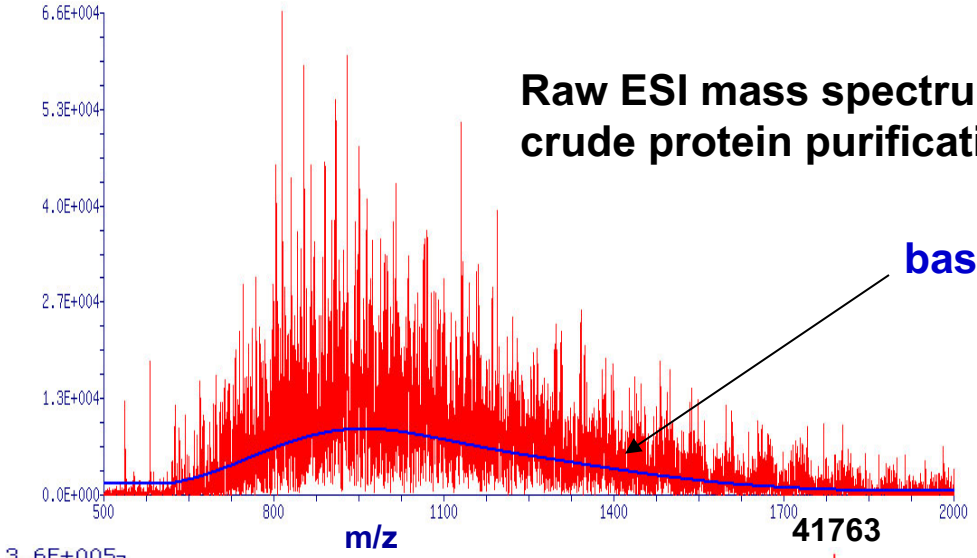
Assume +4 (MW 3000) : +5 = 601, +3 = 1001, +2 = 1500, score = 4

Assume +5 (MW 3750) : +6 = 626, +4 = 938.5, +3 = 1251, score = 1



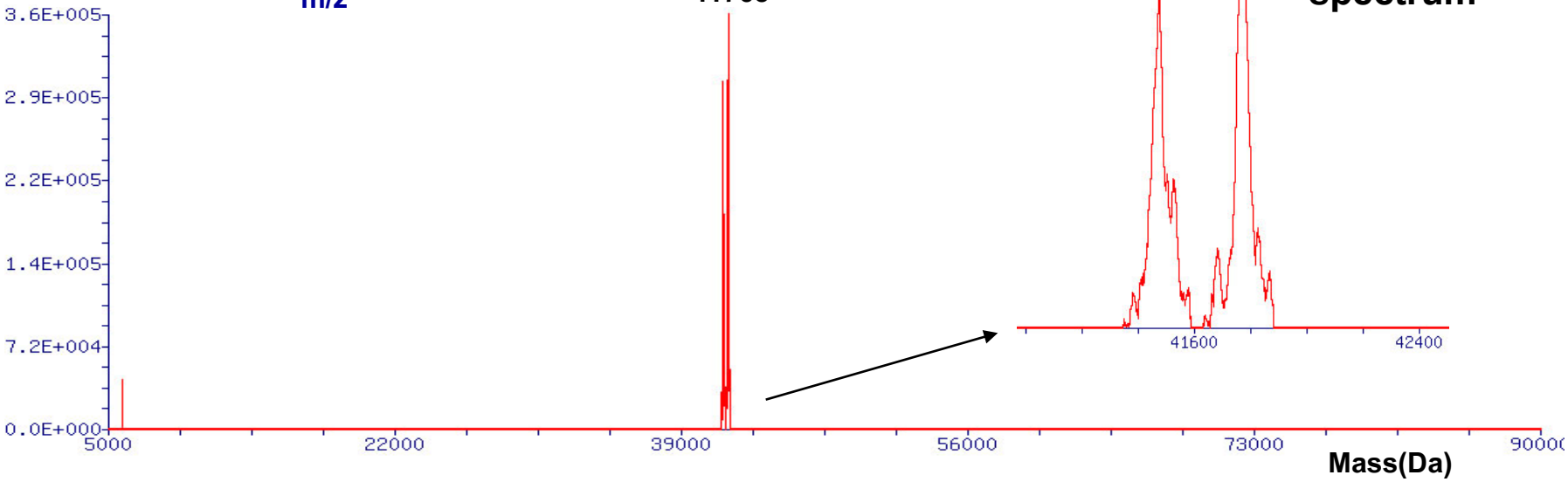
- Scoring based on accumulated S/N of all peaks in a charge series.
- Highest scoring test charge state represents the correct charge state.
- Once correct charges are determined, only those signals are transformed to the zero-charge (deconvoluted) spectrum.

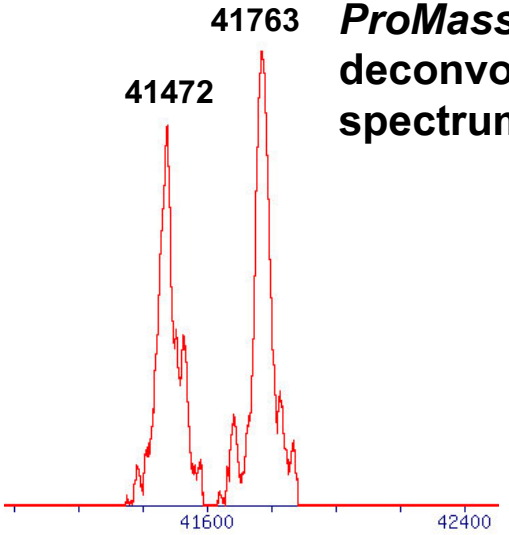
ProMass Deconvolution of Low S/N ESI Data



