Targeted Proteomics Using Skyline

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Preface

This tutorial describes basic workflows and parameters for processing Bruker data acquired with the three targeted quantification methods available on Bruker Q-TOF systems:

- Targeted high resolution extracted ion chromatograms (HR-XIC)

- Middle-band CID

- Broad-band CID

The tutorial is based on Skyline v 1.4. Template documents for data analysis of the three different quantification methods can be found on the homepage of the University of Washington, Seattle[1]:

Additional detailed tutorials for other workflows and strategies that can be followed in Skyline (for example, retention time prediction) can also be found at this site.

What Is Skyline?

Skyline is a widely used open-source proteomics software platform for building Selected Reaction Monitoring (SRM) / Multiple Reaction Monitoring (MRM) and Full-Scan (MS1 and MS/MS) quantitative methods and analyzing the resulting data.

In addition, Skyline supports a variety of full-scan chromatography-based quantitative proteomics approaches for Q-TOF and ion trap instruments; such as MS1 filtering, targeted MS/MS, and data-independent acquisition.

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[1]*https://skyline.gs.washington.edu/labkey/project/home/software/Skyline/begin.view*

1   Targeted Proteomics Strategies on a Q-TOF System

Three different targeted proteomics strategies can be applied using Bruker's Q-TOF instruments:

- Targeted HR-XIC

- Middle-band CID

- Broad-band CID

These methods use different data acquisition methods and quantification is based either on MS or MS/MS signals.

1.1   Targeted HR-XIC

The Targeted HR-XIC method uses the high resolution and excellent mass accuracy of Bruker's Q-TOF systems in MS mode for quantification. Data acquisition is performed in MS only mode, without any MS/MS acquisition.

In this way, the instrument is set up so that the highest sensitivity in MS mode can be obtained without compromising the number of data points that characterize the chromatographic peak. Subsequent targeted quantification is based on precursors.

1.2   Middle-Band CID

The Middle-Band CID method is a data-independent acquisition method, which is based on the sequential isolation and fragmentation of relatively large (for example m/z = 25 Da) precursor windows (Venable et. al, Nature Methods, 2004). Quantitative information is directly obtained from tandem mass spectra, enabling quantification based on multiple fragment ions.

Middle-Band CID acquisition parameters are set in the instrument control software using the **MRM** option in the MS/MS tab (see Figure 2-1).

Figure 2-1   Middle-Band CID acquisition parameters in the MRM option of the MS/MS tab

Parameters should be set according to the experimental setup. Typically, the precursor mass range from *m/z* = 400 to 1200 is acquired using sequential isolation windows of 26, allowing a slight overlap between the windows.

1.3   Broad-Band CID

Broad-Band CID is also a data-independent method. In this approach, no precursor selection is done at all. Instead, the MS switches rapidly between low and high collision energy in the collision cell, delivering all MS and MS/MS information independently of a detected precursor.

Broad-Band CID acquisition parameters are set in the instrument control software using the **bbCID** option in the MS/MS tab (see Figure 2-2).

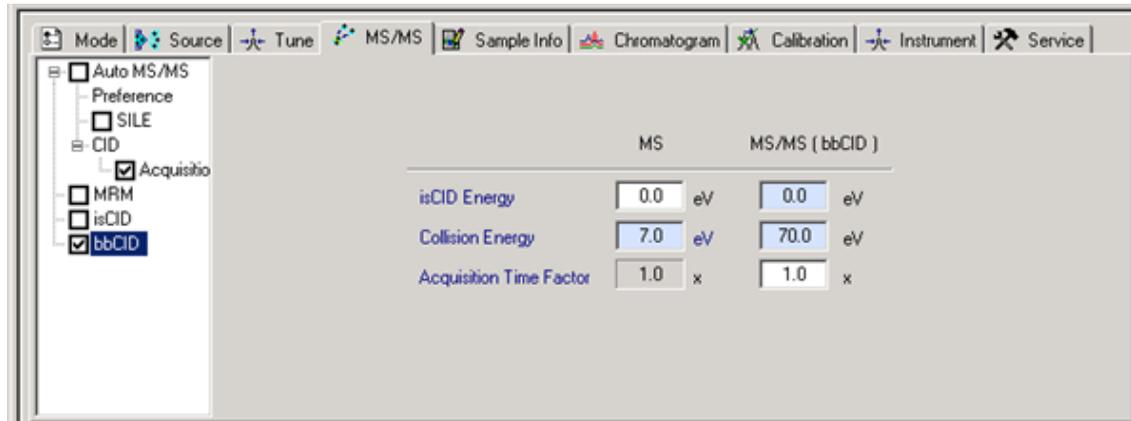**Figure 2-2**   **Broad-Band CID acquisition parameters in the bbCID option of the MS/MS tab**

Parameters should be set according to the experimental setup. An explanation of the different parameters can be found in the instrument's online help.

2   Getting Started

Starting Skyline will open an empty document.

To begin working with targeted quantification experiments in Skyline, a spectral library containing the peptide targets must be created.

Skyline can use any of the major publicly available spectral libraries from the following sources:

- Global Proteome Machine (GPM)

- National Institute of Standards (NIST)

- Institute for Systems Biology (ISB)

- the MacCoss lab

or build a spectral library based on experimental peptide search results.

Building spectral libraries based on experimentally obtained peptide search results requires data preparation steps.

2.1   Data Preparation

To use experimental peptide search results as targets for quantification, the data must first be analyzed using the protein analysis function in DataAnalysis (DA) software to obtain peak lists for database searches.

Retention-time information (in seconds) and file name information are required for Skyline to build a spectral library. This information will be automatically included in the mgf file when using DA 4.1.

Earlier versions of DA require the script *Protein analysis_RT in s* to be used for processing. This script can be found on the Skyline homepage[1].

Subsequent database searches can be performed using a variety of search engines (for example, Mascot or X! Tandem: a complete list can be found in the Skyline tutorial *Skyline Targeted Method Editing*).

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[1]*https://skyline.gs.washington.edu/labkey/project/home/software/Skyline/begin.view*

The results of the database search must then be imported into Skyline to build a spectral library. For Mascot searches, the .dat file will be downloaded from the search engine and imported into Skyline.

For all search engines, it is important that the **RT in s** and data file information is passed to Skyline.

If identification information annotation is required during the quantification process, the names of the exported database search result file and the data file that will be used for quantification must be identical (see Figure 2-3).
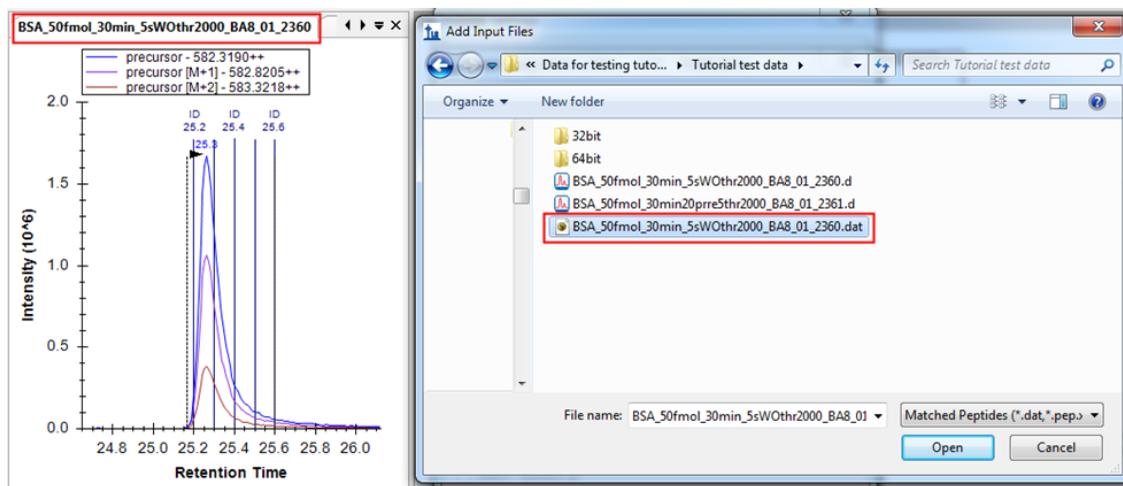

Figure 2-3   Identically named database search result file and quantification data file

ProteinScape 3.0 (patch1) and earlier versions do not pass the required information and therefore cannot be used for creation of spectral libraries based on acquired data.

If mass calibration of data for quantification must be performed, data must be opened in DA and corresponding calibration (for example, lockmass) must be performed. After data is saved, it is ready to be loaded into Skyline.

After performing the data preparation step, all data required for quantification using Skyline are available and include:

- Database search files (.dat in the case of Mascot searches)

- Data for quantification (.d files), recalibrated in DA if necessary

2.2   Building a Spectral Library in Skyline

Configuring Peptide Settings

Before building a spectral library, peptide settings must be adjusted for the document.

