A generic solution for quantifying cross-linked peptides using software Skyline

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Method Article

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Abstract

Quantitative cross-linking/mass spectrometry \(QCLMS) probes protein conformational dynamics in solution. It compares different conformers by quantifying cross-linked peptides obtained by digesting the proteins after them having been cross-linked in solution in their native fold, conserving structural details that may be inaccessible by alternative technologies. Computational solutions that automate quantitation of cross-linking data would promote applications to a wider range of users, beyond the technical development field. Here we present a strategy for quantifying cross-linking data using a well-established quantitation proteomics software, Skyline. Our approach can be applied to label-free quantitation, SILAC based quantitation and quantitation using isotope labelled cross-linkers. The workflow can be adapted to extend across a wide range of existing computational pipeline, from identification to quantitation of cross-linked peptides.

Introduction

Quantitative cross-linking/mass spectrometry \(QCLMS) is a relatively young technique for studying structural dynamics of proteins and protein complexes. It is developed on the base of cross-linking/mass spectrometry \(CLMS). In CLMS, spatial proximities between amino acid residues in protein chains are converted into covalent linkages via cross-linkers, whereby the length of cross-links are redefined as distance constraints between cross-linked residue pairs. These linkages are detected by mass spectrometry in the form of cross-linked peptide pairs \(referred to as cross-linked peptides) and identified through database search. Data produced by CLMS provide information on protein folding, topologies of protein complexes and protein-protein interactions[1–3]. In QCLMS, cross-links obtained at different conformational states of a protein or a protein complex are compared in a quantitative manner based on their MS signals. In general, structural rearrangements are expected to be reflected as yield changes of cross-links, and locations of cross-links with major changes highlight the structural regions that are changing. In parallel with standard quantitative proteomics, there are two major strategies for quantifying cross-links: isotope-labelling and label–free approaches. Thus far, isotope labelled quantitation has only been applied to compare two structural states of a protein/protein complex. For quantitation purposes, one of the two protein samples is labelled using heavy isotopes \(for example ^{2}H, ^{13}C, ^{15}N and ^{18}O), and a 1:1 mixture of the two protein samples is analysed by mass spectrometry. For each cross-linked peptide, the signals derived from the two protein samples are distinguished by their mass and compared based on their signal intensities. For example Huang _et al._[4] applied ^{18}O-labelling mass spectrometry for quantitation of cross-links. In this study, following cross-linking, one of two protein conformers was digested by trypsin in normal water, while the other was digested in $H_2{}^{18}O$. Each cross-linked peptide digested in $H_2{}^{18}O$ was 8 Da heavier than the same peptide derived from the sample digested in normal water. Chavez _et al._ applied stable isotope labelling by amino acids in cell culture \(SILAC) in their study on a protein interaction network, to quantify changes on both yields of cross-links and abundance of proteins[5]. An alternative strategy is to use isotope-labelled cross-linkers[6]. Two different conformational states of a protein/protein complex are cross-linked separately using the non-labelled and the heavy

