Analytical utility of mass spectral binning in proteomic experiments by SPectral Immonium Ion Detection (SPIID)

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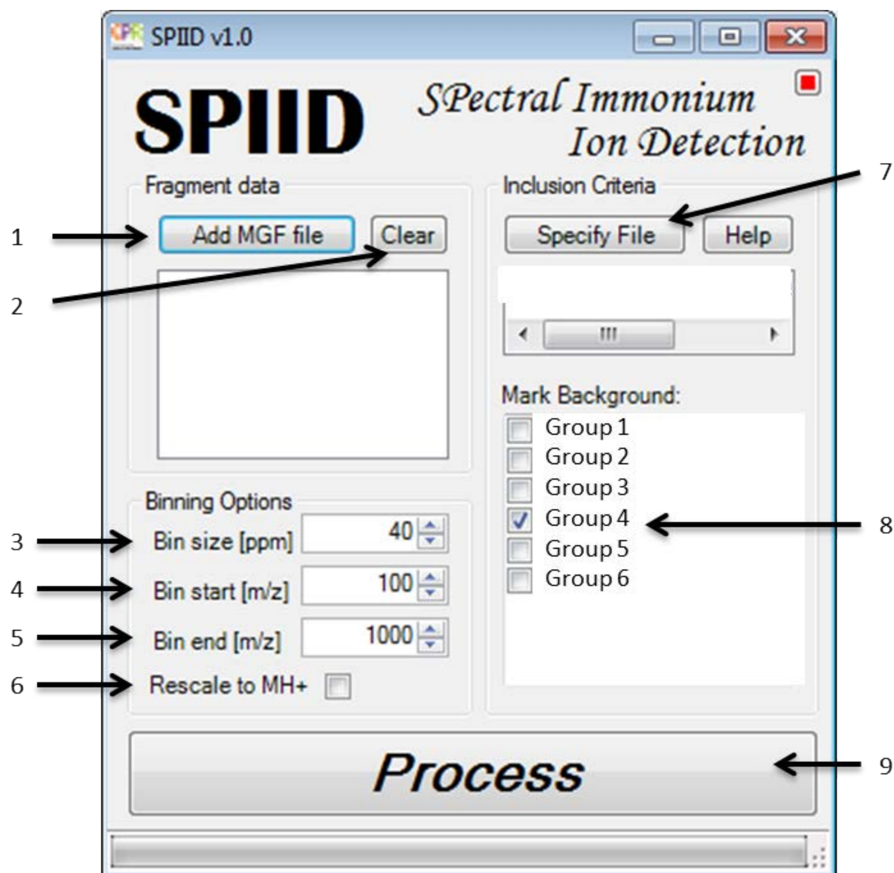
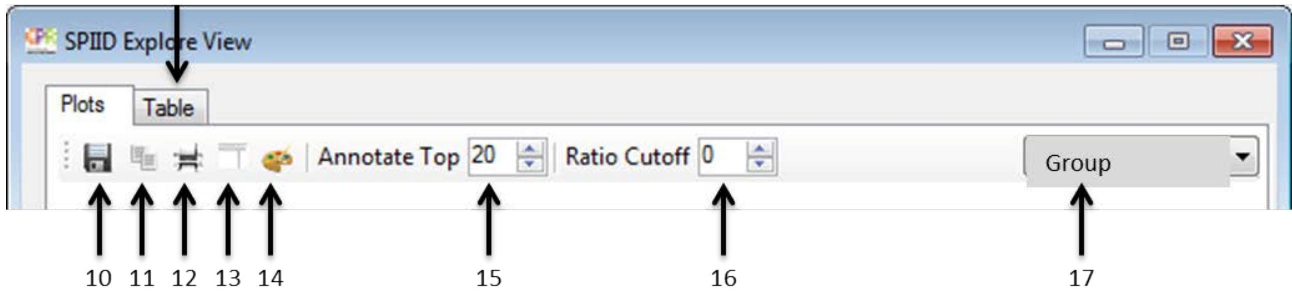
SPIID
User Interface Description