Raw ESI mass spectrum from crude protein purification

baseline calculated for removal

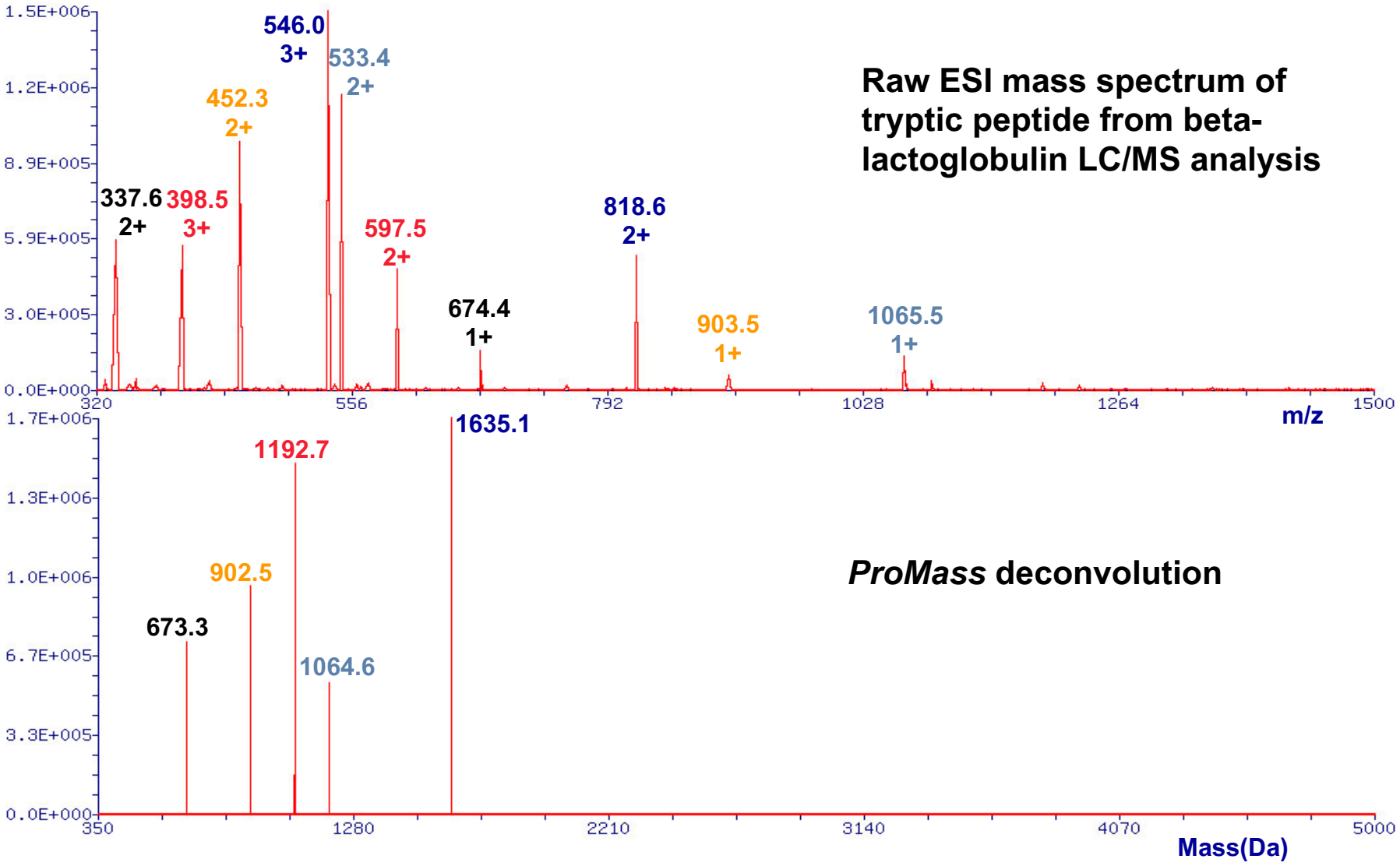




ProMass **deconvoluted spectrum**

ProMass Deconvolution of Peptide Mixtures

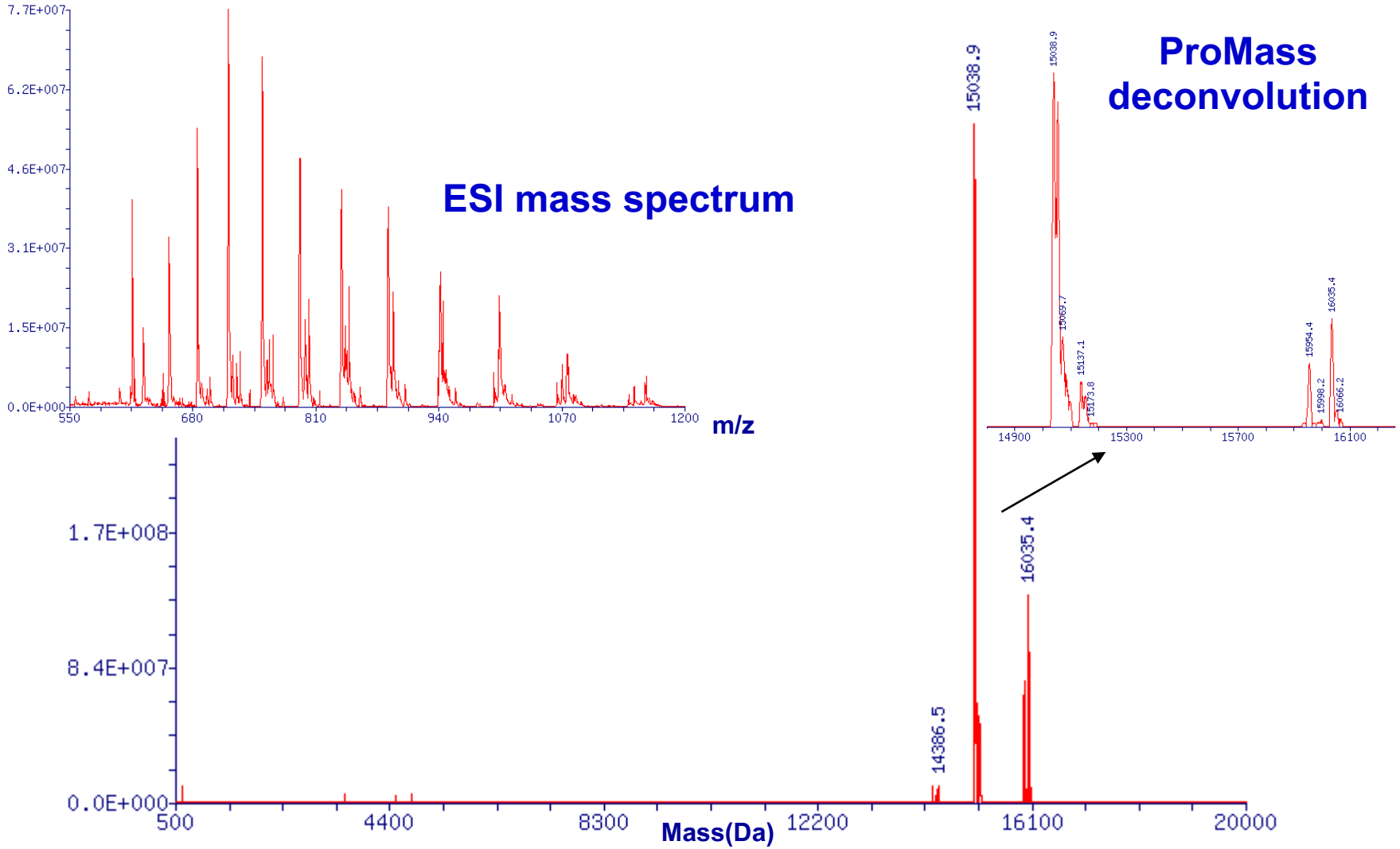
ProMass works on low charge state spectra too



ProMass Deconvolution of Hemoglobin Mixture

Example of overlapping, closely-related mixture components

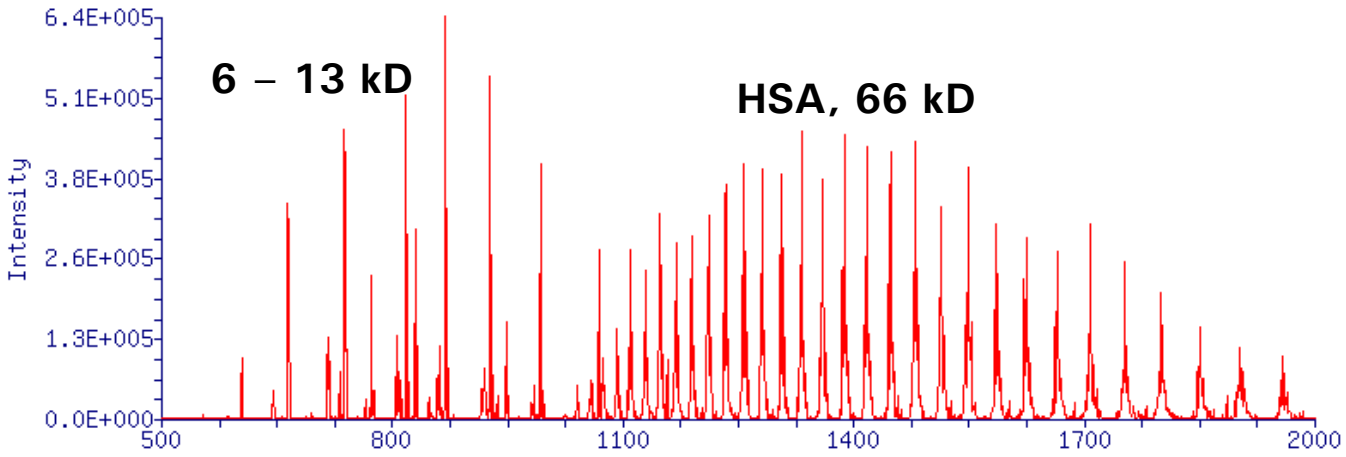
alpha & beta chains, bovine & porcine proteins



ProMass Deconvolution of Coeluting Components

Example of overlapping mixture components over wide MW range

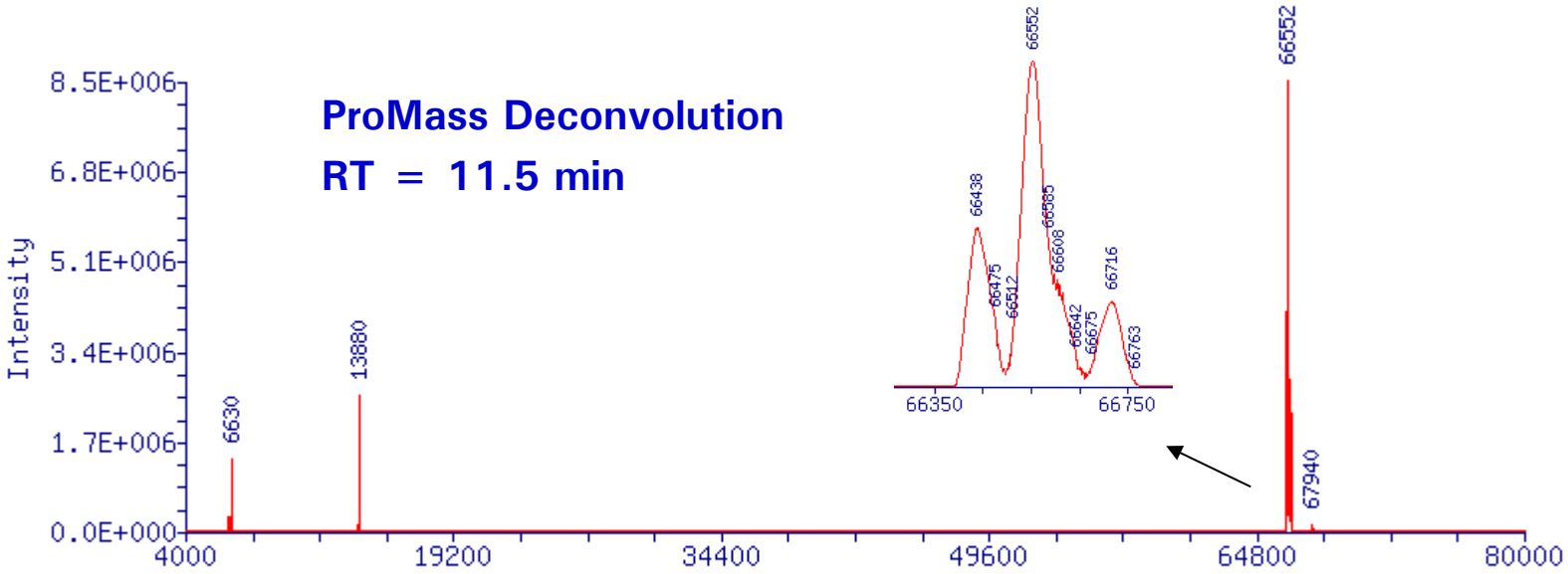
mixture is from supernatant from precipitated human serum



ESI mass spectrum

RT = 11.5 min

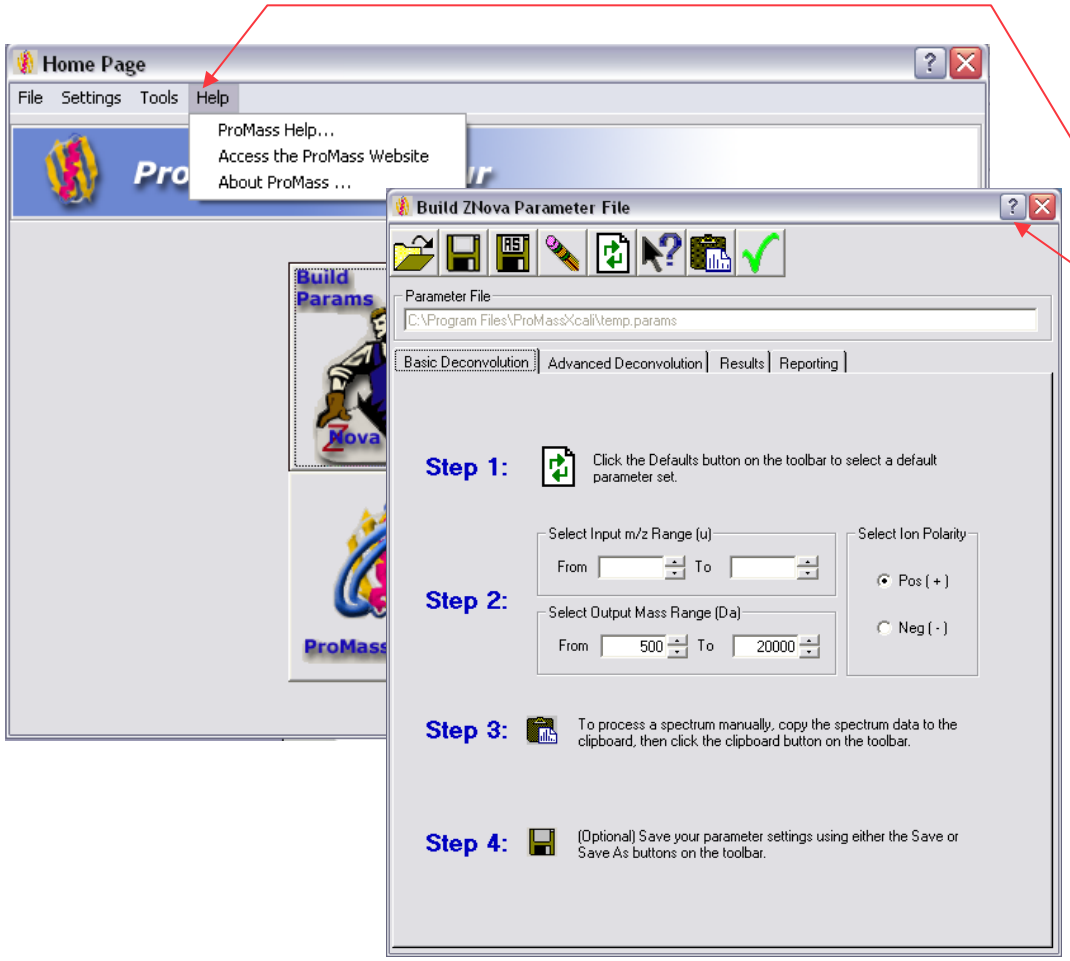
m/z



Mass

ProMass Documentation

A full set of on-line help is available for ProMass



Getting Help!