isotope-labelled analogues of a cross-linker. This cross-linking specific strategy has been applied in most reported QCLMS studies[7–15], including the first protocol for QCLMS[16]. Compared to a global peptide-labelling scheme, applying isotope-labelled cross-linkers only labels cross-linker-containing peptides, minimizing the increase in sample complexity. However, this approach requires pre-separation of different stable protein/protein complex conformers prior to cross-linking. Furthermore, its application is also limited by the cross-linking chemistry used, to cross-linkers available in isotope-labelled forms. In contrast, label-free quantitation tolerates any cross-linking chemistry. In label-free quantitation workflows, protein samples of different structural status are not mixed at any step. They are cross-linked, digested and analysed by LC-MS/MS as separate samples but following identical procedures. For a given peptide of interest, abundance across the set of samples is compared based on the signal intensities across individual MS acquisitions. Therefore, label-free quantitation can compare more than two protein samples in one analysis. Moreover, label-free quantitation does not necessarily require pre-separation of conformers before cross-linking, as long as they can be resolved after cross-linking, for example as two separate bands on SDS-PAGE. Hence it has been successfully applied to reveal, in solution, the dynamic equilibrium of an open and a closed conformations of the complement factor H[17]. Currently, the vast majority of reported QCLMS workflows use MS data acquired in data dependent acquisition \(DDA) mode, with quantitation carried out using the extracted ion chromatographic intensities of full scan \(MS1) signals. Although quantifying cross-linked peptides using targeted manner has also been reported[18,19], it is beyond of scope of this protocol. So far, applications of QCLMS have been restricted to those pioneering labs that have been developing QCLMS. A major reason preventing QCLMS from finding wider application is that the only published protocol is based on manual quantitation[16]. Manual quantitation of mass spectrometric data requires user expertise and often involves subjective criteria which are hard to transfer accurately between users and between groups. Furthermore, manual quantitation is time consuming, and becomes unmanageable when dealing with complex and large datasets. Great efforts have been put to automate the data processing. So far the reported solutions have focused in two directions: one is to develop customized software tools for quantifying cross-linking data such as XiQ[20] and xTrac[21], the other is to exploit established quantitative proteomics software tools that routinely do not accommodate cross-linking data, for example vendor software Pinpoint and open software MaxQuant[15] and Skyline[22]. Applying these solutions to automate data processing offers opportunities for a transferable QCLMS protocol, allowing for more general application of QCLMS. Here we describe a generic solution for automatedly quantifying cross-linked peptides using software Skyline, which is compatible with quantitation workflows using isotope-labelled cross-linkers, SILAC and label-free approach. We use the cross-linker BS^3 as an example for demonstration as it is one of the most commonly used cross-linkers for CLMS analysis and is applicable for all three workflows list above.

Development of the protocol In the past decade, numerous software have become available for identifying cross-linked peptides \(such as xQuest[23], MassMatrix[24], pLink[25], StavroX[26], Kojak[27], Protein Prospector[28],XlinkX[29], and MeroX[30]. Although a good number of analysis pipelines have been established and optimized using these different software, in almost all cases, MS data were acquired in data dependent acquisition mode and high resolution MS1 data was acquired for accurate identification

of cross-linked peptides. Therefore, a generic quantitation solution would be possible and would allow for easy extension of many existing workflows. In QCLMS analysis, structural interpretation of the data is based on the quantitation of individual cross-links \(cross-linked residue pairs). The mass spectrometric data is on cross-linked peptides, where ideally multiple support one cross-linked residue pair. This is due to the existence of modifications or missed proteolytic cleavage sites. Nevertheless, it is important to accurately quantify each cross-linked peptide and be able to view and validate the quantitation easily. We have explored a few solutions for automated quantitation of cross-linking data. XiQ[20] and MaxQuant \(version 1.5.4.1 and higher)[15] quantify cross-linking data in similar ways. They accept lists of MS2 features of cross-linked peptides \(including identified m/z, charge and retention time/scan number of the identified MS2 events) as input, giving an easy way to feed-in cross-linking data. However, both software packages have two major limits. First, isotope-labelling based quantitation requires cross-links to be quantified in replicates with label-swapping and in label free quantitation, the signals of cross-linked peptides need to be compared across the set of samples. The current version of both XiQ and MaxQuant do not support transferring identifications of cross-linked peptides between MS runs, which means only cross-linked peptides that are identified in all samples will be quantified. This fact largely limits the coverage of quantified data. Second, both software packages do not provide an interface for visualizing the quantitation results. Skyline outshines by overcoming both limits. Skyline is able to project identifications of cross-linked peptides between MS runs by aligning the detected features, which means as long as a cross-linked peptide is identified in one of samples, its signal will be quantified across the whole set of samples. Furthermore, Skyline provides nice interfaces for easy and fast visualizing, validating and correcting quantitation results of individual cross-linked peptides. Last but not least, Skyline is freely available. As one of the most commonly used quantitative proteomics software tool, Skyline is under continuous development and support. Detailed user tutorials were offered by Skyline developers, which can be easily followed by users with little experience. Skyline requires the sequences of peptides as part of the input for calculating the mass of the peptides, however it does not routinely accommodate cross-linked peptides. Similar to an approach we previously employed to identify cross-linked peptides, we established a strategy to convert the sequence of cross-linked peptides into a non-cross-linked linear form that has identical mass and retains information on cross-linking sites. This strategy is a general solution for software tools that require peptide sequences for mass calculation but do not accept sequences of cross-linked peptides. Using linearized sequences for cross-linking data, one can construct an input text file using standard Skyline format. Therefore, most existing computational setups for identification of cross-linked peptide can be adopted to our quantitation workflow with a single reformatting step for the identification list of cross-linked peptides. **Limitations of the protocol** When quantifying cross-linking data using Skyline, we manually inspect the peptide signal selection and correct the peak isolation window, which improve the accuracy of quantitation results. Although the process time has been largely reduced compared to quantifying cross-linking data manually \(in average 15 second per cross-linked peptide versus approximately 5 minutes), this procedure can still become a limiting factor when working with datasets containing thousands of cross-linked peptides. **Experiment design** To introduce cross-linking data into the full-scan filtering pipeline of Skyline, we devised a strategy to linearize the sequence of cross-linked peptides **Fig. 1a**. The key element in this strategy is to keep the

