Scaffold Quick Manual

Scaffold files (Proteome Software) can be used for the visualization of the protein and peptide identification results. A free scaffold viewer can be downloaded from:
http://www.proteomesoftware.com/products/free-viewer/

Please find below a brief summary of the most relevant Scaffold features. Refer to the official Scaffold manual for detailed information or use the Scaffold Help function.

Views

Samples view

- Define protein & peptide thresholds
- Change display options
- Search for proteins
- Add GO annotations
- Link to protein databases
- Adjustment of taxonomy preferences

Once opened the file, you should set the filter so that only the confidently identified proteins are shown. Please set the filters (at the top) to:

Protein Threshold: 1% FDR (#1)
Min # of peptides: 2 (#2)
Peptide Threshold: 0.1% FDR (#3)
Display options: Total Unique Peptide or Spectra Count (#4)
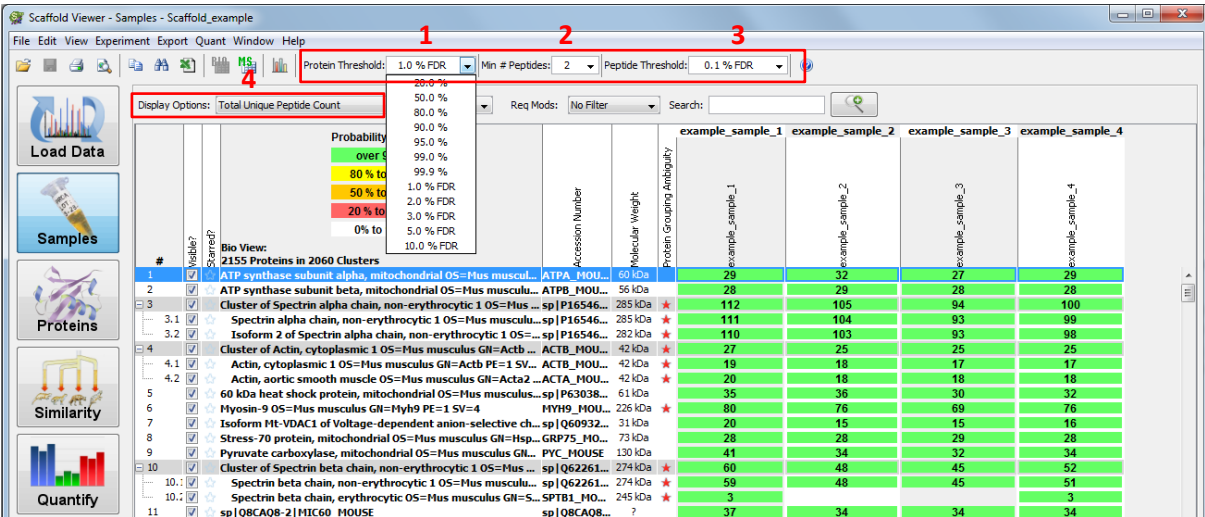
Once the Scaffold file is opened, you may change these settings to view more protein identifications.

The Scaffold search box allows the user to type in search terms to identify specific proteins by protein names or accession numbers, but it can also filter on peptide sequences and/or spectra information. The Search field accepts regular expressions and filters the results based on accession number or protein name.


The Advanced Protein Filter dialog allows to filter for, e.g., Protein Sequence Motifs (N.[ST], ...), or presence/absence from 1 or more categories.

If the GOs are not already added to your experiment, you can add them as follows:
Scaffold allows adding Gene Ontology annotations to the results. From the menu Experiment select "Configure GO annotation sources", select NCBI annotation and add them to the samples (Experiment → Add NCBI annotation).