- Main help menu from ProMass HomePage
- Drag **?** at upper right window to any control to get help about that particular control OR hit F1.
- Hit the button to get context-sensitive help about the current tab or screen.
- Documentation – ProMass User's Guide PDF is available from the Windows Start Menu at **Start | Programs | ProMass for Xcalibur | ProMass User's Guide.** *However, User Guide is not being updated as frequently as on-line help.*

ProMass Example Data Files

- Example data files automatically installed with ProMass application:

  See: C:\Program Files\ProMassXcali\TestData

- All examples include pre-configured sample lists and processing methods to allow testing of automated features.
- Examples include:
  - *myolcmsdata.raw: Myoglobin LC/MS run – useful for demonstrating manual or automatic processing. This is a simple example with a single chromatographic peak. This is the file used in **Getting Started with Automated Processing** topic in the on-line Help.*
  - *protein lcms folder: LC/MS run of 5 protein standard mixture, useful for demonstrating automatic processing of multiple chromatographic peaks.*
  - *oligos folder: Example of oligonucleotide MW confirmation using ProMass. Includes 5 data files. Demonstrates target mass summary and Excel reporting features. Described in **Application Example: Confirming Masses of Oligonucleotides using ProMass** on-line help topic.*

ProMass HomePage

***ZNova* Parameter Setup And Manual Processing**

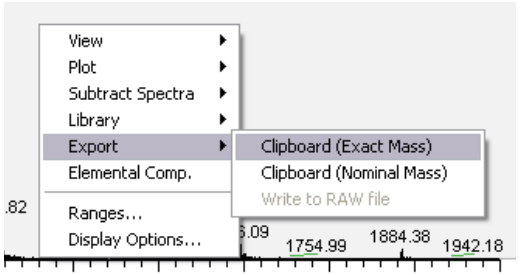
Add *ZNova* Parameters to Xcalibur Sequence

Browse the latest *automated* processing event

Validate Xcalibur Processing Method to ensure proper format

Basic *ProMass* Manual Operation Mode

- Export Xcalibur spectrum to clipboard from *Qual Browser*
  - *Right click spectrum and select **Export** | **Clipboard (Exact Mass)***

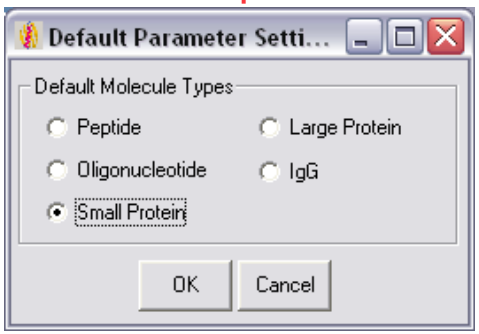


- Launch ProMass and click Build Params

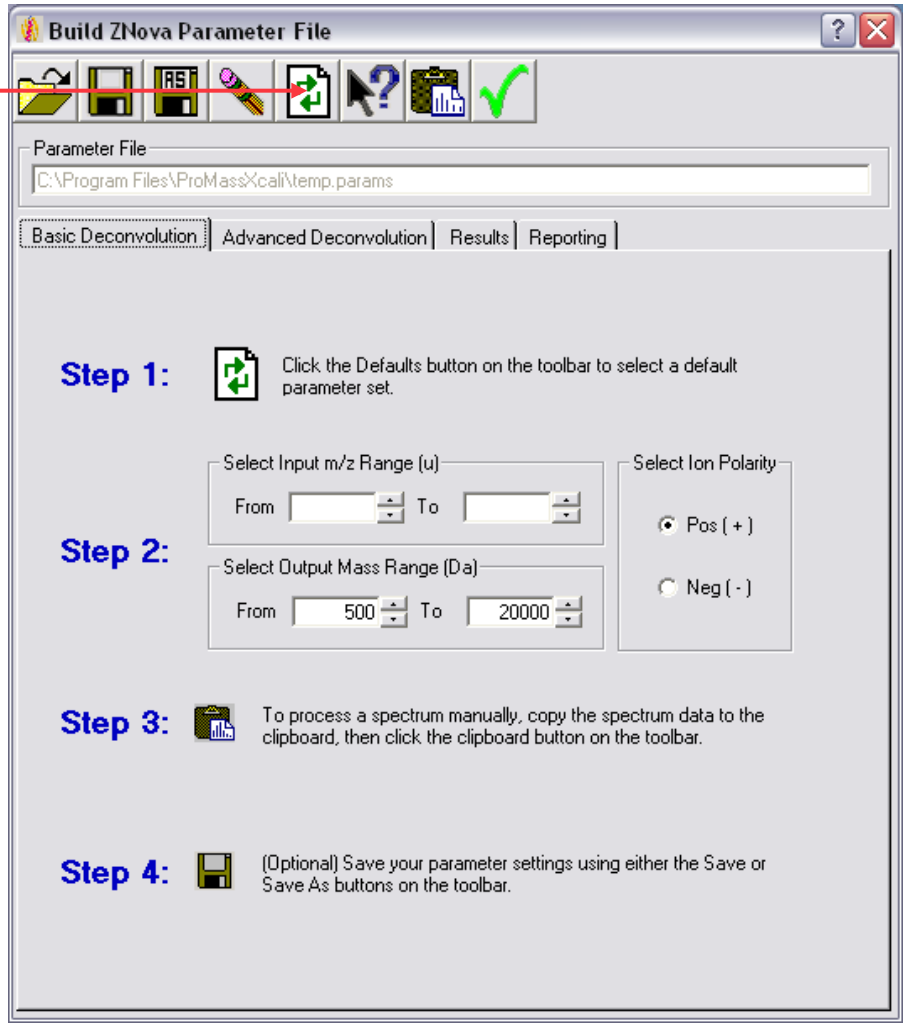
- Set or restore a default set of parameters

- Click the paste/process spectrum button

ProMass Basic Deconvolution Settings

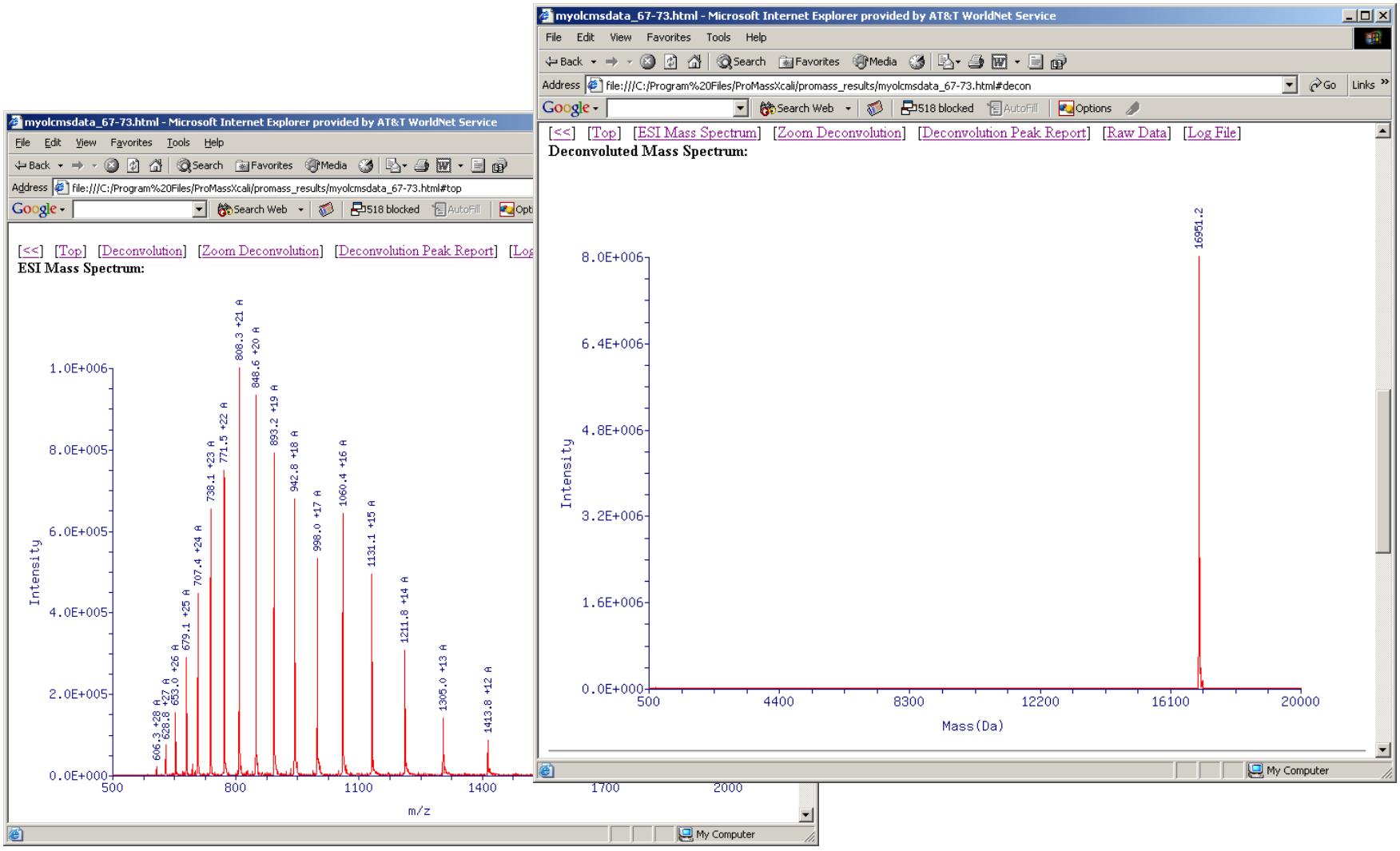


A new user can easily process spectra by restoring a set of default parameters and setting input and output mass ranges.