mass of the linearized the sequence identical to the mass of the cross-linked peptide \(**Fig. 1b**). This strategy can be applied to any cross-linker. The example in **Fig.1c** shows one way of doing this. Using linearized sequences, cross-linked peptides are added to a tab-delimited text file referred to as ssl \(spectrum sequence list). The .ssl file includes the sequence of peptides, the MS file name, scan number of the matched MS2 spectrum, and the charge state of the identified cross-linked peptide. This information allows Skyline to build a library of identified cross-linked peptides and track down their signals in the mass spectra. The sequence and the identified charge state\(s) are used to calculate the "m/z" value\(s) of the cross-linked peptide, and the scan number of the matched MS2 spectrum defines the retention time. A “m/z” value in combination with a retention time serves as a coordinate for Skyline to identify the signal of a cross-linked peptide at a given charge state \(often referred to as a “feature”). The signal intensity of a feature is measured as the area of its chromatographic peak. The signal intensity of a cross-linked peptide pair is the sum of signal intensities of all its detected features. In our approach, isotope labelling through cross-linkers is encoded in structural modifications. Therefore, the identifications of both the light and the heavy versions of each cross-linked peptide feature should be included in the input library. If either version of the cross-linked peptide feature is not identified, a pseudo identification entry is created by transferring the acquisition file name and identified MS/MS from the identified counterpart \(**Fig. 2**). In Skyline, one defines global isotopic labelling including SILAC through "isotope modifications". For SILAC based quantitation, only the heavy labelled version of each cross-linked peptides need to be present in the input file \(**Fig. 3**) and Skyline would generate quantitation transitions for both the light and the heavy version of cross-linked peptides in the spectra library. To maximize the accuracy of quantitation, manual inspection is carried out to ensure that the correct MS1 signals are isolated \(**Fig. 4**). In addition, few Skyline inbuilt measurements of isolated MS1 signals have been used as quality control for quantitation results. Isotope dot product compares isotope distribution of measured MS1 signal against the theoretical distribution calculated based on the peptide sequences. Low isotope dot product value is considered as an indication of incorrect signal isolation. At least three isotope peaks of each cross-linked peptides should be used for quantitation to provide accurate isotope dot product calculations. In label-free quantitation, the coefficient of variation \(CV%) is used to measure the reproducibility of peptide signals between replicates. False signal isolation may lead to high CV%. In isotope labelling based quantitation the light and the heavy versions of each cross-linked peptides are expected to coelute, although a small shift is allowed when deuterium is applied for labelling. The quantitation results from Skyline can be exported as .csv file. One can customize information that need to be included in the result report. For SILAC based quantitation, the signal ratio of the light and the heavy version of each cross-linked peptides is calculated in Skyline and can be included in the report. For label-free quantitation and quantitation using isotope labelled cross-linkers, signal changes between samples \(_i.e._ conformers) need to be calculated post exporting the data. This process can be conducted using any spreadsheet applications such as Microsoft Excel.