NOTE: downloading the GO annotation of the whole NCBI database takes around 4h. Updates are performed every time you add annotation to your samples.
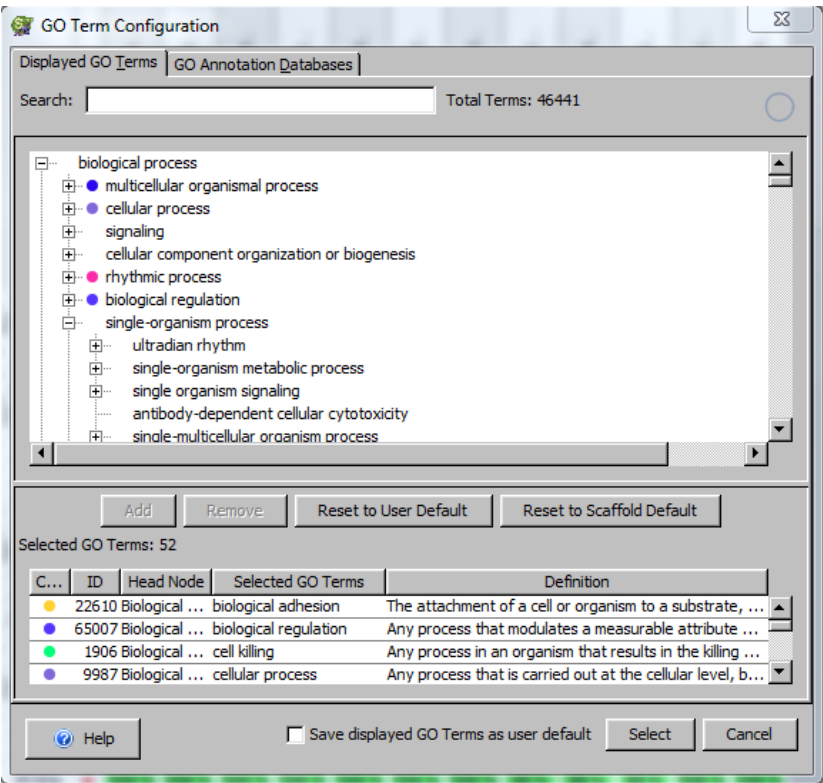
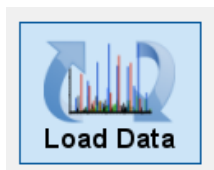The GO terms can be visible by selecting View → Show GO annotations
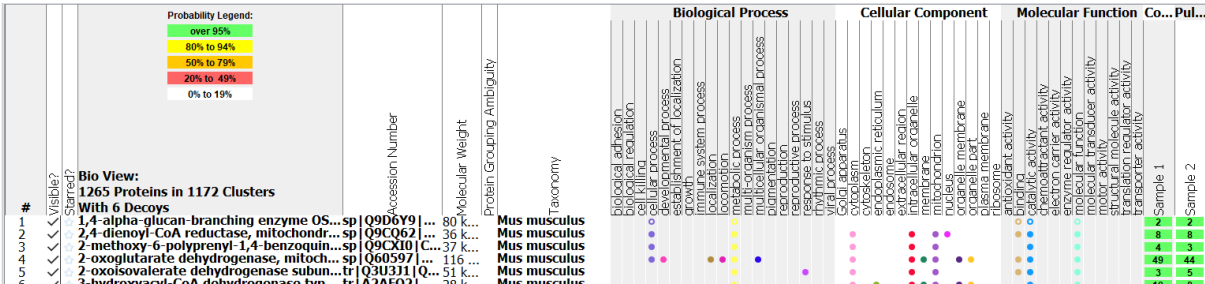