1. Select **Settings > Peptide Settings**.
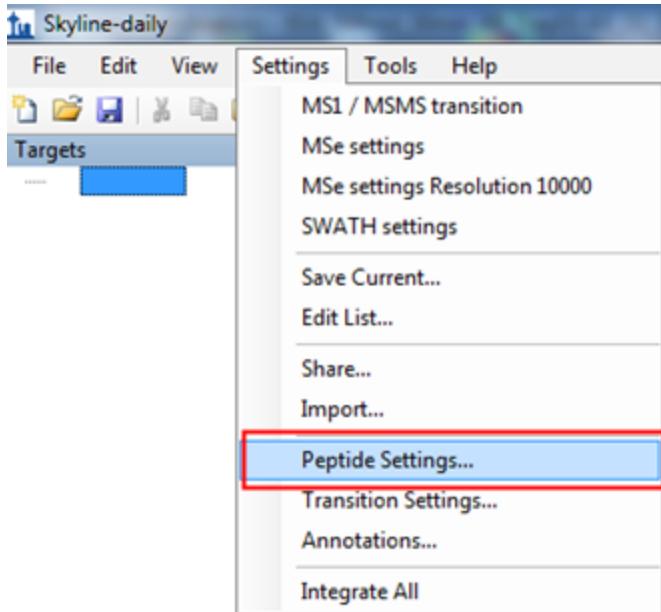
2. Click the **Digestion** tab and select the settings appropriate to the experiment performed. Typical settings are:

- **Enzyme**: Trypsin [KR | P]

- **Max. missed cleavages**: 1

- **Background proteome**: None

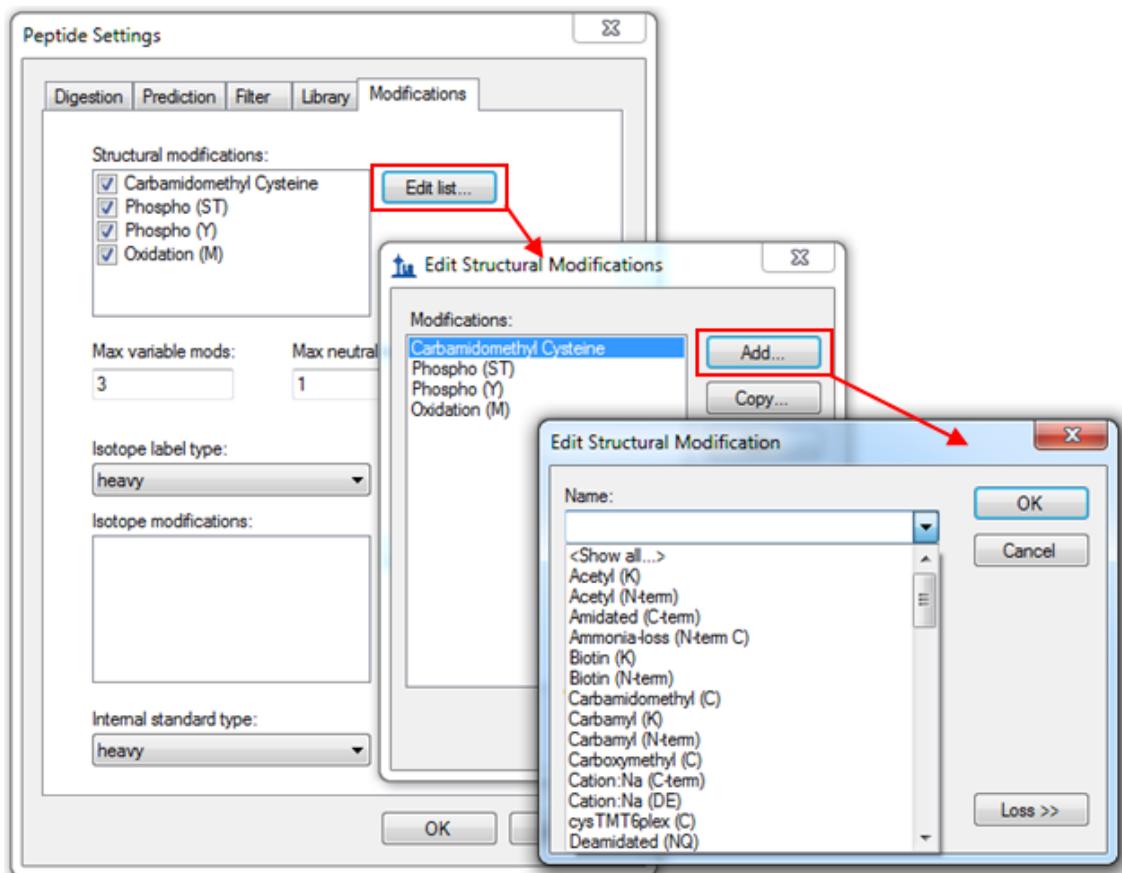The **Background proteome** parameter defines the background matrix of the experiment using the corresponding FASTA sequence files. The background proteome can be made up of a FASTA file for one or more organisms or proteins. For more information about importing a background proteome, please refer to the Skyline "Targeted method editing" tutorial on the Skyline web page[1].

3. Click the **Modifications** tab and select the modifications appropriate for your sample.

    a. Add modifications by clicking **Edit list > Add** and selecting modifications from the list in the **Edit Structural Modification** dialog.

    b. Click **OK** to add modifications to the list.

All modifications are set as fixed modifications, unless the "variable" check box in the **Edit Structural Modification** tab is selected. A wide variety of different modifications are already available in Skyline. Missing modifications can be easily added as described in the Skyline *Spectral Library Explorer* tutorial[1].

Building a spectral library

Spectral libraries can either be imported using available libraries or built based on database search results. For targeted approaches on the Q-TOF system, spectral libraries will typically be created from the output of database search results from standard AutoMS/MS experiments. To use public libraries, please refer to the Skyline tutorial *Targeted method editing* on the Skyline web page[2].

Building a spectral library based on previously acquired data requires the following:

- AutoMS/MS runs or the use of scheduled precursor lists for MS/MS runs of the peptides of interest

- Subsequent data processing using the "protein analysis" function in DataAnalysis

Skyline supports creation of spectral libraries from many different peptide search engines (for example, Mascot, X! Tandem, OMSSA, Scaffold). When using the Mascot search engine, the .dat file can be easily exported from the Mascot result page for subsequent use in Skyline (see Figure 2-4).

[1]*https://brendanx-uw1.gs.washington.edu/labkey/wiki/home/software/Skyline/page.view? name=tutorial_library_explorer*
[2]*https://brendanx-uw1.gs.washington.edu/labkey/wiki/home/software/Skyline/page.view? name=tutorial_method_edit*

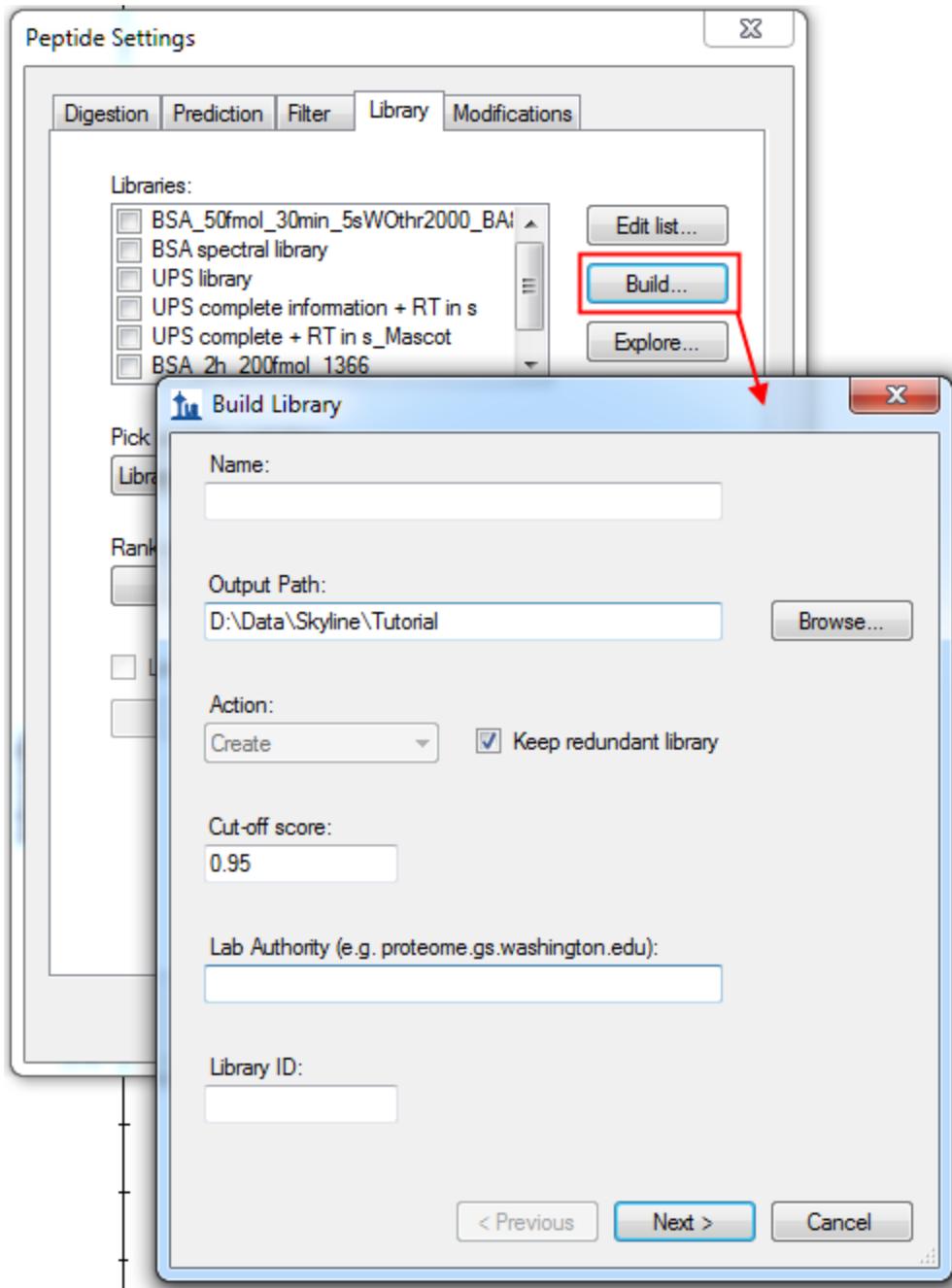**Figure 2-4   Exporting the .dat file from Mascot**

Note   If identification information annotation is required during the quantification process, the names of the exported database search result file and the data file that will be used for quantification must be identical (see Figure 2-3).

►►To build a spectral library

1. Select **Settings > Peptide Settings > Library > Build**.

2. Type an appropriate library name into the **Name** field.

    This name will be entered automatically into the **Library ID** field.

3. Specify the preferred output path by clicking the browse button next to the **Output Path** field.

4. Make sure that the **Keep redundant library** check box is selected.

    Unlike SRM, with targeted proteomics on a Q‑TOF system, it is useful to keep all redundant spectra for each identified peptide. Skyline makes it possible for the user to inspect these spectra during review of the results.

5. Type a unique Domain Name System (DNS) name into the **Lab Authority** field to obtain a unique identifier for the library.

    This is usually a DNS name controlled by your lab. A library cannot be created if this field is not completed.