Equipment

Pre-required data ==*== Raw mass spectra data files. ==*== List of peptide-spectra matches \(PSM) for cross-linked peptides, including raw file name, scan number, identified charge, and sequence of identified cross-linked peptides \(including modifications). _Software_ ==*== MSconvert \(http://proteowizard.sourceforge.net) ==*== Skyline \(https://skyline.ms/project/home/software/Skyline/begin.view) ==*== Spreadsheet applications e.g., Microsoft Excel \(version 1997 and higher) _PC hardware_ Computer workstation with dual core processor, 64-bit Microsoft Windows system \(XP or newer versions), 4GB RAM would meet minimal requirement for installation of all software listed above.

Procedure

1. Convert vendor specific mass spectrometry raw data into the mzML format using MSConvert[31]. i. Open software tool msConvert. ii. Select the file\(s) to be converted. iii. Select the output directory; if not specify, output file would by default be placed in the same directory as the input files. iv. Choose "mgf" as “Output format”. v. Only if the MS2 spectra are acquired in profile mode, select "peakPicking" as a filter. Otherwise, leave the “filter” option empty. vi. Start the process by click on the "start" button. **CRITICAL STEP** A detailed tutorial for such process can be found at \(http://proteowizard.sourceforge.net/IntroductoryTutorial.shtml) _Generating Skyline input. ssl files_ 2. Linearize the sequences of identified cross-linked peptides as demonstrated in **Fig. 2**. This process can be automated using a combination of in-built functions in Microsoft Excel. 3. Generate .ssl files A. Label-free quantitation Convert modifications in the linearized sequences of cross-linked peptides into Skyline format \(**Fig. 1**). A list of identified features of cross-linked peptides in the standard .ssl format \(https://skyline.ms/wiki/home/software/BiblioSpec/page.view?name=BiblioSpec%20input%20and%20output%20file%20formats) can be generated using Microsoft Excel. Save this list as a text \(tag delimited) file, and rename the ==**==.txt file as ==**==.ssl in file explorer. B. SILAC based quantitation Generate .ssl files as described in 3A. In the .ssl files, if a feature is identified as the non-isotope-labelled version of a cross-linked peptides, replace the sequence with the corresponding heavy \(isotope-labelled) version. C. Quantitation using isotope-labelled cross-linkers. Generate a .ssl file containing all identified feature of cross-linked peptides as described in 3A. Generate a separate .ssl file containing pseudo identification information of cross-linked peptide features. **CRITICAL STEP** This procedure requires co-elution of the light and the heavy versions of cross-linked peptides in the reverse phase chromatography in the LC-MS system. It is the case for BS^3 and BS^3-d4, however cross-linkers labelled with more deuterium atoms may lead to shift on elution time compared to their non-labelled analogues. _Quantitation using Skyline MS1 full scan filtering_ 4. Start Skyline and open a new file 5. Set parameters that are relevant to MS1 full scan filtering i. Define modifications \(**Fig. 5**) ==*== Select modifications that are predefined in Skyline Settings>Peptide settings>Modifications Select modifications from the dropdown list, such as Carboxymethyl \(C) and Oxidation \(M). **?TROUBLESHOOTING** ==*== Define user customized modifications Settings>Peptide settings>Modifications>Edit list>Add **Table 1** lists modifications defined for BS^3 cross-linking. ==*== Set "Max variable mods" according to input data. ==**== For SILAC based quantitation define isotope