The default settings do not show all the possible GO annotations. To change the settings go to Edit → Edit GO Term Options…
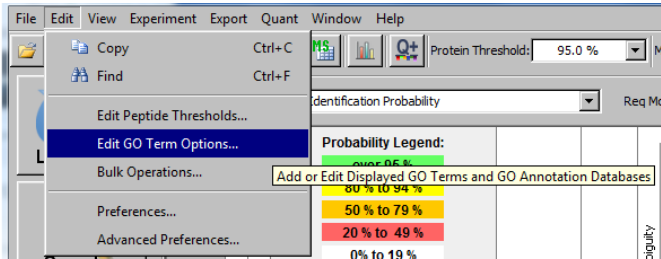
In the Configuration, you can choose which annotations should be displayed in the Samples view.
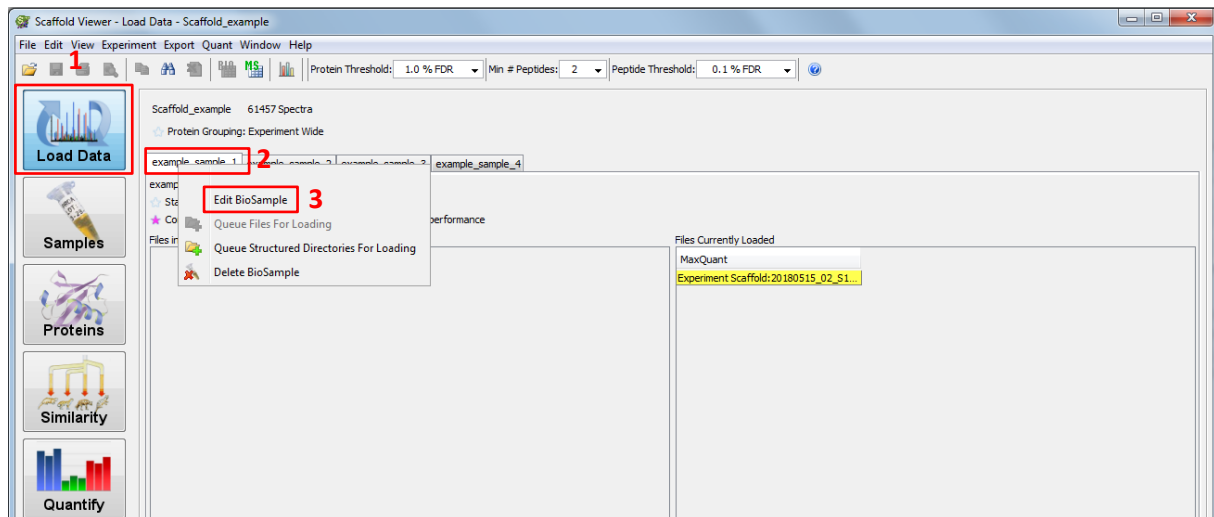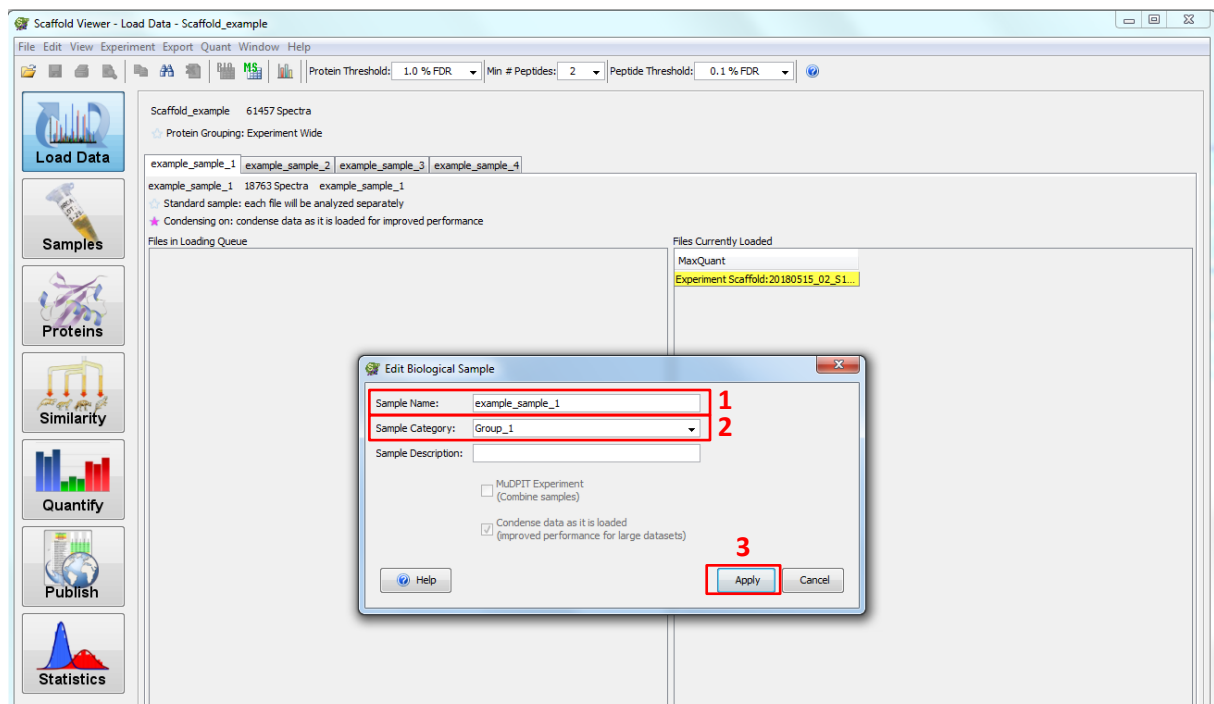
Load Data View