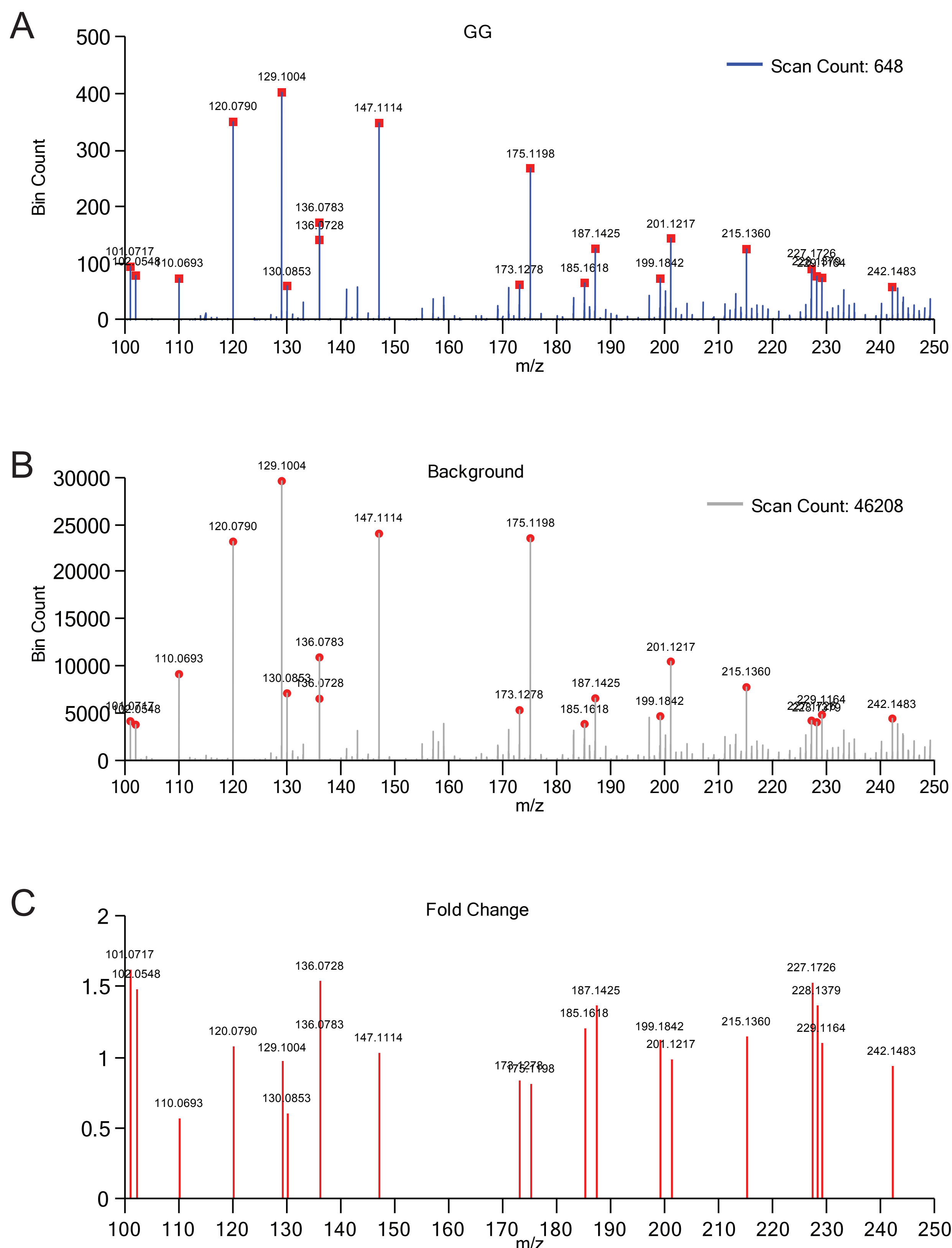


1) Input data are peak lists extracted from the raw data. APL files (MaxQuant/Andromeda peak lists) or MGF (MASCOT generic format) are supported. Multiple files can be specified. When selected they appear in the white box below this button.
2) This button clears the list of files to be processed.
3) The bin size is defined as the center mass +/- half the value specified in this field. A low bin size will generate more bins and therefore require more memory in the analysis.
4) The minimum m/z of the analysis can be specified here.
5) The maximum m/z of the analysis can be specified here.
6) This option specifies if spectra should be rescaled to the singly charged precursor mass prior to binning. This is useful for investigating neutral losses.
7) An optional file specifying subset groups of spectra to analyze can be specified here. The format of this need to be a tab-seperated file with details for each scan that needs to be included specified as rawfile, scan number, and group identifier i.e.
   RawFile112 29309 Unmodified
8) If a file was specified under 7) this shows the identified groups. Groups checked off as background are considered as one and used to construct the reference background histogram.
9) This button starts the analysis and a second window will appear with a graphical output.