6. Enter a cut-off score appropriate for the data set in the Cut-off score field.

    The default ProteinProphet cut-off score value is 0.95.

7. Click **Next** and **Add Files**.

8. Go to the location where the database search result file (for example, Mascot .dat file) was saved, select the file, and click **OK**.

    The file is added to the file list in the **Input Files** dialog (see Figure 2-5).

9. Repeat steps (8) and (9) until all files that should be added to the library are listed in the **Input Files** dialog.

    The library can include identification information from multiple MS files, which will be combined in the library.
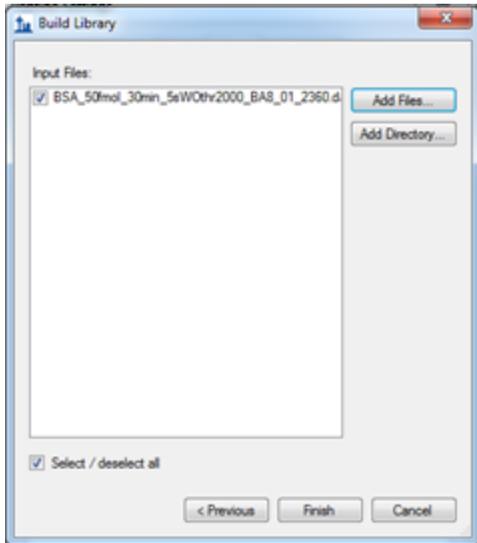
Figure 2-5   Database search result file added to the file list in the Input Files dialog

10. To build the library, select the check box(es) next to the search result file name(s) and click **Finish**.

   The library will be built in Skyline and a notification will appear briefly in the lower left corner when the library has been successfully built (see Figure 2-6).

Figure 2-6   Notification of successfully built library

11. Complete the library configuration by selecting **Library** in the **Pick peptides matching** drop-down list on the **Peptide Settings > Library** tab.

   Two new files — `xxx.blib` (containing the best-matching spectra) and `xxx.redundant.blib` (containing all matching spectra) — should be located in the folder where Skyline saved the new spectral libraries.

12. Check that the resulting library contains the necessary retention time information by clicking **View > Spectral Libraries**.

The Spectral Library Explorer window will open (see Figure 2-7). The **RT** value at the lower right of the spectrum displays the retention time information and the **File** value at the lower left of the spectrum indicates that the correct files are associated.
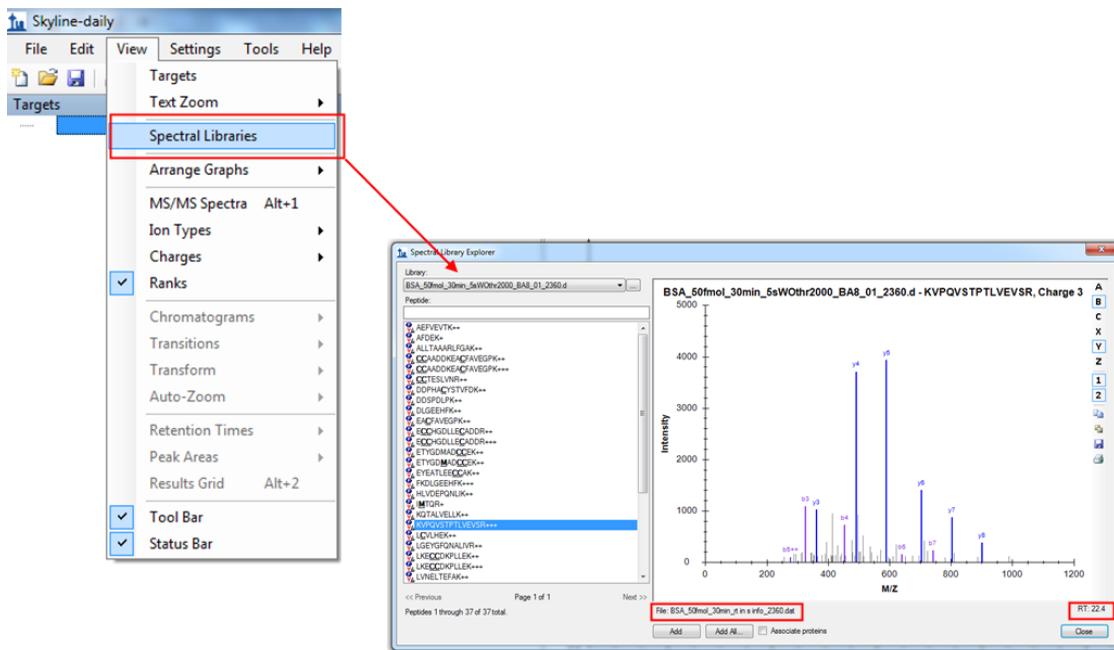

Figure 2-7   **Spectral Library Explorer window**

Note   If identification information annotation is required during the quantification process, the names of the exported database search result file and the data file that will be used for quantification must be identical (see Figure 2-3).

3 Targeted Quantification Using Different Data Acquisition Schemes

3.1 Configuring Transition Settings

Transition settings are used to filter intensity values for the desired ions (*m/z* peaks). Some settings are applicable for all approaches (see section 3.1.1). Other settings are dependent on the approach used for quantification:

- MS1 filtering for DDA acquisition (see section 3.1.2)

- Targeted HR-XIC (see section 3.1.3)

- Middle-band CID (see section 3.1.4)

- Broad-band CID (see section 3.1.5)

All settings for the different approaches can be saved by selecting **Settings > Save current** and used for later analyses.
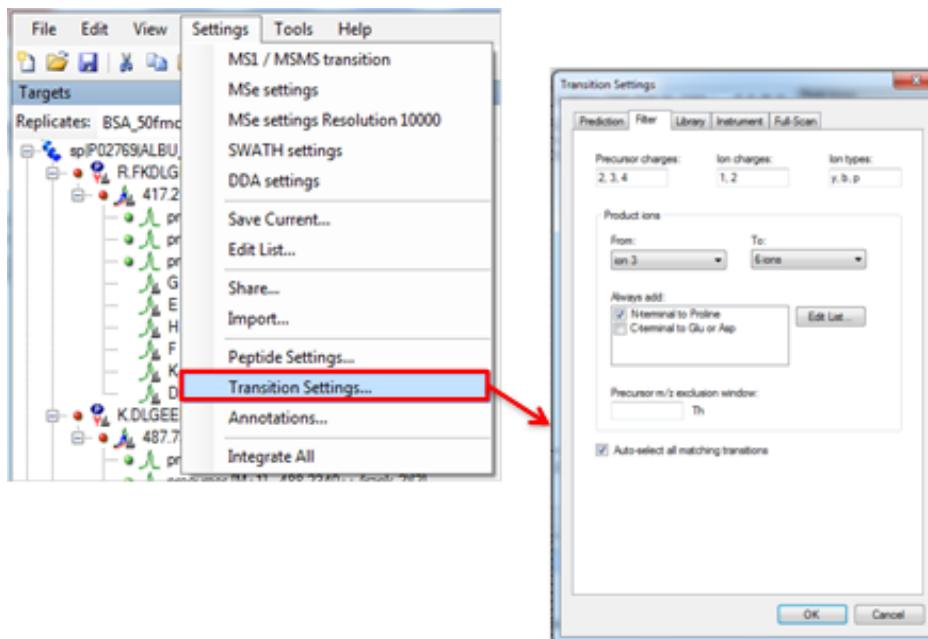

Figure 2-8   Opening the Transition Settings dialog

3.1.1   Global Transition Settings

The following Transition Settings are used for all approaches.

Prediction tab

- **Precursor mass > Monoisotopic**

- **Product ion mass > Monoisotopic**

Collision energy and **Declustering potential** are related to method setups for SRM/MRM-type experiments and do not need to be adjusted for Q-TOF-related approaches.

Filter tab

Settings on the **Filter** tab are dependent on the acquisition mode and are dealt with in the corresponding section.

Library tab

Ion match tolerance must be set according to your instrument and the results obtained from the spectral library data (see typical values below).

The **If a library spectrum is available, pick its most intense ions** check box must be cleared.

Instrument tab

Settings on the **Instrument** tab are related to the specification of your instrument and the method used. Typical values are:

- **Min m/z > 50 Th**

- **Max m/z > 1500 Th**

- **Match tolerance m/z > 0.01 Th**

- **Firmware transition limit >** leave blank

- **Firmware inclusion limit >** leave blank

- **Min time > 10 min**

- **Max time > 120 min**

- The **Dynamic min product m/z** check box should be cleared.

Retention times (**Min time** and **Max time**) can be limited to the times when peptides are eluting instead of using the complete gradient to reduce the amount of data.

The **Match tolerance** should be selected according to the Q-TOF instrument used. The minimum value is 0.001 Th.

Full-Scan tab

Settings on the **Full-Scan** tab are dependent on the acquisition mode and are dealt with in the corresponding section.

3.1.2   MS1 filtering for DDA acquisition

Filter tab

Figure 2-9 shows suitable **Filter** tab settings if samples for quantification were acquired in a typical DDA MS/MS run (either in an AutoMS/MS mode or by using inclusion lists/scheduled precursor lists).

Precursor charges and fragment **Ion charges** appropriate to the experiment performed must be selected. All required charges must be listed and separated using commas.

For quantification based on MS1 ions, **p** (which stands for precursor) must be entered into the **Ion types** box.