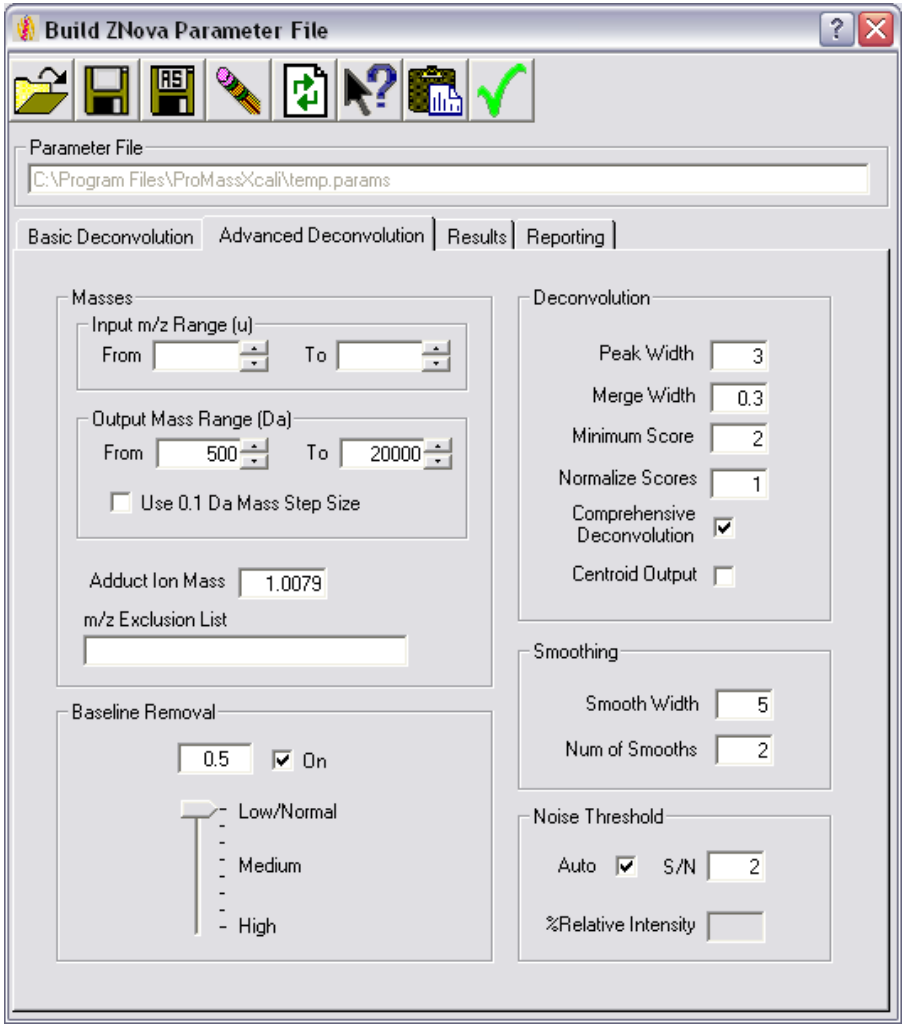


Basic Deconvolution Live Demo

Myoglobin LC/MS example data



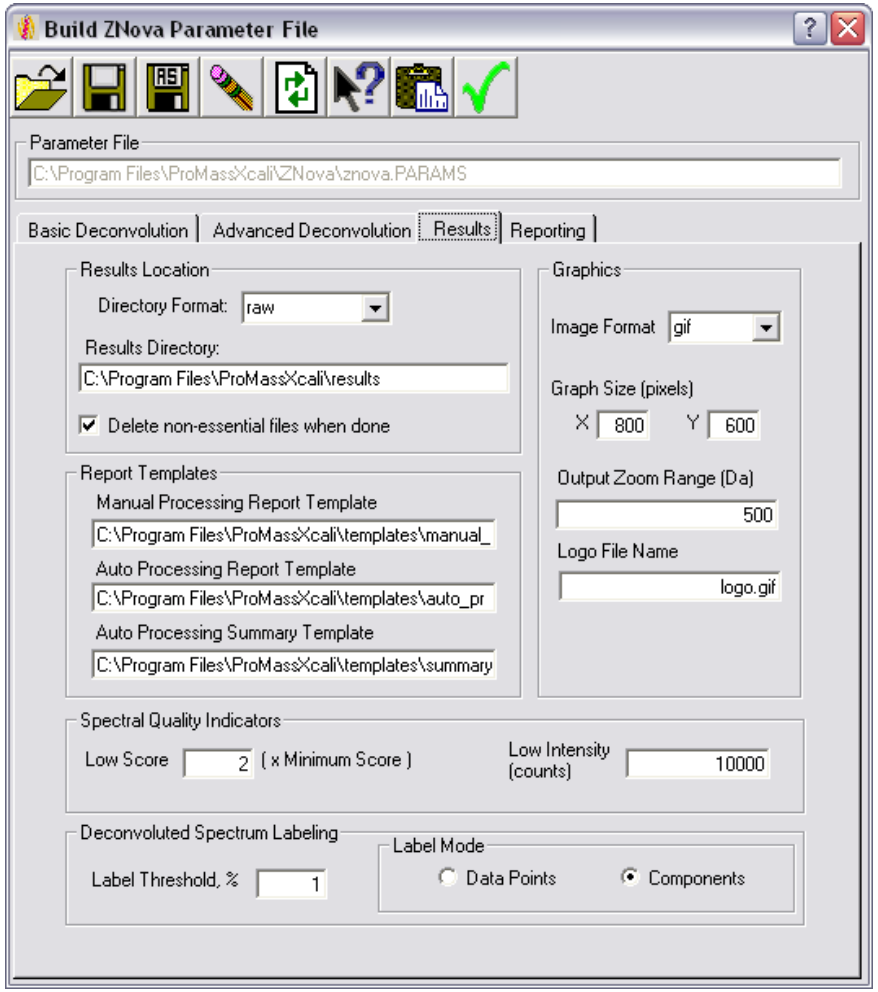
ProMass Advanced Decon Settings



Parameters Guide:

- Set input/output mass ranges
- Use 0.1 Da step size for < 5000 MW
- *Adduct Ion Mass* 1.0079 for pos ions, –1.0079 for neg ions
- Adjust *Baseline Removal* to get a flat baseline (look at raw ESI spectrum in ProMass report)
- Set *Peak Width* to match m/z peak width (at base) in original mass spectrum
- *Merge Width* ~10% of *Peak Width*
- Use *Comprehensive* decon for most applications where there could be mixture overlap
- *Centroid Output* not generally recommended
- Explore settings! Take advantage of the context-sensitive on-line help.

ProMass Results Settings



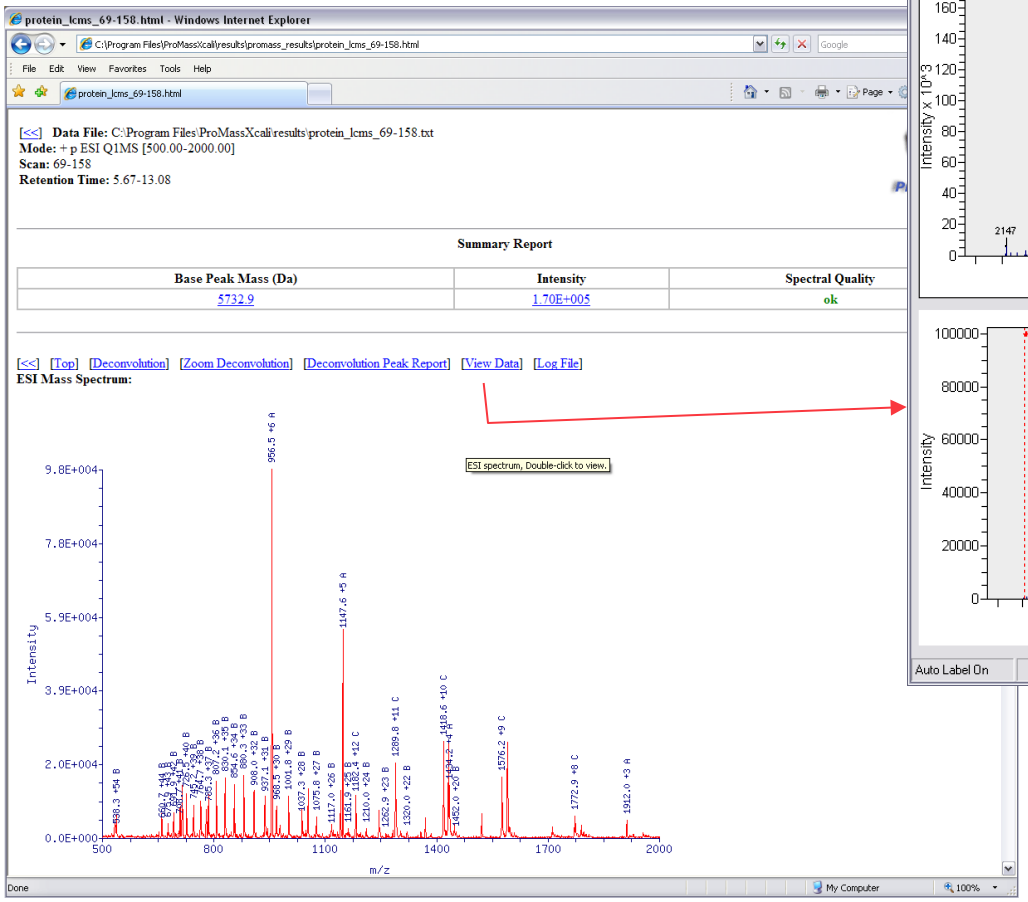
What the settings do:

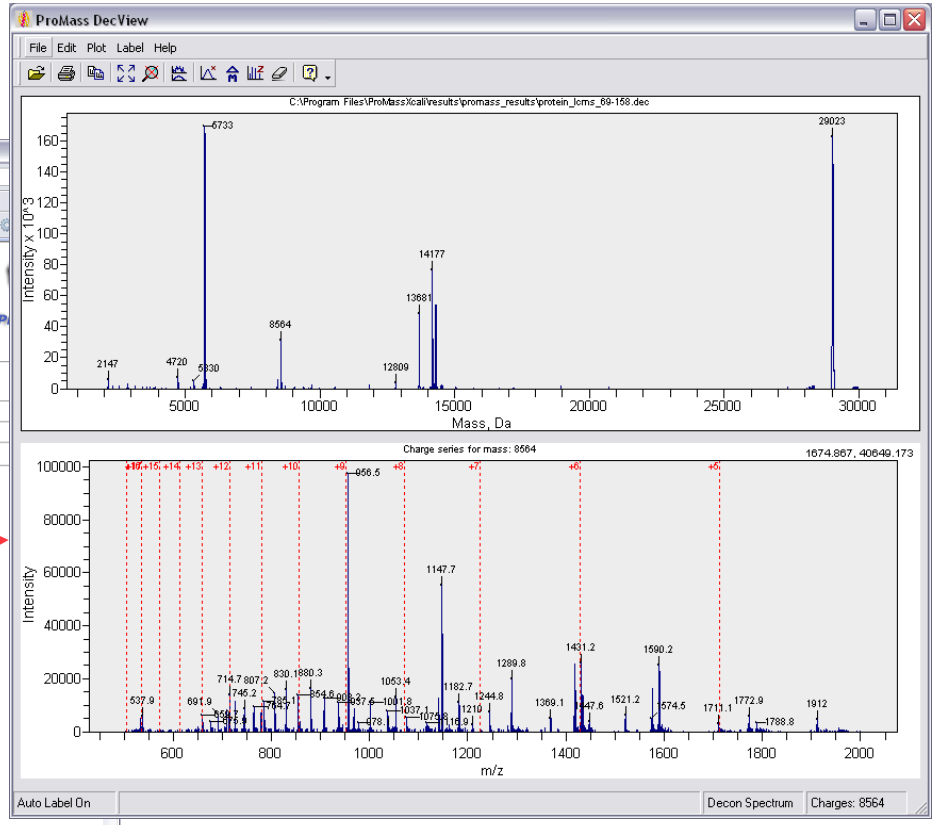
- Determine where results are stored
- Customize the graphics output
- Allow user to set the width of the zoomed spectrum, or to set an explicit zoom range
- Specify report template for web-based output
- Set labeling parameters – use data points labeling mode if components labeling does not give you enough detail, especially when a wide peak width setting is used.

Deconvoluting Difficult Spectra

- Most (>80%) spectra can be deconvoluted successfully with "default" settings
  - *Try default settings first, then modify as needed*
- Noisy spectra, complicated mixtures, or spectra with a high baseline may require some parameter adjustment
  - *Adequately remove the baseline noise*
  - *Choose the correct peak width*
  - *For very noisy spectra, narrow your deconvolution range to focus the algorithm to where you expect masses to be present.*
  - *Noisy spectra may require manually setting the noise threshold*
- A *"blob"* spectrum with a peak at every mass may be impossible to deconvolute with any algorithm
- Use ProMass viewer to validate charge series
- Demonstrated Examples:
  - *Intact IgG's – setting a wide peak width*
  - *Crude 40 kd protein – removing baseline, setting a reasonable noise level*
  - *Hemoglobins, etc*

Interactive Viewing with the ProMass Viewer



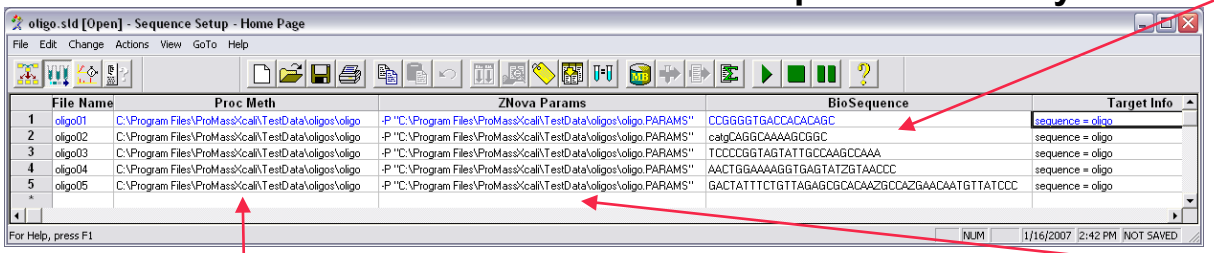


- Double click spectrum or use [View Data] link to launch ProMass viewer
- Use the ProMass viewer to validate charge series and export high quality graphics to Word, Powerpoint, etc.

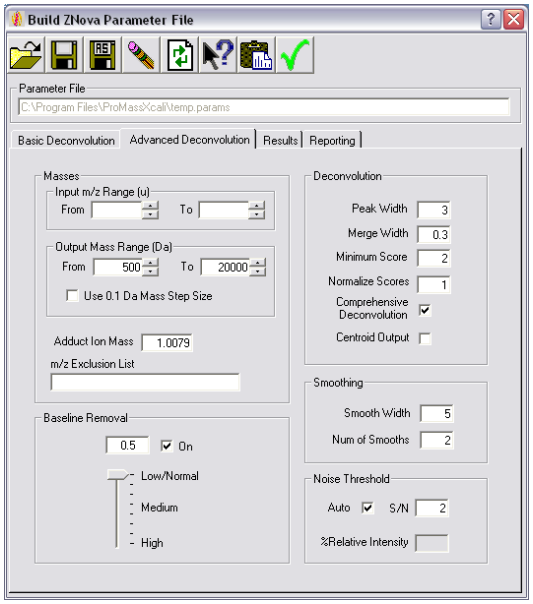
ProMass Automated Processing

Xcalibur Sample Sequence
defines samples to be analyzed

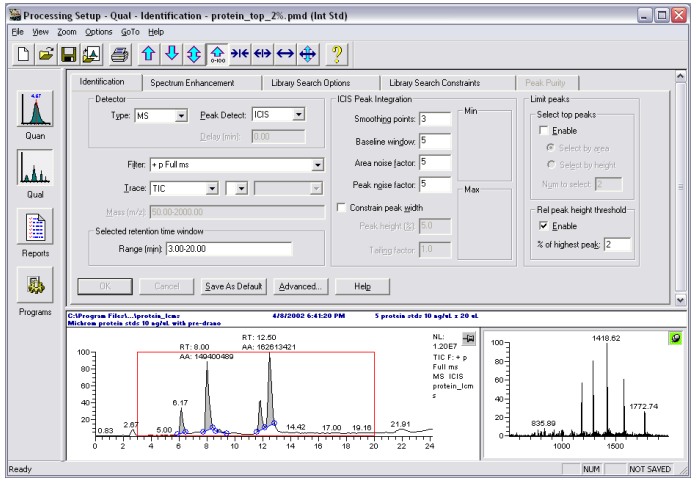
optional amino acid or nucleotide sequence



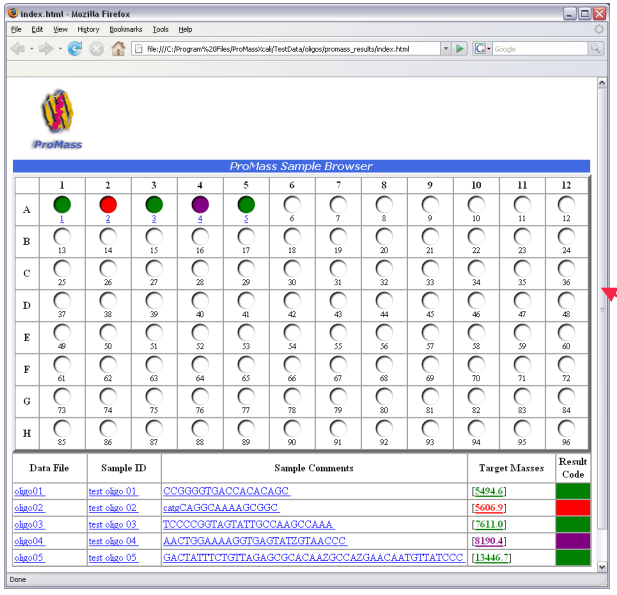
Parameter Setup
defines parameters for deconvolution



Xcalibur Processing Method
defines how peaks are selected



Peak picking, auto deconvolution, and report generation



HTML Results Report
including plate view, spectra, chromatograms, tables, etc.

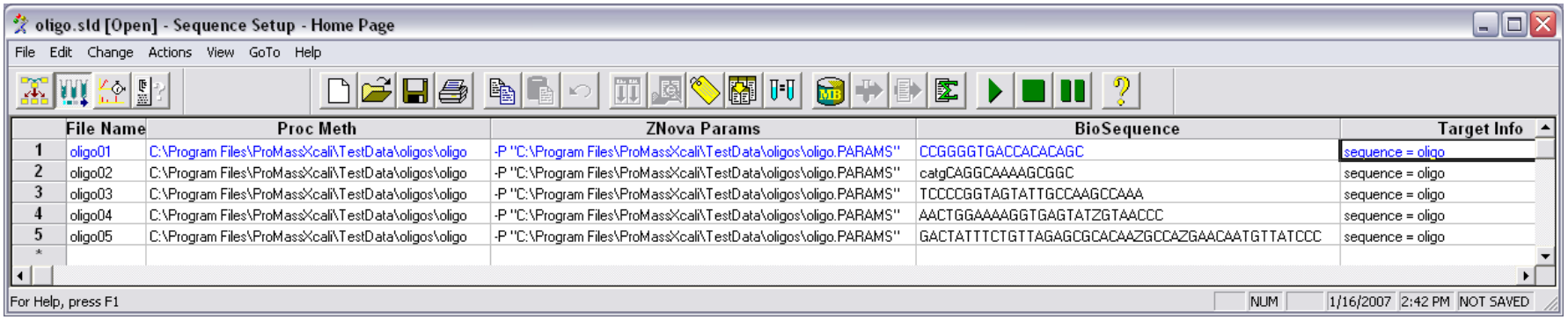
ProMass Automated Processing

- The easiest way to learn is by trying the examples in the ProMass program TestData folder

- Help topics include a Getting Started section and detailed instructions for setting up automated runs

- Basic Requirements:
  - *Xcalibur sequence with data file(s)*
  - *Valid Qual Processing Method*
    - ProMass program is "attached" to Proc Method
    - Proc Method scan type must be set to match data file
    - Proc Method must be able to detect chromatographic peaks
  - *Optional ZNova parameters, target masses, or biomolecule sequence*
  - *Process data using **Batch Reprocess** function or launch automatically after acquistion is completed*