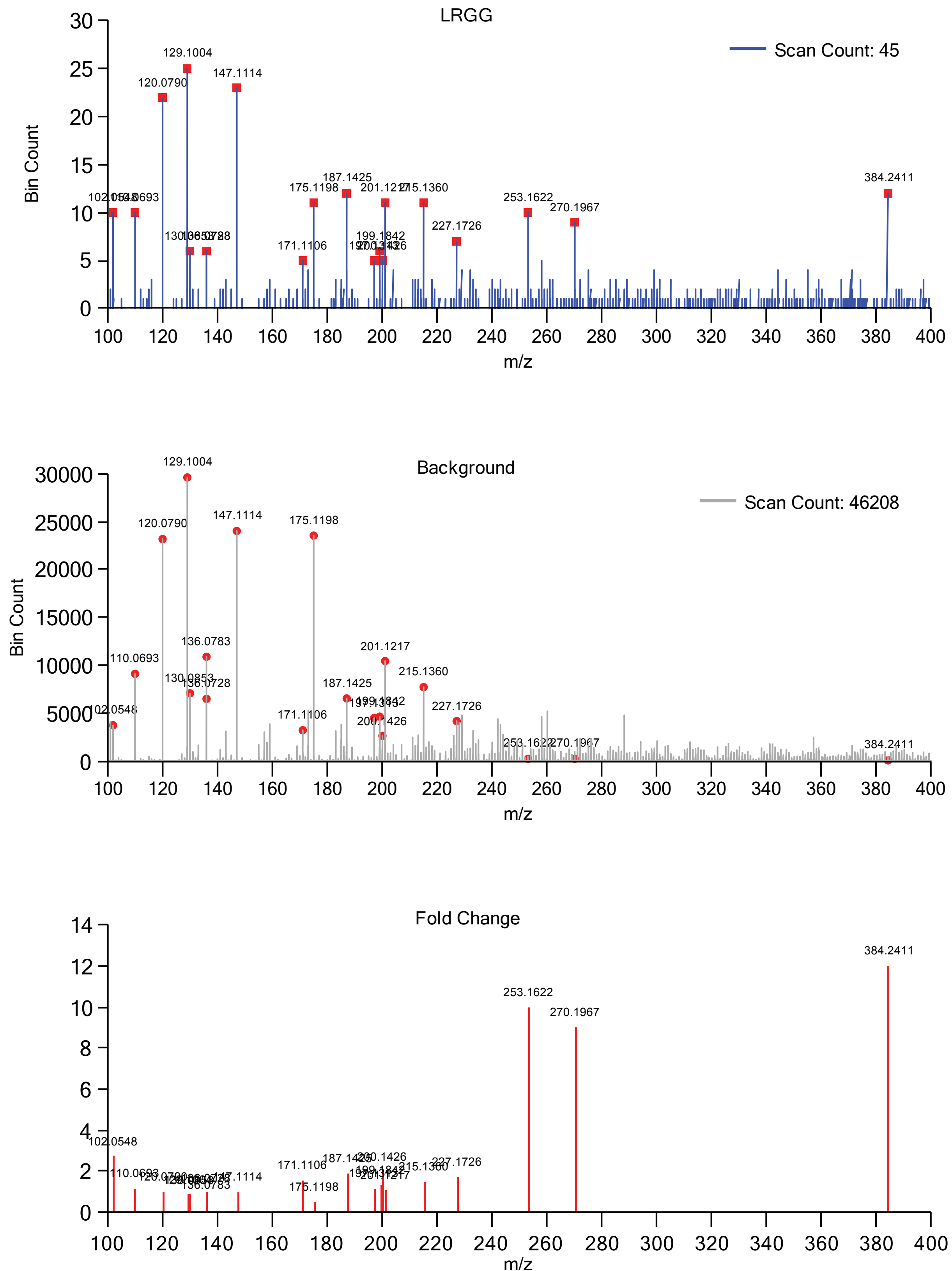
10) This button saves the current plot (Bitmap, JPEG, EMF, PNG, GIF, and TIFF are supported).
11) This button copies the current plot to the clipboard.
12) This button prints the current plot.
13) This button shows a drop down table of the current calculated fold changes
14) This button can change the visuals (color and font) of the current plot
15) This value can change the number of annotated bins. The bins are annotated in descending order based on bin count in the currently viewed range.
16) This enables a lower cutoff on the fold change ratios where lower values can be filtered out.
17) This changes the currently viewed group if a scan grouping file was specified under 7).
18) This table view enables a table view instead of the graphical depiction that can either be copied into a spread sheet program or exported as a tab delimited file.

Supplementary Figure 1



Supplementary Figure 1: SPIID output from an analysis of peptides identified to carry a Gly-Gly modified lysine, typically a remnant after tryptic digest of a ubiqutinylated peptide. (A) The histogram shows a histogram of the low mass region of 648 high resolution HCD spectra identified to carry the Gly-Gly modified lysine. The bins with the highest counts are annotated with their center m/z value. (B) A control histogram, “Background”, of 46,208 HCD spectra from unmodified peptides with the same bins annotated as the previous plot. (C) The last plot depicts the ratio difference between the GG modified spectra and control scaled by the difference in scan counts, with a maximum fold change equal to the bin count of the LRGG modified spectra. Here, no particular peak stands out.

Supplementary Figure 2



Supplementary Figure 2: SPIID output from an analysis of peptides identified to carry a Leu-Arg-Gly-Gly modified lysine, a remnant after tryptic digest of ubiqutinylation with a missed cleavage of the ubiquitin side chain. (A) The histogram shows a histogram of the low mass region of 45 high resolution HCD spectra with the highest counts annotated with their center m/z value. (B) A control histogram, “Background”, of 46,208 HCD spectra from unmodified peptides with the same bins annotated as the previous plot. (C) The last plot depicts the ratio difference between the LRGG modified spectra and control bins scaled by the difference in scan counts, with a maximum fold change equal to the bin count of the LRGG modified spectra. Here, mass bins corresponding to b-ions from the LRGG side chain are found, in particular the b2 ion, the b2 with ammonia loss, and the b4 ion.