The **Auto-select all matching transitions** check box must be checked, so that only transitions (peptides and fragments) that fit to the specific filter and library settings will be chosen for quantification. If this check box is cleared, all transitions will be shown and manual document editing will be necessary to check transition selection.
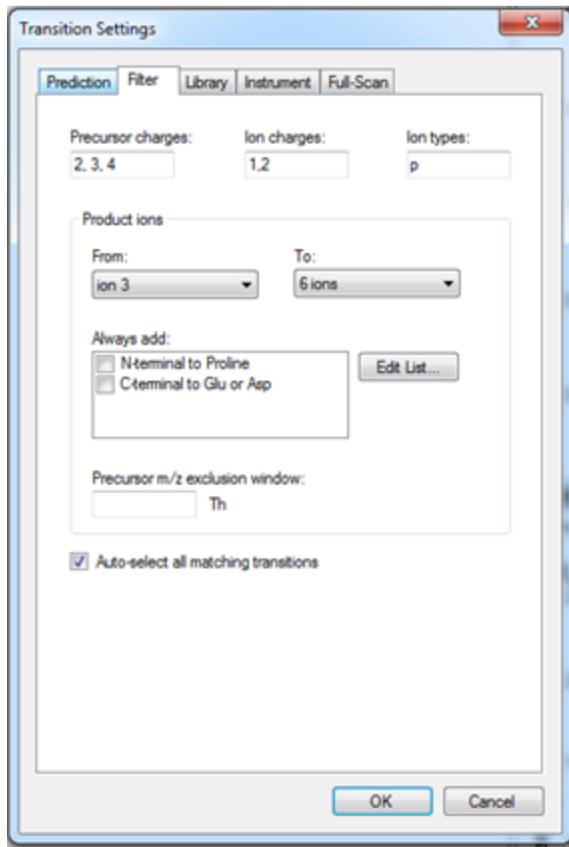
Figure 2-9   Suitable Filter tab transition settings for MS1 filtering for DDA acquisition

Full-Scan tab

Figure 2‑10 shows suitable **Full‑Scan** tab settings if samples for quantification were acquired in a typical DDA MS/MS run (either in an AutoMS/MS mode or by using inclusion lists/scheduled precursor lists).
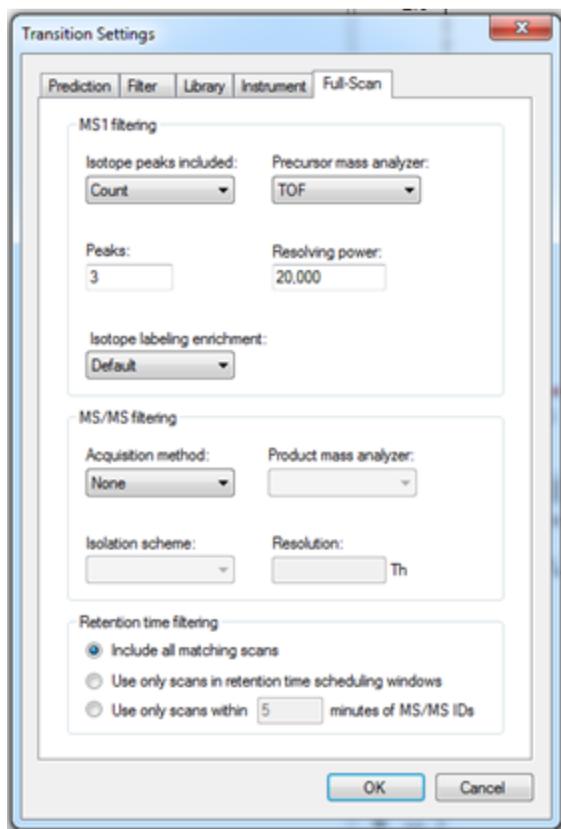
Figure 2-10   Suitable Full-Scan tab transition settings for MS1 filtering for DDA acquisition

If Q-TOF data is used, **MS1 filtering > Isotope peaks included** must be set to **Count** and **Precursor mass analyzer** must be set to **TOF**.

For high-resolution data, **3** can be entered in the **Peaks** field to enable Skyline to filter the first 3 isotope peaks (M, M+1, and M+2). For lower resolution data, type 1 into the **Peaks** field.

The **Resolving power** field defines the width of the MS1 filtering window around each precursor *m/z* to be filtered. Skyline uses this value to predict full width at half maximum (FWHM) for a peak in the *m/z* dimension and uses 2 times that FWHM value as the filtering window (see Figure 2-11).

This value should be set according to your instrument settings. A typical value for the maXis impact is 20,000.
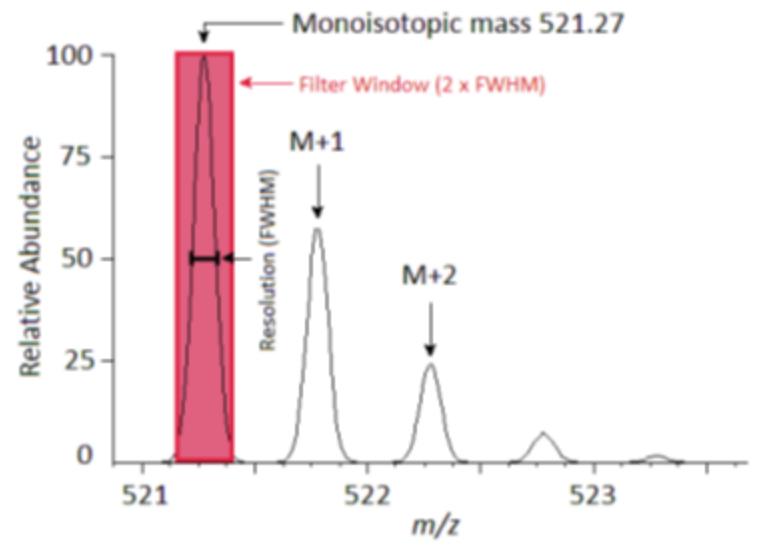
Figure 2-11   Calculation of the filter window based on FWHM of a peak

MS/MS filtering > Acquisition method must be set to **None**, because quantification is based only on MS signals.

3.1.3   Targeted HR-XIC approach

The Targeted HR-XIC approach is characterized by the fact that only MS runs are acquired and thererfore no MS/MS information exists.

Transition settings should be the same as those used in the MS1 filtering approach for DDA acquisition (see section 3.1.2).

Note   Settings for **Ion charges** in the **Filter** tab will have no influence, because no MS/MS data is available.

3.1.4   Middle-band CID approach

Using the Middle-band CID approach, quantification is based on MS/MS signals. Transition Settings on the **Filter** and **Full Scan** tabs must be adjusted accordingly.

Filter tab

Figure 2-12 shows suitable Filter tab transition settings for the Middle-band CID approach where MS/MS and MS scans were acquired for verification of the presence of the precursor.

If only MS/MS scans were acquired, **p** (precursors) can be omitted from the **Ion types** field and the **Precursor charges** and **Ion Charges** fields can be left blank.
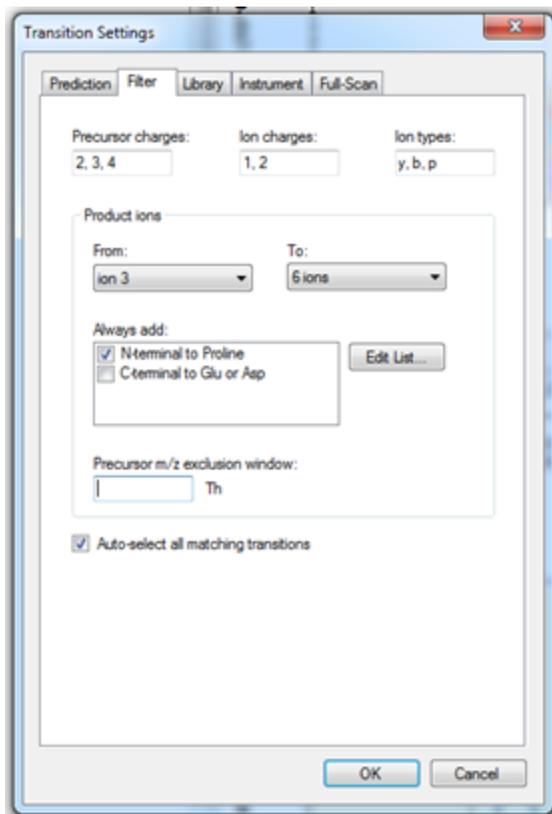

Figure 2-12   Suitable Filter tab transition settings for a Middle-band CID approach where MS/MS and MS scans were acquired for verification of the presence of the precursor

Full-Scan tab

If no MS spectra were acquired, **MS1 filtering > Isotope peaks included** must be set to **None**.

If MS spectra were acquired, **MS1 filtering > Isotope peaks included** must be set to **Count** and **Precursor mass analyzer** must be set to **TOF** if a Q-TOF system was used. **Peaks** must be set to **3** and the **Resolving power** set according to your instrument (see section 3.1.2).

For this approach, a new **Isolation scheme** must be created for MS/MS filtering (see Figure 2-13).

1. In the **MS/MS filtering** group box select **Acquisition method > DIA**.

2. Create a new **Isolation scheme**.

   a. Select **Isolation Scheme > Add**.

   b. Type a name into the **Name** field of the **Edit Isolation Scheme** dialog.

   c. Click **Calculate**; type an appropriate value into the **Start m/z**, **End m/z**, **Window width**, and **Overlap**[1] boxes; and click **OK**.

      Skyline will automatically write the isolation scheme to the **Prespecified isolation windows** table.

      Alternatively the **Start** and **End** *m/z* values can be entered manually into the **Prespecified isolation windows** table.

      After the isolation scheme is saved it will be available for later analyses.