Configuring Xcalibur Sample List

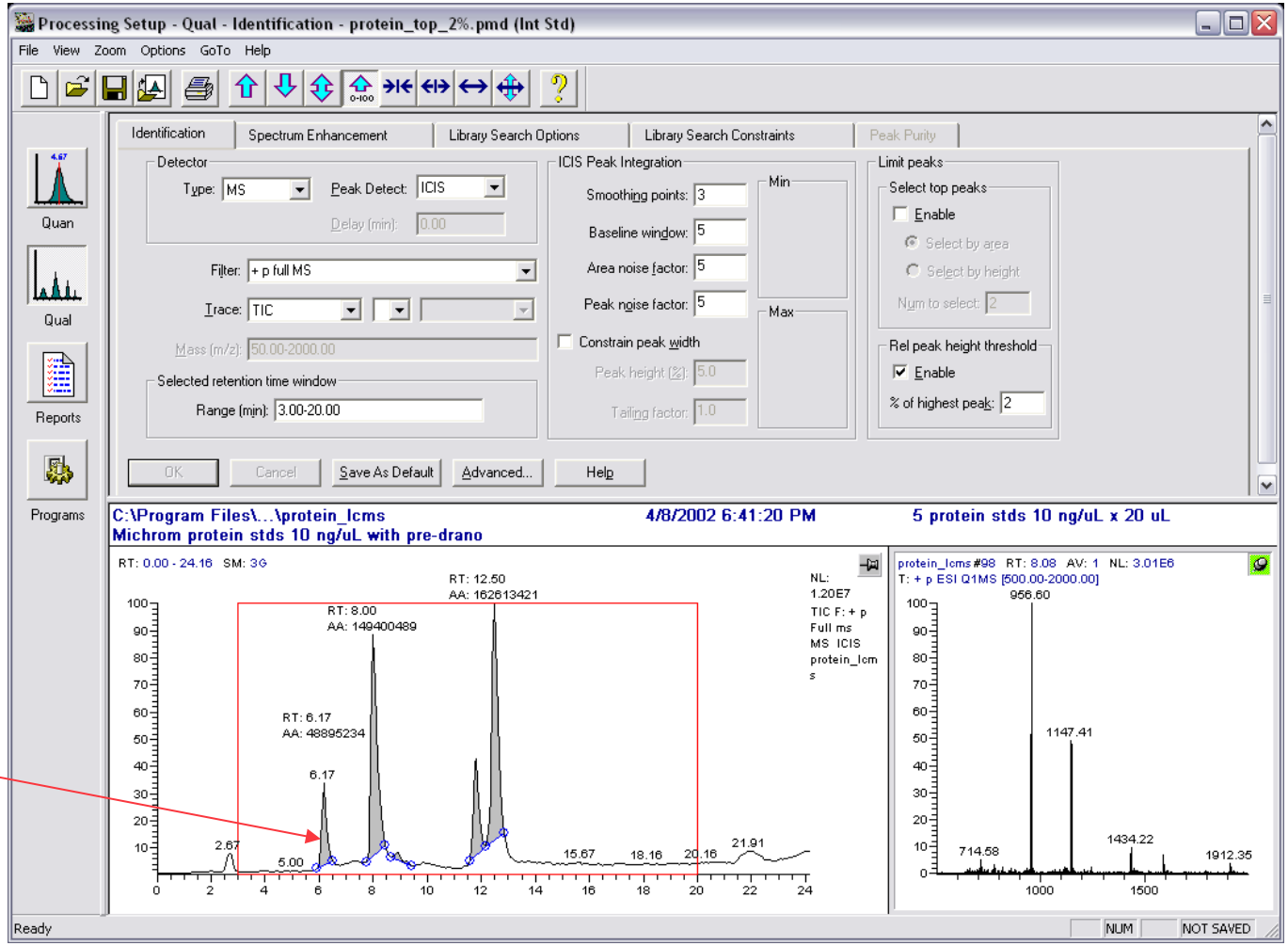


	File Name	Proc Meth	ZNova Params	BioSequence	Target Info
1	oligo01	C:\Program Files\ProMassXcali\TestData\oligos\oligo	-P "C:\Program Files\ProMassXcali\TestData\oligos\oligo.PARAMS"	CCGGGGTGACCACACAGC	sequence = oligo
2	oligo02	C:\Program Files\ProMassXcali\TestData\oligos\oligo	-P "C:\Program Files\ProMassXcali\TestData\oligos\oligo.PARAMS"	catgCAGGCAAAAGCGGC	sequence = oligo
3	oligo03	C:\Program Files\ProMassXcali\TestData\oligos\oligo	-P "C:\Program Files\ProMassXcali\TestData\oligos\oligo.PARAMS"	TCCCCGGTAGTATTGCCAAGCCAAA	sequence = oligo
4	oligo04	C:\Program Files\ProMassXcali\TestData\oligos\oligo	-P "C:\Program Files\ProMassXcali\TestData\oligos\oligo.PARAMS"	AACTGGAAAAGGTGAGTATZGTAACCC	sequence = oligo
5	oligo05	C:\Program Files\ProMassXcali\TestData\oligos\oligo	-P "C:\Program Files\ProMassXcali\TestData\oligos\oligo.PARAMS"	GACTATTTCTGTTAGAGCGCACAAZGCCAZGAACAATGTTATCCC	sequence = oligo

- **Required Fields: *File Name, Proc Meth***
- *ZNova Params (optional):* for entering parameter strings
  - *if not specified, default parameter set is used (znova.params in znova program directory)*
  - *Parameter file specified with –P option, many other options available*
- *BioSequence (optional):* amino acid or nucleotide sequence
  - *Masses automatically calculated and treated as "target masses"*
  - *Sequence type must be specified in Target Info field*
  - *Can specify a path to a sequence text file*
- *Target Info (optional):* information about target masses
  - *Specify BioSequence string type*
  - *Enter explicit comma-separated target masses*

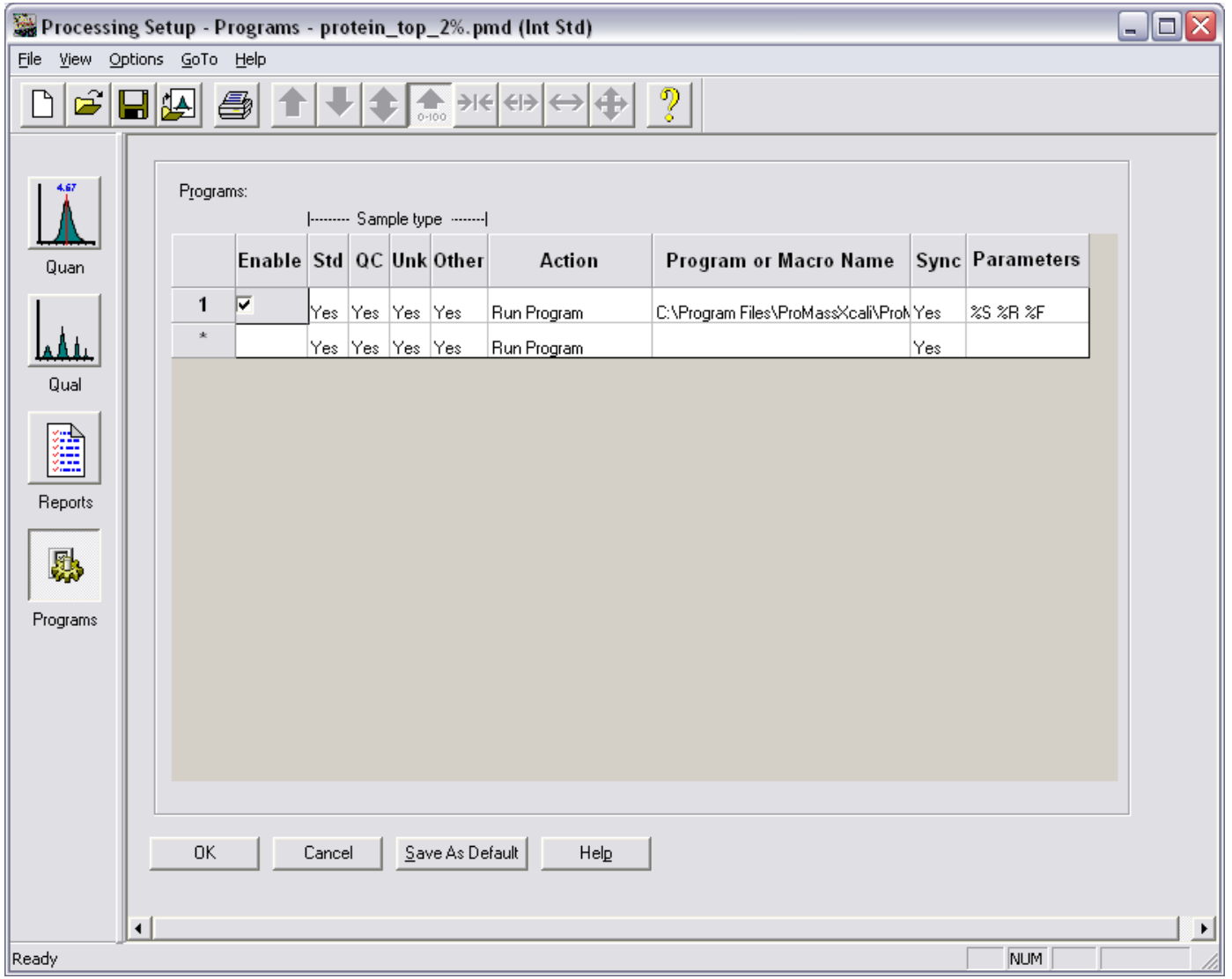
Configuring a Qual Processing Method

- Select Qual
- Open Raw file
- Set Scan Filter
- Set Chrom. Time range
- Set integration options
- Limit peaks if necessary
- Greyed area of peaks indicates what scans will be averaged for ProMass decon