- Rename samples
- Group samples into categories
- Delete samples

To group the samples go to "Load Data" (#1), do a right mouse click on the sample that you want to change (#2) and select "Edit BioSample" (#3).


In the "Edit Biological Sample" window, the "Sample Name" (#1) and the "Sample Category" (#2) can be changed. To assign a sample to a certain group change the name of the "Sample Category" to your preferred group description (free text). With "Apply" (#3) the changes are saved.

After saving the view changes automatically back to the "Samples" table, change back to "Load Data" (#1) to edit the other samples. Choose the next sample to edit (#2) and with a right mouse click, the "Edit Biological Sample" window opens again. If the description of the group was added already for another sample, the description can now be chosen from the drop-down menu of the "Sample Category" (#3). With "Apply" (#4) the changes are saved.
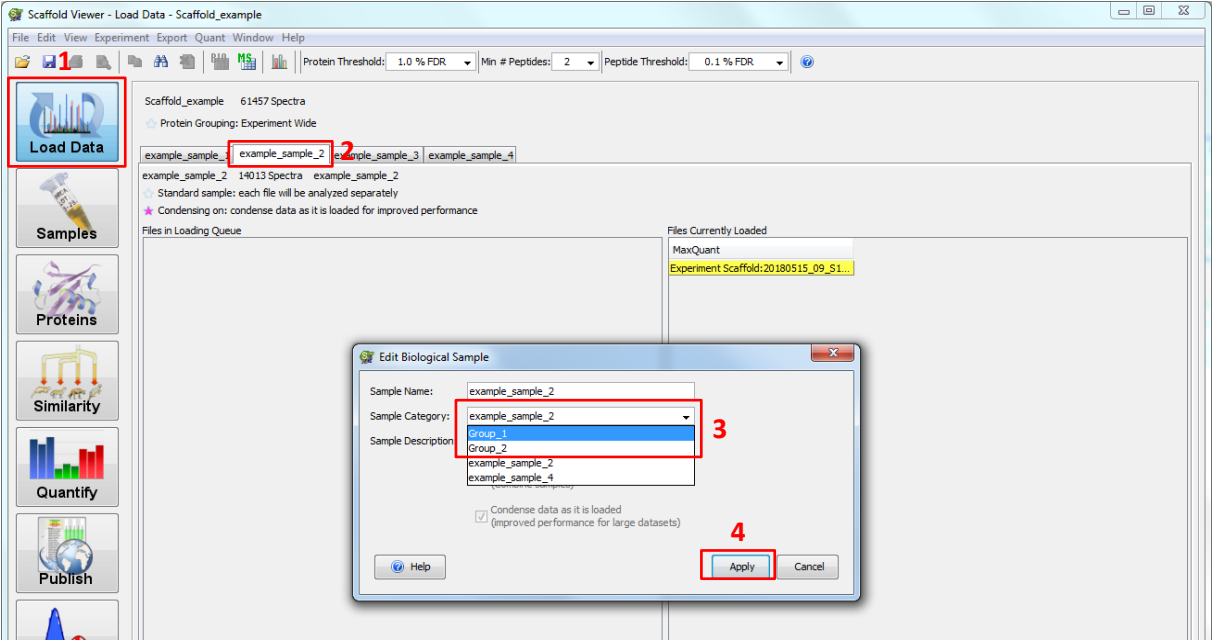
After all samples are assigned to their group, in the "Samples" (#1) tab you can see which samples belong to which group (#2).
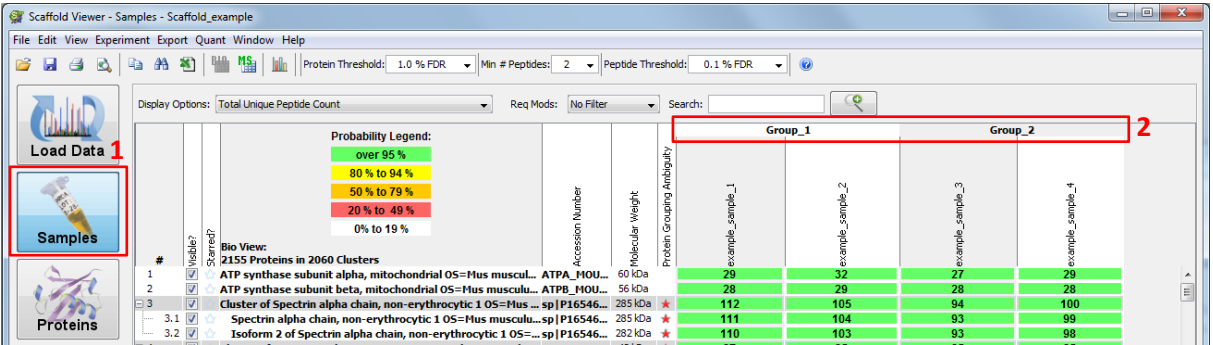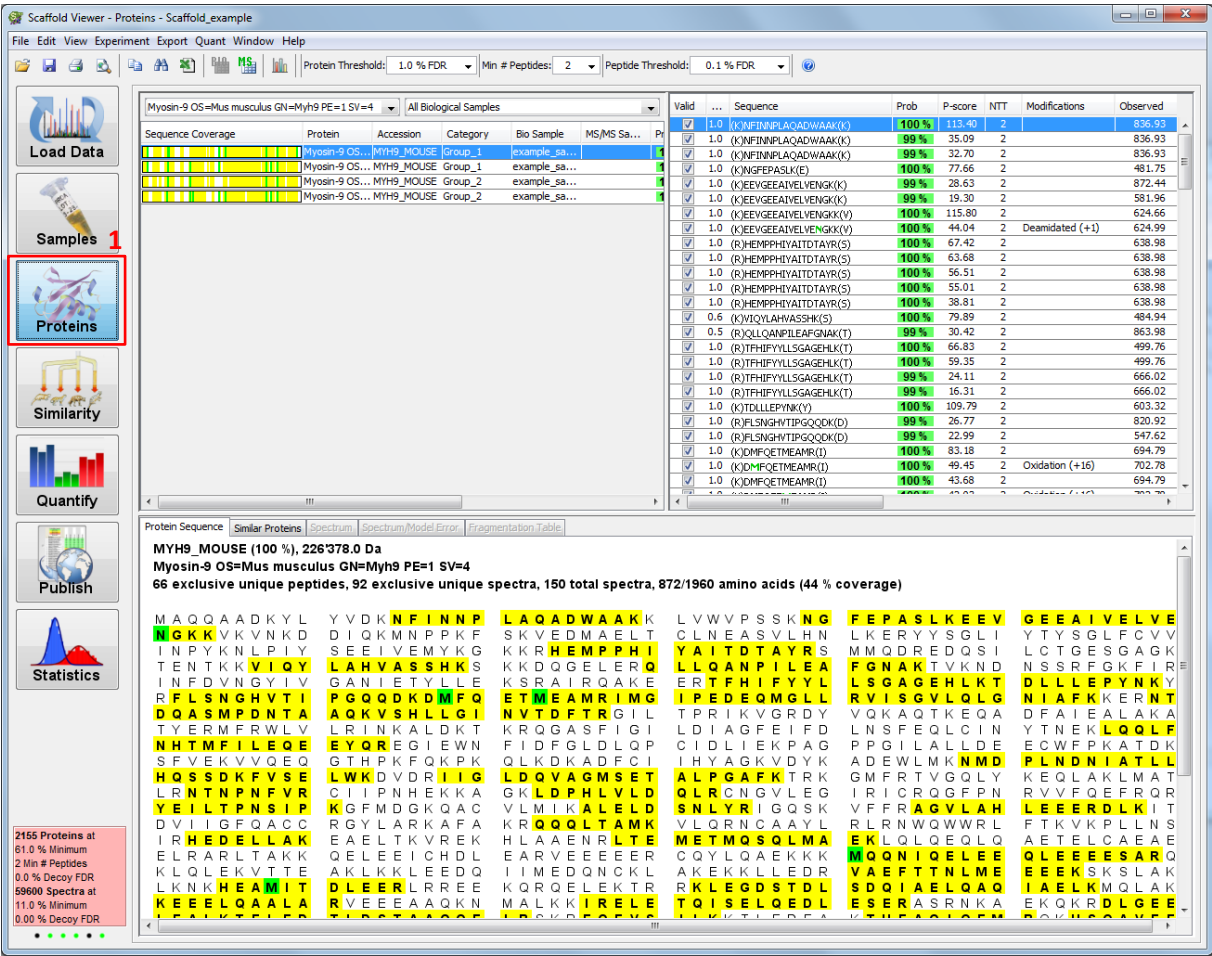
Proteins View