labelling scheme \(**Fig. 6*) Settings>Peptide settings>Isotope modifications>Edit list>Add Select predefined isotope modification from the dropdown list or defined customized isotope modifications. ii. Peptide filters Settings>Peptide settings>Filter "Min length", “Max length” and should be set accordingly to include all identified peptides that need to be quantified in the peptide library. iii. Transition full scan parameters \(**Fig. 7a**) Settings>Transition settings>Full scan ==*== "Isotope peak included" = Count ==*== "Peaks" = number of isotope peaks used for quantitation. **CRITICAL STEP** At least three isotope peaks are recommended for use in quantitation. However, this number should be less than the mass difference between the light and the heavy version of a cross-linked peptide when isotope-labelled cross-linkers are applied for quantitation. ==*== "Precursor mass analyzer" = analyzer applied for acquiring MS1 spectra. ==*== "Resolving power" = resolution applied for acquiring MS1 spectra. **CRITICAL STEP** Precursor mass analyzer and Resolver power should be defined based on the mass spectrometer and acquisition method used. ==*== "Retention time filtering", select “use only scans within \(X) minutes of MS/MS IDs”. The default time-window is set to five minutes, however it can be customized. iv. Transition filter \(**Fig. 7b**) Settings>Transition settings>Filter ==*== "Precursor charge" should include all identified charge state of cross-linked peptides. ==*== "Ion type" = p \(for precursor) 6. Build a peptide library \(**Fig. 8**) Settings>Peptide settings>Filter>Build Select the .ssl files as "input files". **CRITICAL STEP** A separate library should be built for pseudo identifications of cross-linked peptide features when use isotope-labelled cross-links for quantitation. **?TROUBLESHOOTING** 7. Select spectra libraries for quantitation. Settings>Peptide settings>Library>Libraries ==*== Select all spectra libraries that should be used for quantitation for a given dataset from the list. ==*== Select "Pick peptide matching library" 8. Add peptides in the libraries into the list for quantitation \(calculating transitions for quantitation). ==*== View spectra libraries in the spectra library explorer View>spectra libraries ==*== Select "Add all" to add all peptides in the library to the target list for quantitation. Based on the transition parameters set in Step 5, transitions are calculated for each cross-linked peptide. For SILAC labelled sample, transitions are calculated for both non-labelled and SILAC labelled version of every cross-linked peptide. **CRITICAL STEP** If input data consists of multiple libraries \(for example, a separate library is built for pseudo identifications of cross-linked peptide features when use isotope-labelled cross-linkers), after adding peptides from one library, switch view to another library using the dropdown menu and add peptides. Repeat the process until peptides in all libraries are added. **?TROUBLESHOOTING** 9. Import mass spectrometry raw data for quantitation. File>import>results Upload data in .mzML format. **CRITICAL STEP** For label-free quantitation, MS data of all conformers \(including replicates) should be uploaded and quantified together. Similarly, when quantifying using isotope-labelled cross-linkers or SILAC, MS data from the forward-labelled and the reverse-labelled experiments should be uploaded and quantified together. 10. Quality control of automated quantitation results. **CRITICAL STEP** Like any other software, errors do exist in the automated quantitation results from Skyline which are mainly resulted from incorrectly isolated MS1 signals. This happens particularly more often to those low intensity signals. Skyline provides very user friendly interface for manually inspecting, and correcting the isolate peptide signals for quantitation. This process can dramatically improve the accuracy of quantitation results. A. In a typical example, it takes less than 20 seconds to correct isolation windows of a cross-linked peptides quantified across six samples \(two conformational states, each with triplicated MS