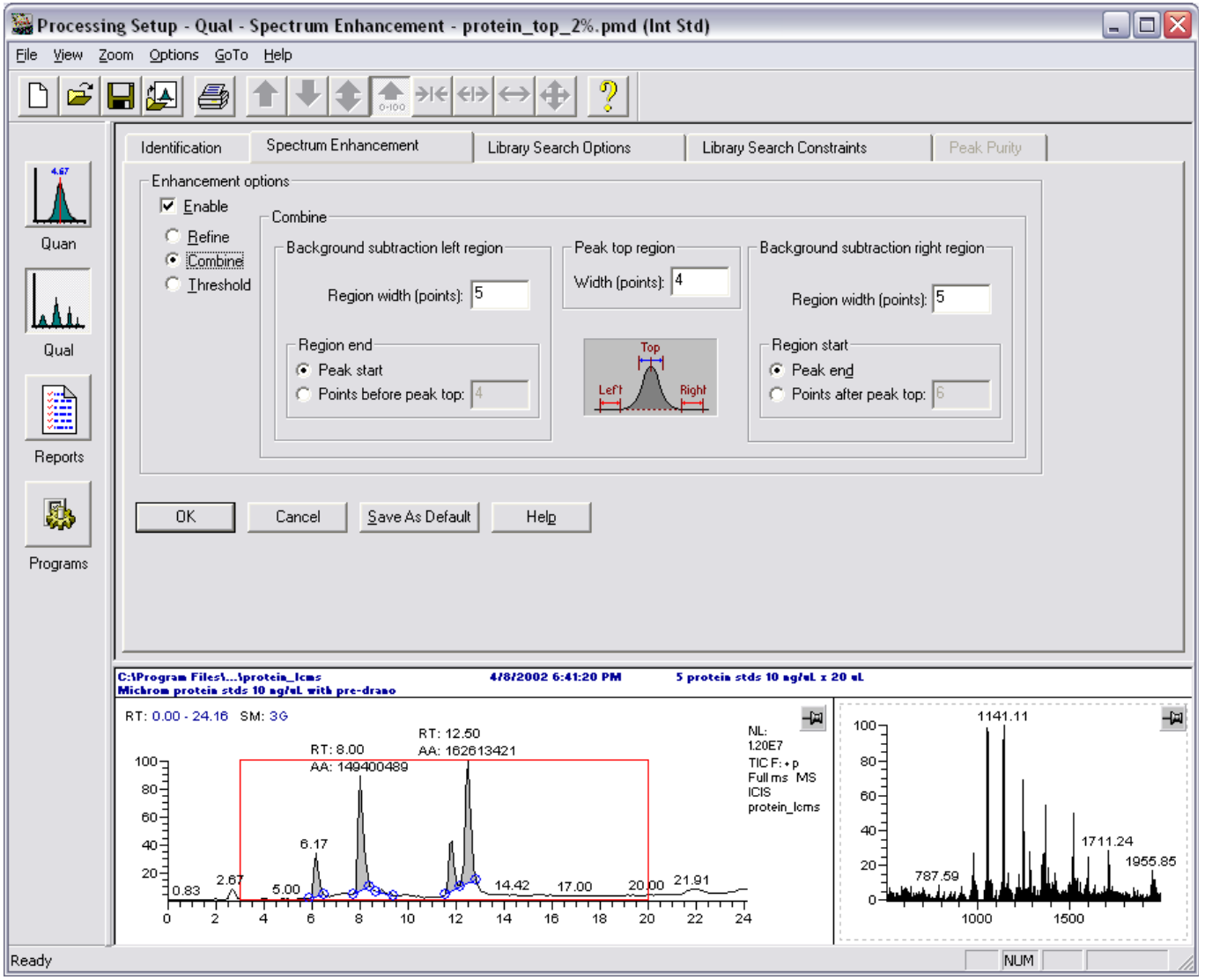
Configuring a Proc Method, Programs Section

- ProMass program is attached to Proc Method
- Must be configured as shown
- Use Check Proc Method tool on ProMass Home Page to add these parameters

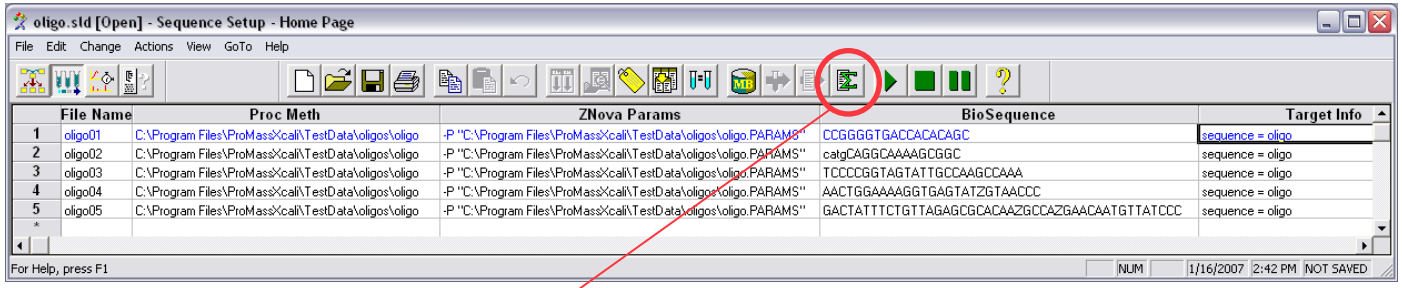


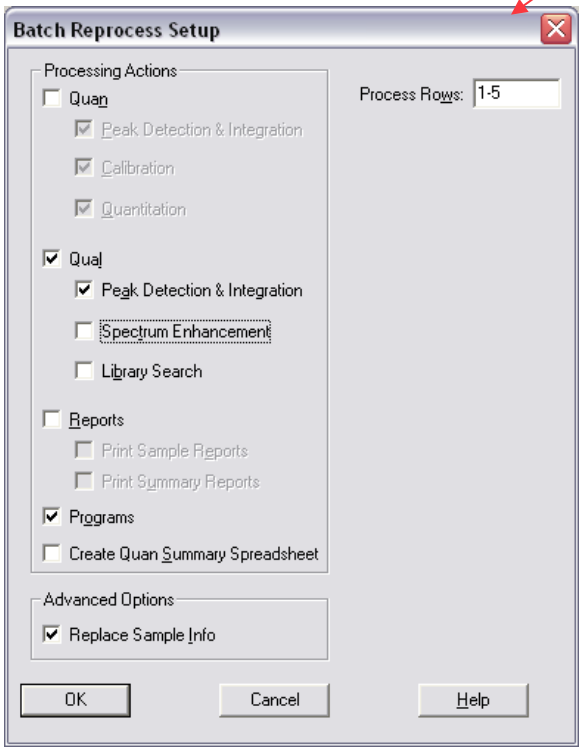
Configuring a Proc Method for Background Subtraction

- Select Spectrum Enhancement Tab
- Select *Enable*
- Only *Combine* option is supported for bkgnd subtract
- Set regions for peak top and before and after peak



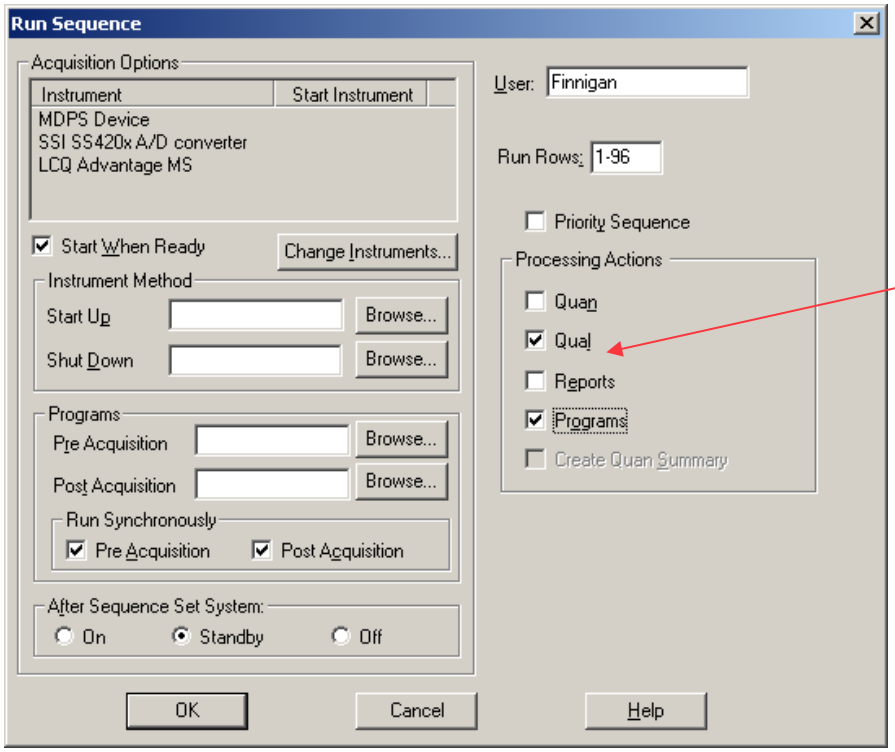
Batch Reprocessing with ProMass





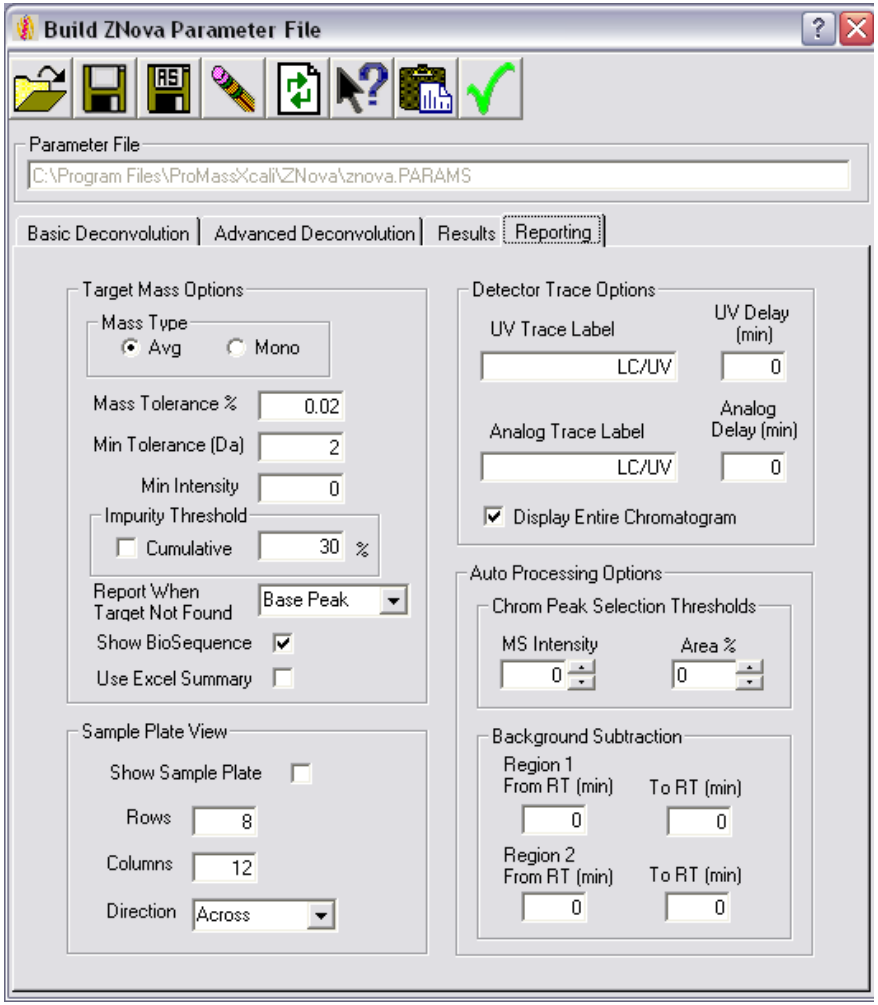
- Acquire data
- Configure sample list
- Hit the Batch Reprocess button
- Check the options shown at the left
- *Spectrum Enhancement option may also be checked to allow for background subtraction (must also be configured in Proc Method)*

Automatic Post-Acquisition Processing



- ProMass can also be run automatically following acquisition through the Run Sequence… dialog.
- Select the *Qual* and *Programs* options in the *Run Sequence* dialog.
- Ensure that the sample list is fully configured to run ProMass if you select these options at run time.