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[1]*If isolation windows were chosen in a slightly overlapping fashion.*
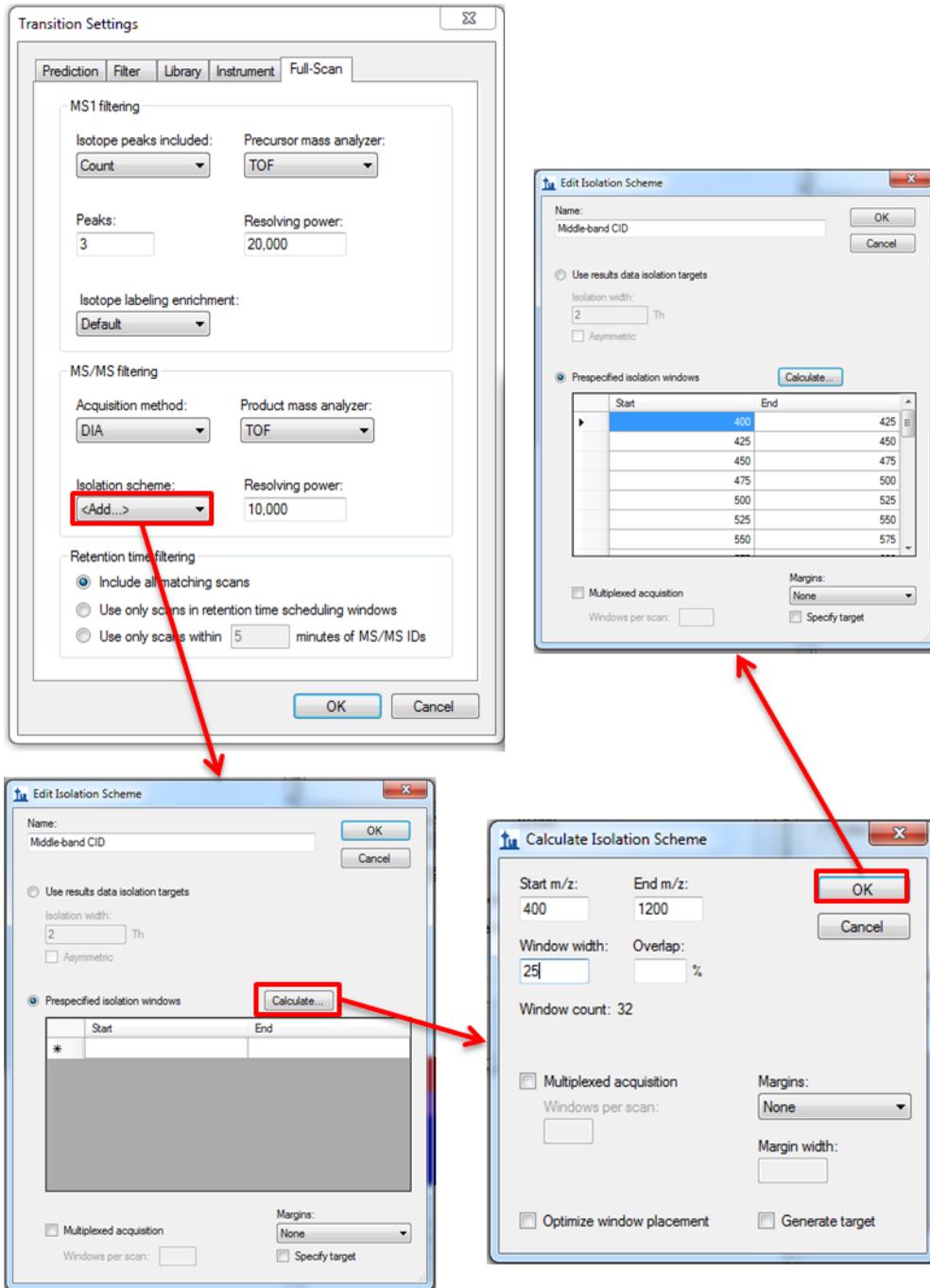
Figure 2-13   Creating an Isolation Scheme

3.1.5   Broad-band CID

For the Broad-band CID approach, quantification is based on MS signals and identification verification is based on typical fragment ions ("qualifier ions").

Filter tab

Figure 2-14 shows suitable Filter tab transition settings for the Broad-band CID approach.

y, b, p must be entered into the **Ion types** box and the expected precursor and ion charges must be typed into the respective box.
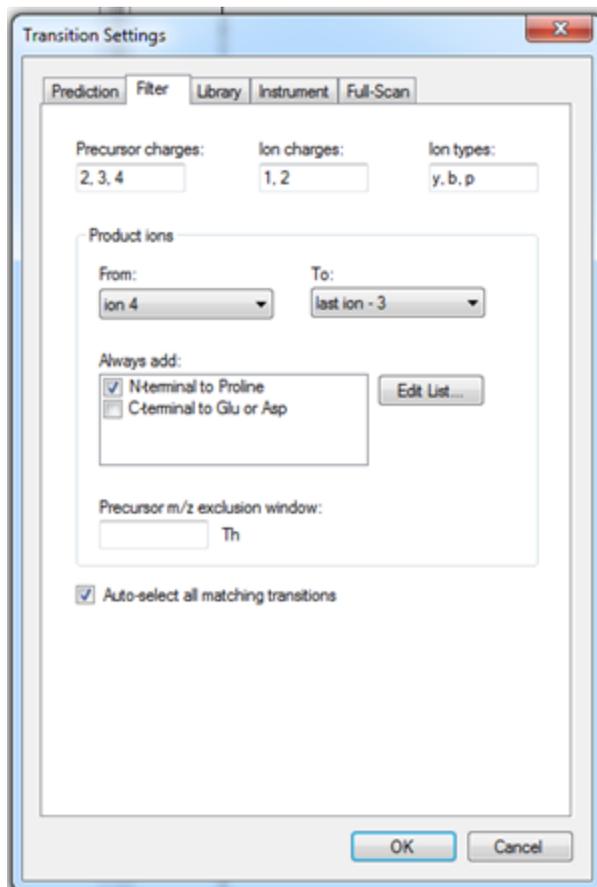

Figure 2-14   Suitable Filter tab transition settings for a Broad-band CID approach

Full-Scan tab

MS1 filtering > Isotope peaks included must be set to **Count** and **Precursor mass analyzer** must be set to **TOF** if a Q-TOF system was used.

For high-resolution data, **3** can be entered in the **Peaks** field to enable Skyline to filter the first 3 isotope peaks (M, M+1, and M+2). For lower resolution data, type 1 into the **Peaks** field.

The **Resolving power** field defines the width of the MS1 filtering window around each precursor *m/z* to be filtered. Skyline uses this value to predict full width at half maximum (FWHM) for a peak in the *m/z* dimension and uses 2 times that FWHM value as the filtering window (see Figure 2-11).

This value should be set according to your instrument settings. A typical value for the maXis impact is 20,000.

In the **MS/MS filtering** group box, select :

- **Acquisition method > DIA**

- **Product mass analyzer > TOF**

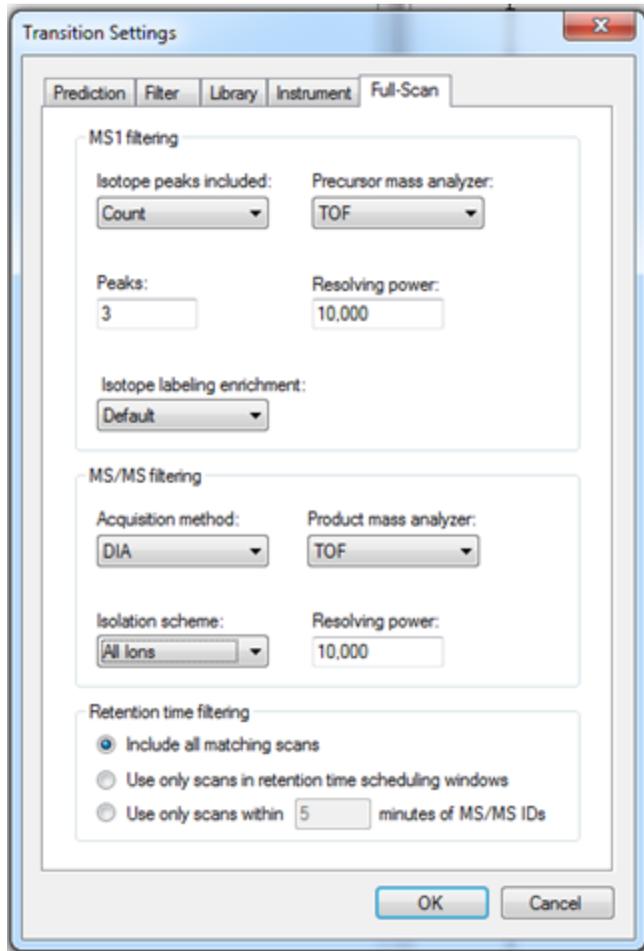- **Isolation scheme > All ions**

Figure 2-15   Suitable Full-Scan tab transition settings for a Broad-band CID approach

3.2   Populating the Skyline Peptide Tree

After configuring the transition settings, the peptides for which Skyline should extract chromatograms and produce quantitative data must be added.

The fastest way to add all of the proteins and peptides for which quantitative data should be extracted, is to import a FASTA file (**File > Import > FASTA**). Go to the folder where the FASTA file is located and start the import by double-clicking the FASTA file.
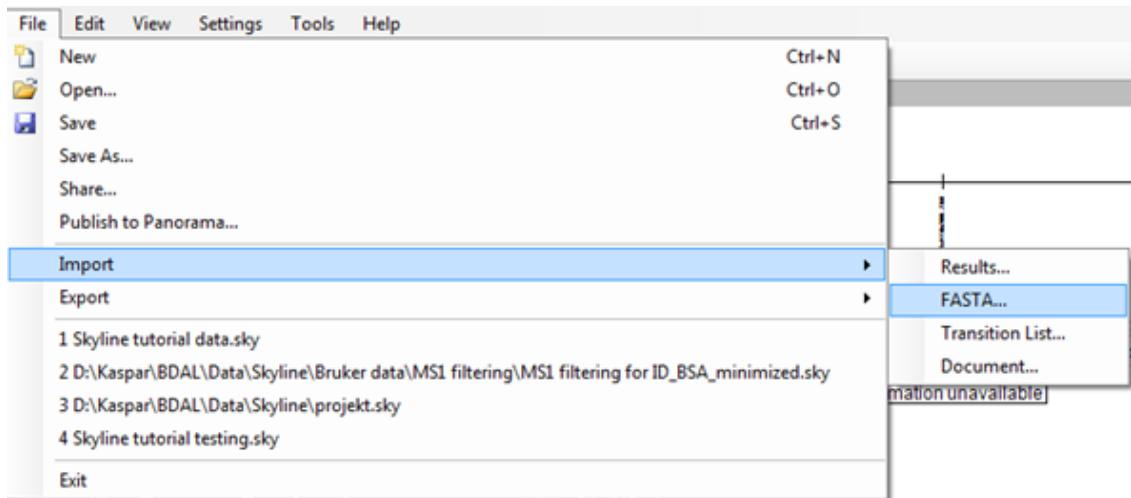

Figure 2-16   Importing a FASTA file

Alternatively, information from a FASTA file can be imported by copying complete FASTA sequences using a text-editing program and then pasting them directly into Skyline. Further options for adding peptides for quantification, for example, using **Add All** in the **Spectral Library Explorer** are covered in the Skyline tutorial.