- Explore supporting identification data for a specific protein.
- Sequence coverage
- Mass spectra

In the "Proteins" (#1) tab, you get an overview about the sequence coverage, number of peptides, sequence of peptides and the protein sequence. The protein, which is selected in the "Samples" tab, and the proteins from the according cluster are shown.

Quantify view

- Venn Diagrams pane
- Gene Ontology Terms pane

In the "Quantify" (#1) tab, different Venn Diagrams can be shown. One can choose between displaying individual proteins or protein clusters (#2). Once the samples are grouped, one can only compare groups to each other (#3). If single samples need to be compared it either has to be done before grouping or the grouping has to be deleted. It's possible to compare proteins, total unique peptides or total unique spectra in the Venn diagrams (#4). The panel next to the Venn diagram (#5) shows all proteins (with the given filter criteria). The number of normalized total spectra of the selected protein in #5 is shown in the «Quantitative Value» (#6) view. The values are given for all samples (groups are indicated in same colours).
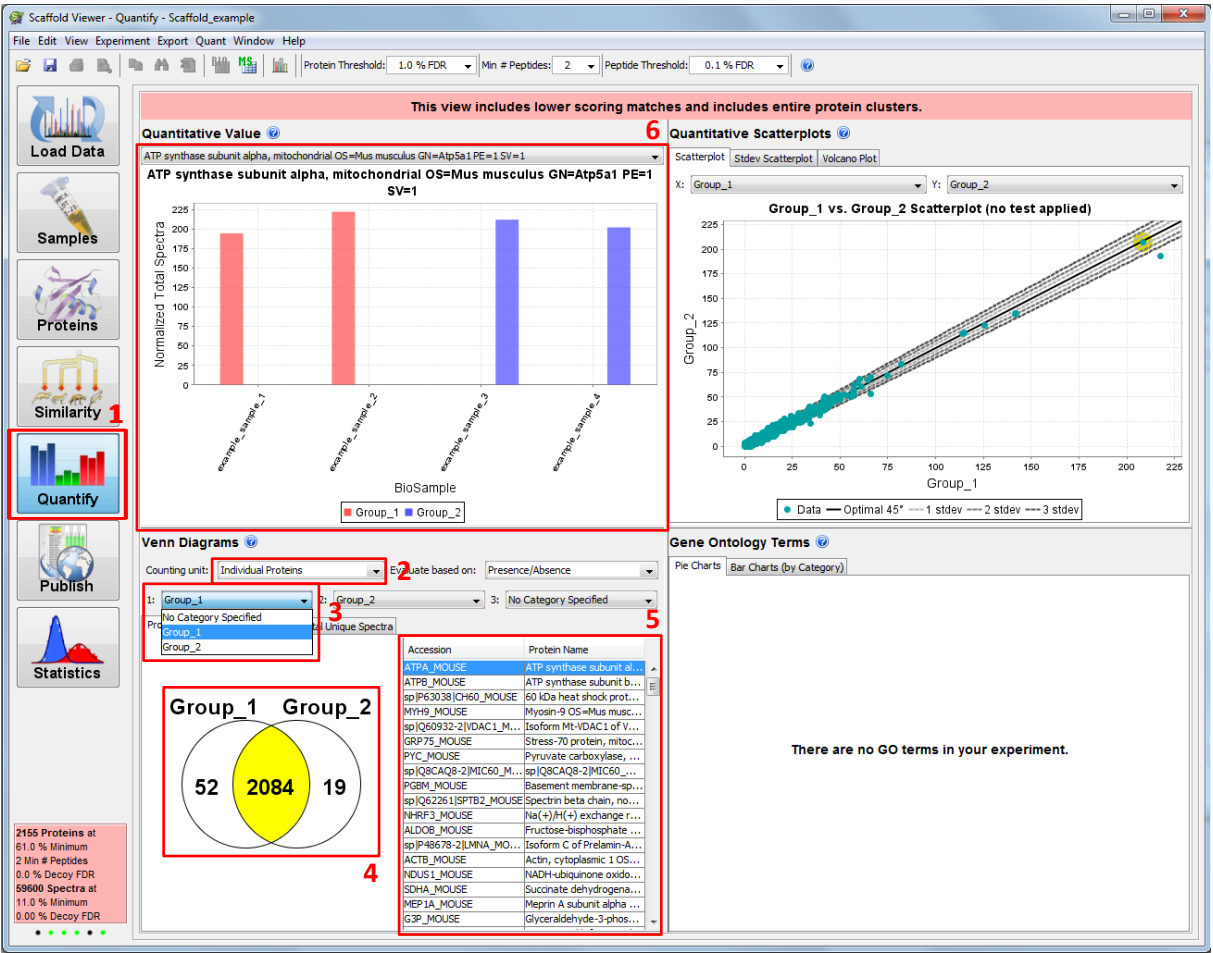
The Gene Ontology Terms pane gets populated only when GO terms have been searched and found.