ProMass Reporting Settings



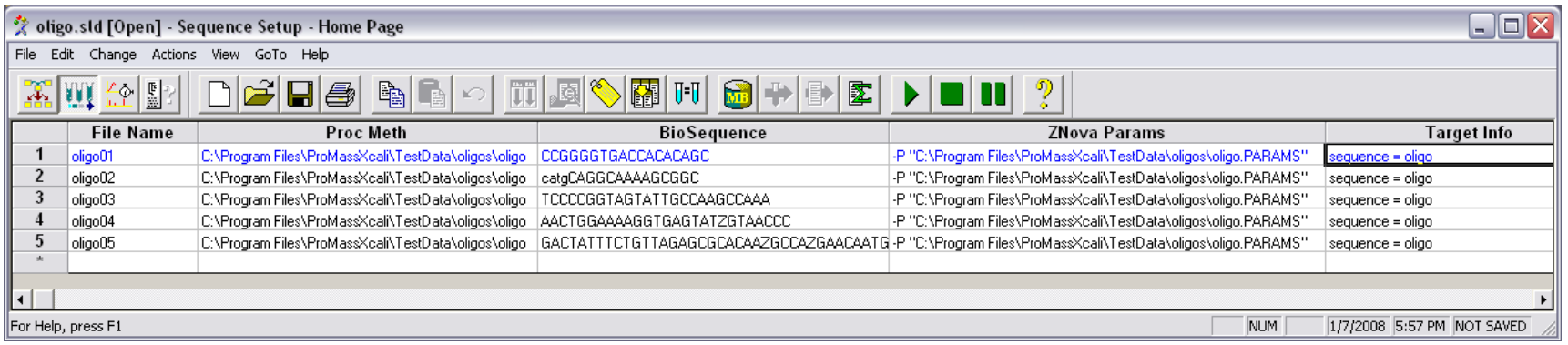
What the settings do:

- Reporting Tab ONLY affects automated processing report does nothing to manual processing report
- Determines Target Mass behavior
- Enables Sample Plate View or Excel Summary
- Set labeling behavior of chromatogram traces
- Allows for setting of thresholds for chromatographic peaks selected for deconvolution (overriding proc method settings)
- Allows explicit background subtraction regions to be set (overriding proc method settings)

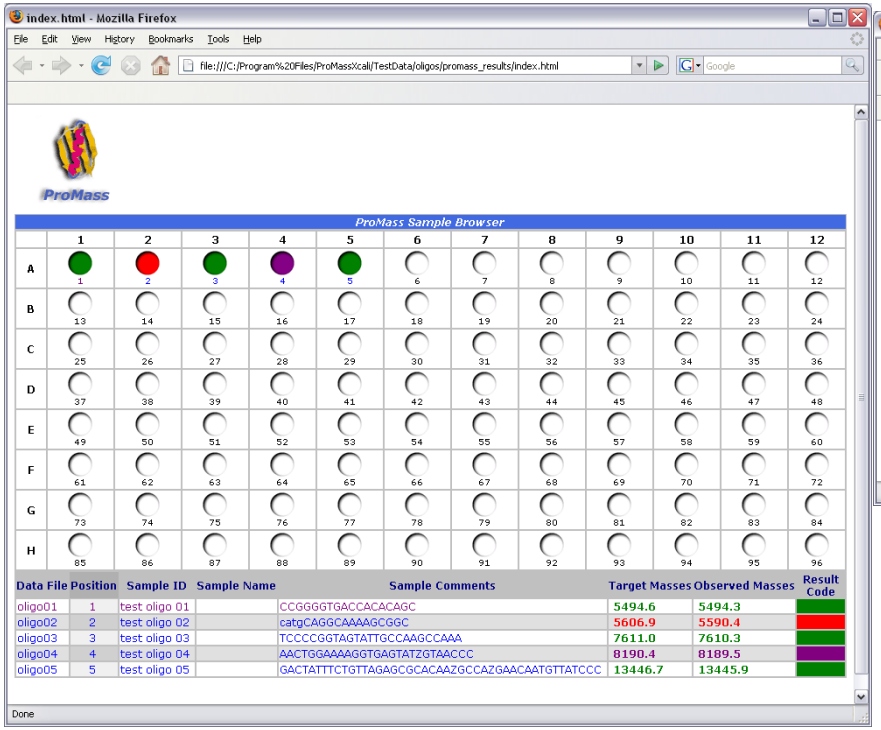
Automated Processing Demo

- Examples are included with ProMass installation
- Myoglobin LC/MS – simple example with single chromatographic peak
- Oligonucleotide mass confirmation – target mass features, plate view, Excel summary
- Protein LC/MS mixture – deconvoluting multiple chromatographic peaks, multiple target masses

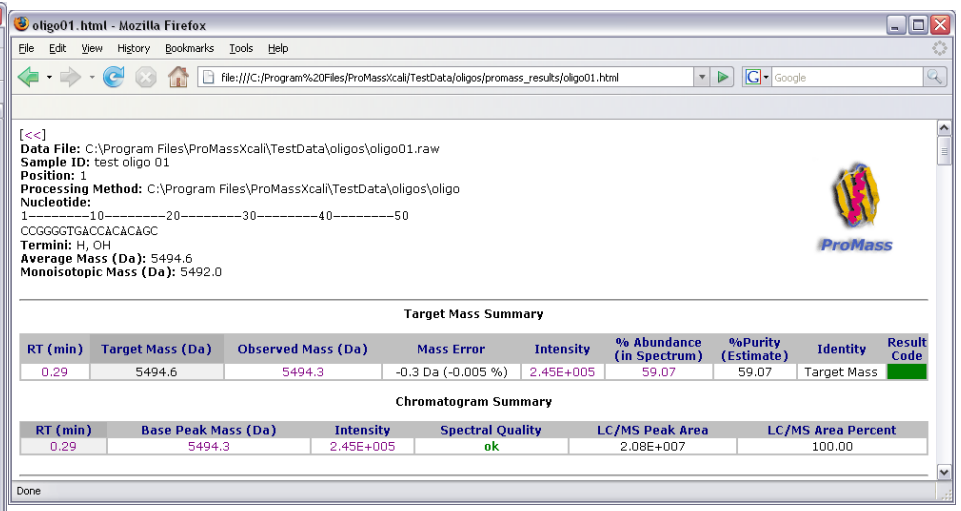
Oligo Application Example



	File Name	Proc Meth	BioSequence	ZNova Params	Target Info
1	oligo01	C:\Program Files\ProMassXcali\TestData\oligos\oligo	CCGGGGTGACCACACAGC	-P "C:\Program Files\ProMassXcali\TestData\oligos\oligo.PARAMS"	sequence = oligo
2	oligo02	C:\Program Files\ProMassXcali\TestData\oligos\oligo	catgCAGGCAAAAGCGGC	-P "C:\Program Files\ProMassXcali\TestData\oligos\oligo.PARAMS"	sequence = oligo
3	oligo03	C:\Program Files\ProMassXcali\TestData\oligos\oligo	TCCCCGGTAGTATTGCCAAGCCAAA	-P "C:\Program Files\ProMassXcali\TestData\oligos\oligo.PARAMS"	sequence = oligo
4	oligo04	C:\Program Files\ProMassXcali\TestData\oligos\oligo	AACTGGAAAAGGTGAGTATZGTAACCC	-P "C:\Program Files\ProMassXcali\TestData\oligos\oligo.PARAMS"	sequence = oligo
5	oligo05	C:\Program Files\ProMassXcali\TestData\oligos\oligo	GACTATTTCTGTTAGAGCGCACAAZGCCAZGAACAATG	-P "C:\Program Files\ProMassXcali\TestData\oligos\oligo.PARAMS"	sequence = oligo
*					



Data File	Position	Sample ID	Sample Name	Sample Comments	Target Masses	Observed Masses	Result Code
oligo01	1	test oligo 01		CCGGGGTGACCACACAGC	5494.6	5494.3	
oligo02	2	test oligo 02		catgCAGGCAAAAGCGGC	5606.9	5590.4	
oligo03	3	test oligo 03		TCCCCGGTAGTATTGCCAAGCCAAA	7611.0	7610.3	
oligo04	4	test oligo 04		AACTGGAAAAGGTGAGTATZGTAACCC	8190.4	8189.5	
oligo05	5	test oligo 05		GACTATTTCTGTTAGAGCGCACAAZGCCAZGAACAATGTTATCCC	13446.7	13445.9	



Target Mass Summary

RT (min)	Target Mass (Da)	Observed Mass (Da)	Mass Error	Intensity	% Abundance (in Spectrum)	%Purity (Estimate)	Identity	Result Code
0.29	5494.6	5494.3	-0.3 Da (-0.005 %)	2.45E+005	59.07	59.07	Target Mass	

Chromatogram Summary

RT (min)	Base Peak Mass (Da)	Intensity	Spectral Quality	LC/MS Peak Area	LC/MS Area Percent
0.29	5494.3	2.45E+005	ok	2.08E+007	100.00

Additional Tools and Tips for Using ProMass

- Tools:
  - *The **ZNova-ize Sequence** utility will add ProMass processing parameters to an Xcalibur sequence*
  - ***Check Processing Method** utility will add the ProMass program to a Proc Method*
  - *Deconvoluted spectrum viewer*
- Settings
  - *Minimizing the console, setting task priority, suppressing error messages*
- Browsing previously processed files
- ProMass directory structure
- Where to find results
- *ZNova* mass configuration file
- Sharing ProMass results with colleagues

Resources and Feedback

- ProMass website: http://www.enovatia.com/products/promass/
  - *Includes the latest ProMass news*
  - *Downloads and updates*
  - *FAQ*
  - *Support information and links*
  - *Application examples*

- Novatia is continually working to improve ProMass and wants to be aware of any problems or suggestions to enhance the product.

- Please forward any feature requests and suspected software problems to Novatia.

- Contact Novatia at info@enovatia.com or (267) 385-8355