Figure 2-17 shows how the fully configured document should appear.

The information shown will depend on the settings entered in the preceding sections. The document is now fully configured for the different targeted proteomics approaches.
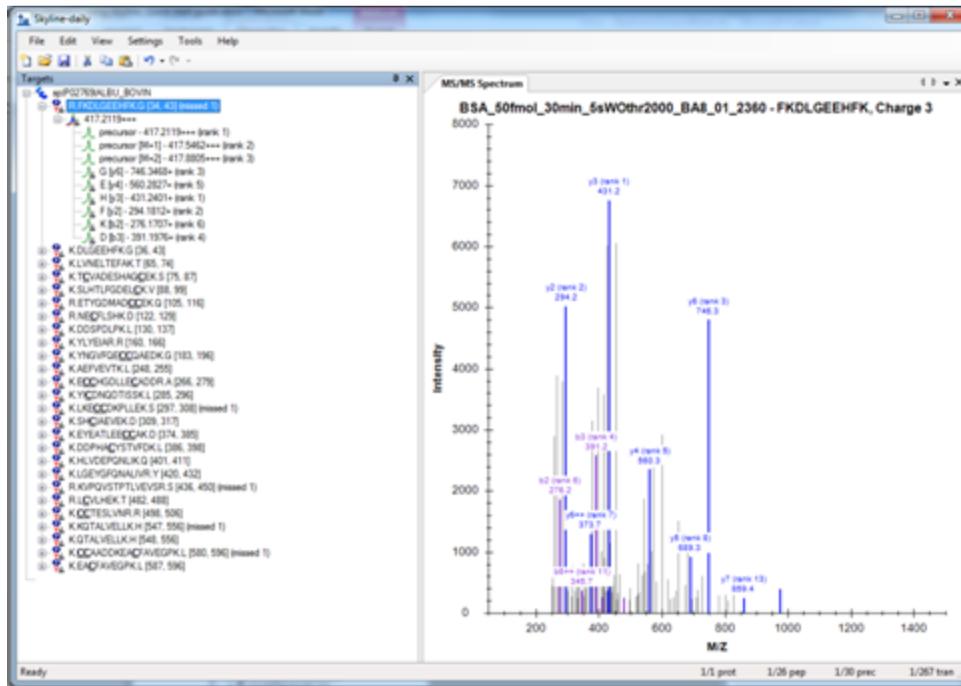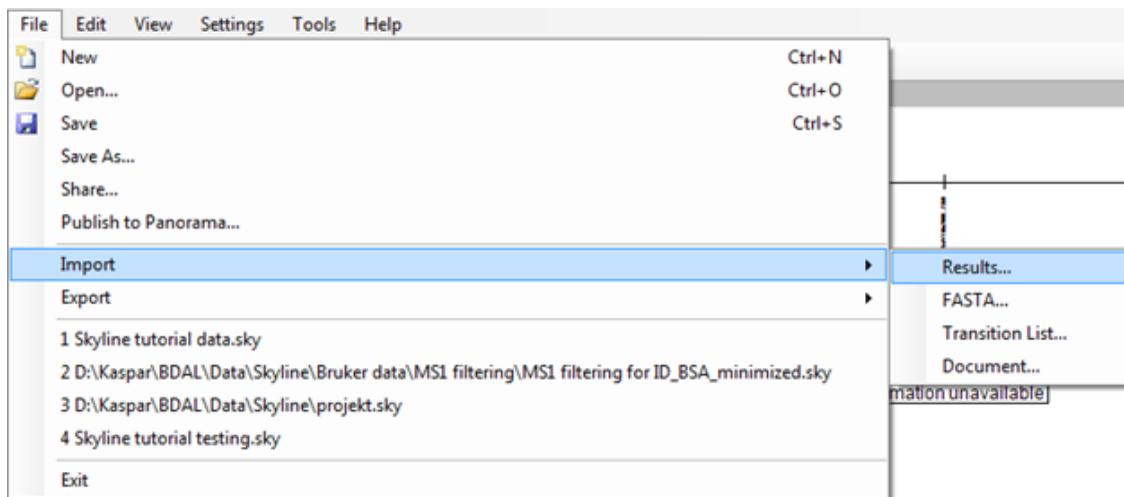
Figure 2-17   Fully configured document

3.3 Importing Raw Data into Skyline and Subsequent Filtering

After setting up the filtering options for the corresponding targeted quantitative methods, raw data files can be imported by selecting **File > Import > Results**.

The **Import Results** dialog will open. Select **Add single-injection replicates in file** to retain the default setting. Click **OK** and go to the folder where the raw data files are located.

Select raw data file(s) and click **Open**. Skyline will start to import the file(s).

While the data is being imported, Skyline can be configured to optimize visualization of the filtered data.

- Select **Settings > Integrate All**.

- Select **View > Peak Areas > Replicate Comparison** to open the Peak Areas pane.

- Right-click within the Peak Areas pane and select **Normalize To > None**. **Normalize To > Maximum** will set the most intense sample peak to 1 and normalizes all other peaks accordingly. **Normalize To > Total** will set the peaks of

all samples to 100% and therefore allows comparison of experimentally obtained isotopic distributions to theoretical values.

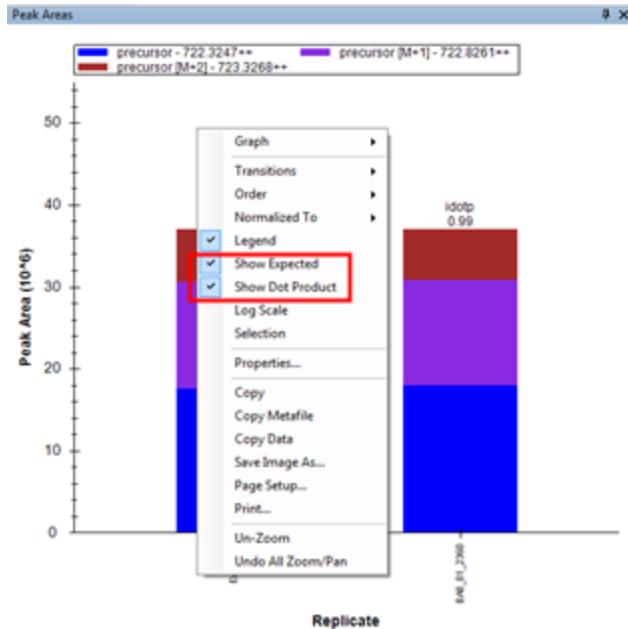- Right-click within the Peak Areas pane and select **Show Expected** and **Show Dot Product**.

Figure 2-18   Optimize visualization of the filtered data

Selecting **Show Expected** displays the expected precursor area distribution, which can be directly compared to the observed precursor ion peak area distribution for the selected precursor isotope ions.

Selecting **Show Dot Product** displays the isotope dot product ("idotp") above the peak area bar in the peak area view. This value is a comparison observed and theoretical distributions and should be 1.0 for a perfect match.

Figure 2-19 shows how the different panes can be arranged by dragging and dropping to display data after import is complete.
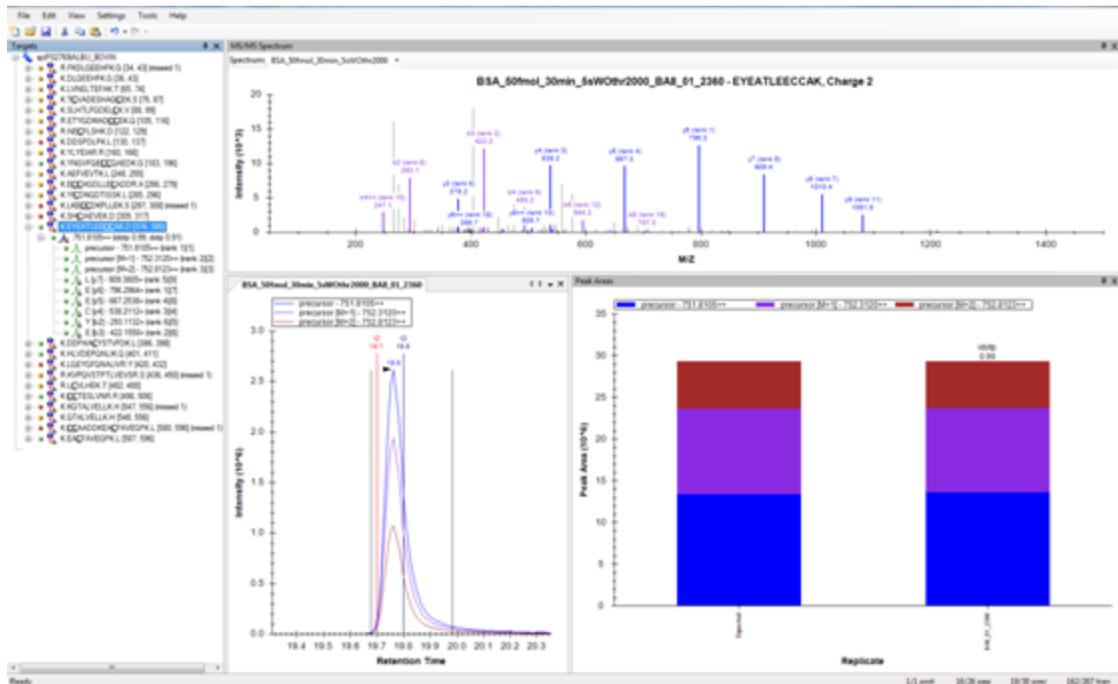
Figure 2-19   Optimized Skyline user interface after data import

In the Targets view on the left of the interface, green, yellow, and red dots to the left of the peptide sequences indicate peak quality. These color-coded dots enable fast evaluation of the quality of peptide targets.

A green dot indicates that all filtered transitions contribute a co-eluting peak to the peak Skyline picked as the best target fragment or peptide (based on intensity and idotp value).

A yellow dot indicates that at least half of the filtered transitions contribute to a co-eluting peak.

A red dot indicates that less than half of the transitions contribute to a co-eluting peak.

The chromatogram view shows the extracted ion chromatograms (in this case, for all the precursor isotope ions). If product ion information is also available, extracted ion chromatograms can also be shown for products.

Select the desired product in the Targets tree at the left of the user interface, right-click within the chromatogram view, and select **Transitions > Single**.

If quantification is performed for precursor and fragment data (set using Transition settings), the option **Transitions > Precursors and Transitions > Products** becomes available, allowing separate display of precursor and fragment ions.
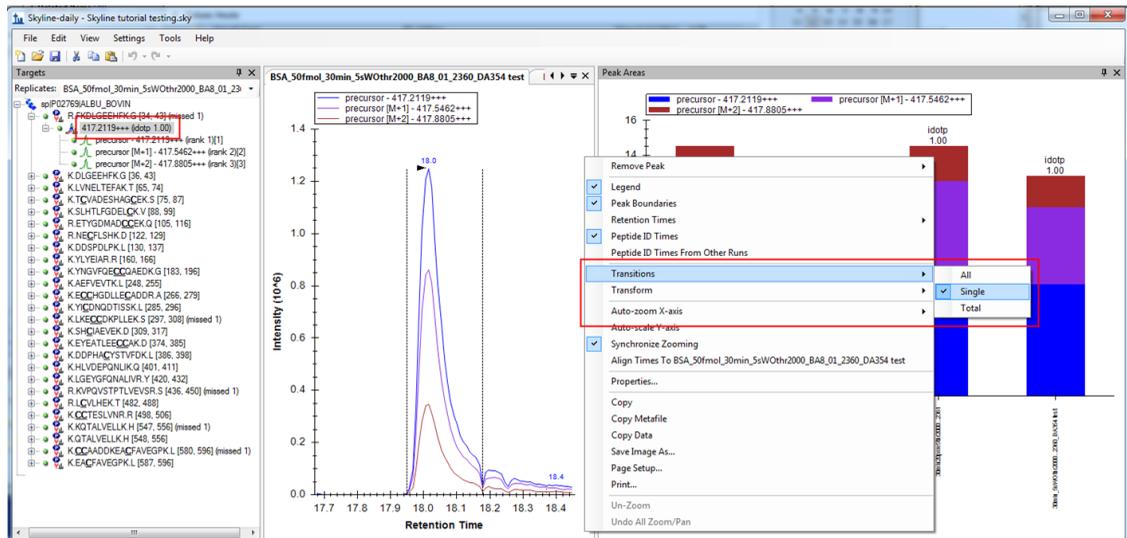

Figure 2-20   **Extracted ion chromatograms with ID annotations**

A vertical line labeled with **ID** and a retention time value indicates the retention time of a sample MS/MS spectrum with a confident identification for this particular peptide.

For runs without identification information, retention time information can be copied from runs with available identification information. Right-click within the chromatogram view and select **Peptide ID Times from Other Runs**. Retention time information will be displayed as a light blue line in the chromatogram view.

Click the **ID** label to display the identified spectrum from the acquisition replicate in the MS/MS spectrum view. This spectrum is stored in the redundant library that was created during the library build.

Note   Identification information will only be available if the .dat file used to create a spectral library and the file for quantification have identical names (see Figure 2-3).

Because database searches for Targeted HR-XIC (due to missing MS/MS information), Middle-band CID, and Broad-band CID are typically not available, no **ID** information will be obtained. In these cases precursor, fragment, and retention time information can be used for manual verification of peptide identity.

After all raw data files for quantification have been imported, the data must be reviewed to ensure correct peak picking throughout all samples and replicates. All information for all replicates and samples can be reviewed at the same time by selecting **View > Peak Areas > Replicate Comparison**. and **View > Retention Times > Replicate Comparison**.

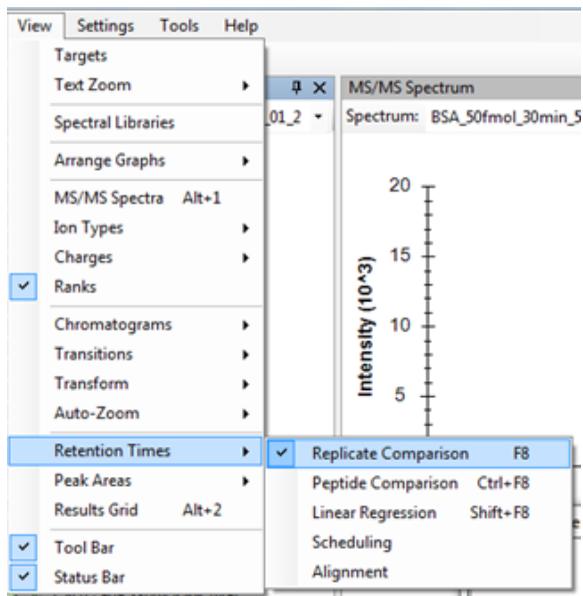Using these settings provides a detailed overview of all imported samples and allows data to be reviewed manually.

Figure 2-21   **Selecting View > Retention Times > Replicate Comparison**
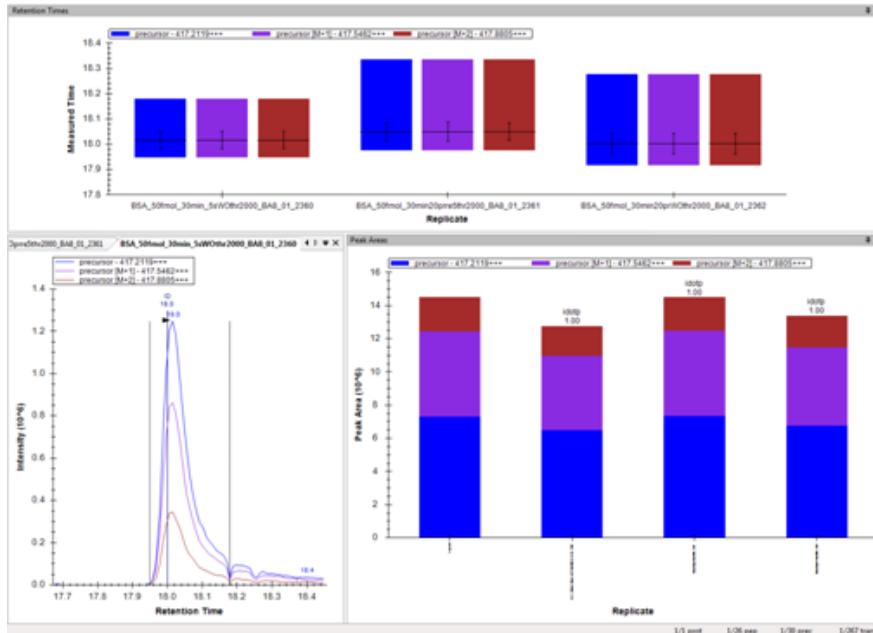
Figure 2-22   Viewing retention time and peak area replicates in the Skyline user interface

Retention times and integration boundaries (box sizes) can be easily reviewed in the Retention Times pane. Peak areas and idopt values can be compared in the Peak Areas pane. Peak selection can therefore be easily evaluated based on the matching of retention times and relative ion abundances.

Thanks to these capabilities, Skyline can also be used for regular quality control of the instruments by monitoring retention time and signal intensity stability.

For a time-based view of the data, which is essential for example for instrument quality control over time, retention time and peak area view can be sorted by acquisition time. Right-click on the corresponding field and selecting **Order > Acquired Time**. This will sort the data according to the acquired time information in the corresponding data file.

The order of samples in the document can also be changed by selecting **Edit > Manage Results** and selecting **Up** or **Down** to move the sample to the desired position (see Figure 2-23).
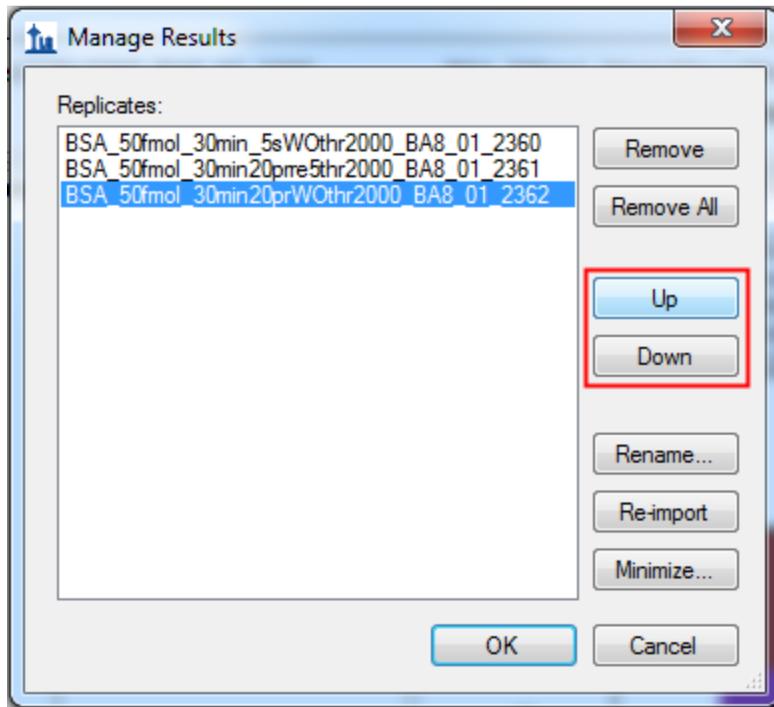
Figure 2-23   Changing the order of samples

During inspection of the data, targeted peptides, precursors, and product ions can be modified within the document using Skyline pop-up pick-lists (see Figure 2-24).
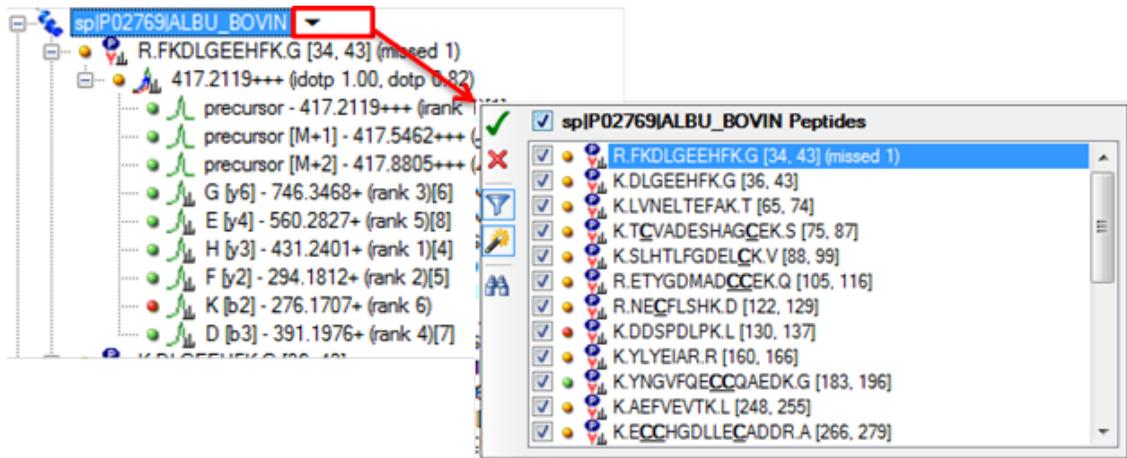

Figure 2-24   Skyline pop-up pick-lists

To change a list, point to the protein until a drop-down arrow appears next to the protein name. Click the drop-down arrow to open a pop-up pick-list that is filtered according to the settings of the document.

Using this list, peptide targets can be manually selected or deselected for quantification. To apply changes, press the Enter key or click ✔.

This function can be used to limit the contents of the document to peptides with an unambiguous quantification and identification.

Peak boundaries can be adjusted by dragging in the Chromatogram pane.

If an incorrect peak is annotated, the correct peak can be selected by:

- Clicking on the correct peak (if it already contains integration boundaries)

- Setting peak boundaries by dragging existing annotated boundaries.

3.4   Re-Importing Raw Data

If inspection of the data determines that changes must be made to the Transition Settings, the data must be re-imported into the existing Skyline document for changes to be applied.

To re-import data, select **Edit > Manage Results**, select the files to be re-imported, and click **Re-import**. Selected files will be marked with an asterisk (see Figure 2-25).

Note   Re-importing files will overwrite the existing project! If the previous version of a project is required, the new project must be saved under a different name using the **File > Save As** command after re-importing.

Click **OK** to start re-importing the selected files.

The advantage of re-importing over deleting and importing data is that any manual edits made to integration boundaries and Result Grid annotation will be maintained.
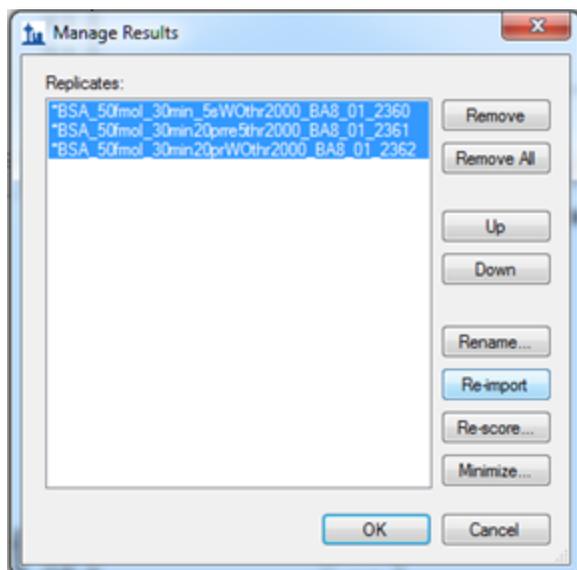

Figure 2-25   Files selected for re-import are marked with an asterisk

3.5   Exporting Results and Customizing Reports

Skyline provides informative visual displays of the raw data and allows data evaluation with regard to peak picking and integration boundaries.

Further statistical analysis data requires tools such as Microsoft® Excel® and R. Skyline's export function enables customized result grids to be exported.

Select **File > Export > Report** to open the Export Report dialog, which contains several Skyline default report methods (see Figure 2-26).
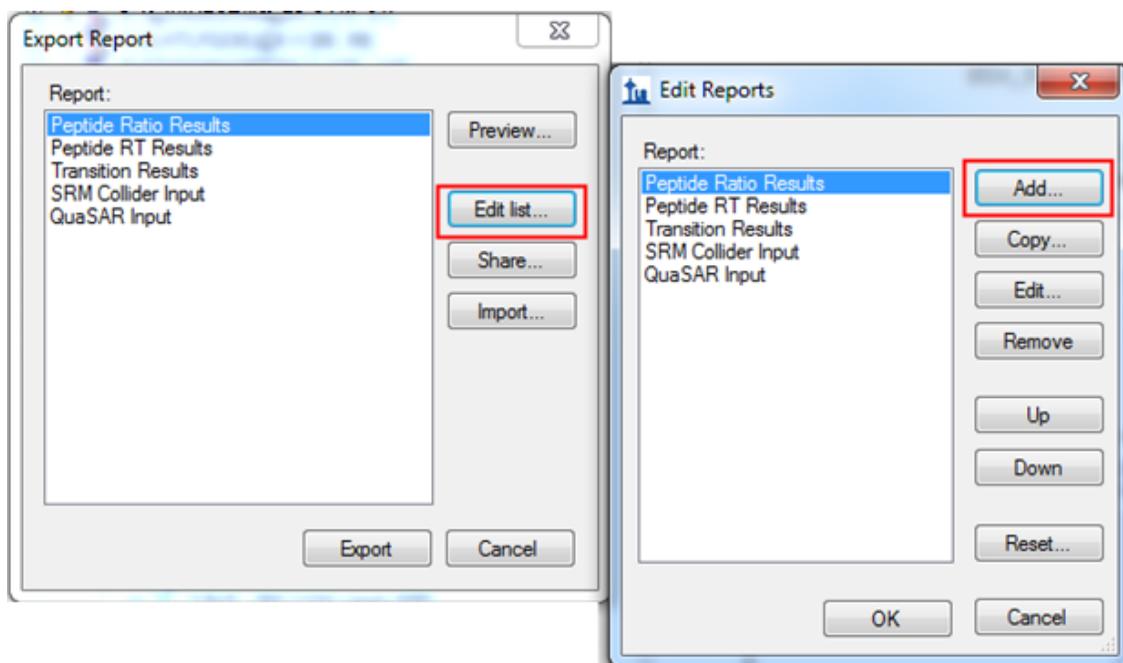

Figure 2-26   Export Report and Edit Reports dialogs

A new Skyline custom report template can be created by clicking **Edit List > Add** to open the Edit Report dialog (see Figure 2-27).
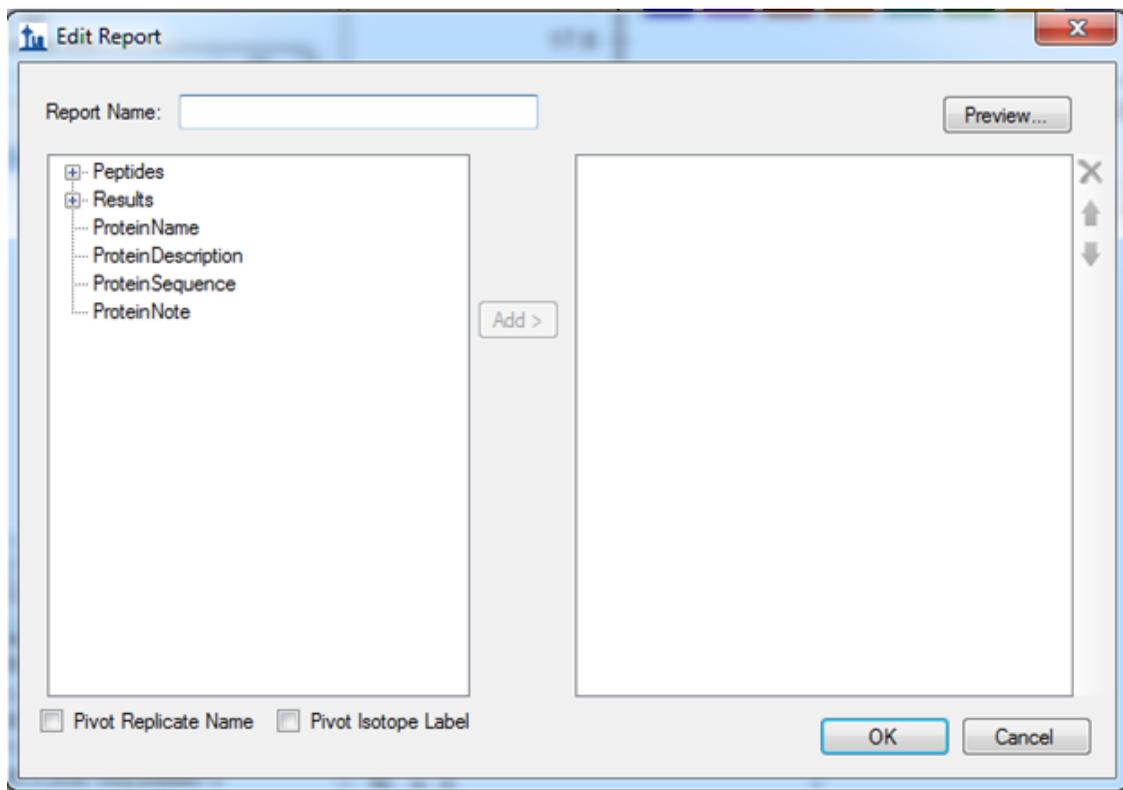
Figure 2-27   Edit Report dialog

Type an identifier into the **Report Name** box. Select the components that should be included in the report template from the tree on the left of the dialog and add them to the template by clicking **Add**.

Click **Preview** to preview the report.

Click **OK** to save the new template. The new template will be added to the template lists in the **Export Report** and **Edit Reports** dialogs.

Saving report templates enables them to be shared (using the **Share** button) and imported into new installations of Skyline.

Detailed information on report creation and a glossary of the custom report fields can be found in the Skyline webpage in the tutorial *Custom Reports & Results Grid[1]*.

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[1]*https://brendanx-uw1.gs.washington.edu/labkey/wiki/home/software/Skyline/page.view? name=tutorial_custom_reports*