analysis). Therefore, it is feasible to carry out manual inspection for every cross-linked peptide when quantifying dataset of few hundred cross-linked peptides. i. View>Arrange Graphics>Tiled Display chromatogram of all quantified samples in a tiled view \(each MS acquisition in a separate window). ii. Click on one cross-linked peptide listed in the "targets" list to display the extracted MS1 signals of this peptide in each MS acquisition. iii. Right click on one chromatogram, under "peptide ID times" option select “matching” and “aligned” to display retention time of identified MS2 events and/or aligned identification of this peptide in each MS acquisition. iv. View>Peak areas>Replicate comparison. The abundance of isolated MS1 signals of the selected peptide in each MS acquisition are shown in a bar chart with isotope dot products labelled \(in a new window). The isotope dot product compares the intensities of isolated isotope peak signals against the theoretical isotope abundance distribution calculated based on peptide sequence. A dot product value can range between 0 and 1, and 1 indicates a perfect match. v. Inspect the isolated peptide signal according to following criteria: ==*== Isolation window includes the whole elution peak. ==*== Isolated elution window agrees with the retention time \(RT) of identified MS2 events or aligned identification RT. ==*== Isotope dot product value is 0.9 or higher ==*== If Isotope dot product value is lower than 0.9, exam the MS1 spectrum of the selected peptide by clicking on the elution peak in the chromatogram \(a new window will appear with MS1 spectrum). vi. Correct the isolation window if not correct. vii. Move to the next cross-linked peptides until going through all peptides in the "target" list. B. When working with larger datasets, it becomes unfeasible time-wise to manually inspect quantitation results of each individual cross-linked peptide. In such cases, one could utilize Skyline’s inbuilt measurements for isolating peptide signals to filter out cross-linked peptide that are likely to be incorrectly quantified and carry out manual inspection and correction only on this subset of data. i. Export the automated quantitation result following Step 11 and 12 with two additional data columns: ==*== Isotope Dot Product \(Proteins>Peptides>Precursors>Precursor Results) ==*== Peptide Retention Time \(Proteins>Peptides>Peptide results) ii. View the automated quantitation results in Microsoft Excel \(version 1997 and higher). iii. Any isolated MS1 signal of a cross-linked peptide with isotope dot product value below 0.9 should be manually inspected. iv. For label-free quantitation, calculate the coefficient of variation \(CV) value on "Normalized Area" for the triplicate of each sample as standard deviation divided by mean. Cross-linked peptides with CV% higher than 20% within a sample should be manually inspected v. For SILAC based quantitation, calculate the retention time difference between the light and the heavy signals of a cross-linked peptide. If it is larger than 20 seconds, manually inspect the isolated signal of this cross-linked peptides vi. For quantitation using isotope-labelled cross-linkers when the retention time difference between the light and the heavy signals of a cross-linked peptide is larger than 40 seconds, manually inspect the isolated peptide signal vii. Follow the procedures list in Step 10A, manually inspect the isolate MS1 signals of cross-linked peptides if it fulfils any conditions listed above \(iii-vi) viii. Export manually corrected quantitation results following Step 11 and 12. 11. Customize contents \(data columns) in the quantitation results report View>Document Grid>Views>Edit view Following data columns should be included in the results report. ==*== Peptide Modified Sequence \(Proteins>Peptides) ==*== Replicate Name \(Replicates) ==*== Normalized Area \(Proteins>Peptides>Peptide results>Quantitation) ==*== Isotope Label Type \(Proteins>Peptides>Peptide results) \(only for SILAC based quantitation) ==*== RatioLightToHeavy \(Proteins>Peptides>Peptide

results) \(only for SILAC based quantitation) 12. Export report for automated quantitation results File>Export>Report The reports are in .csv format.

Timing

Step 1, Converting MS data to mzML format \(1-5 minutes, per raw MS file). Step 2-3, Linearize the sequence of cross-linked peptides and generate .ssl file \(==~== 1 hour with excel). Step 4-5, Setting up Skyline parameters. \(5-10 minutes) Step 6, Build peptide library \(30 minutes – few hours depending on the size of the .ssl files and the number of mzML files) Step 7-8, Generating list of cross-linking with calculated transitions for quantitation \(30 minutes -few hours, depends on number of cross-linked peptides. Step 9, Import MS raw data for quantitation \(==~== 10 minutes per raw file with 180 minutes acquisition time). Step 10, Manual inspection and correction of quantitation results. \(In average 15 second per cross-linked peptide). Step 11-12, Export Skyline quantitation results. \(==~== 30 minutes)

Troubleshooting

Troubleshooting advice can be found in Table. 2

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Figures

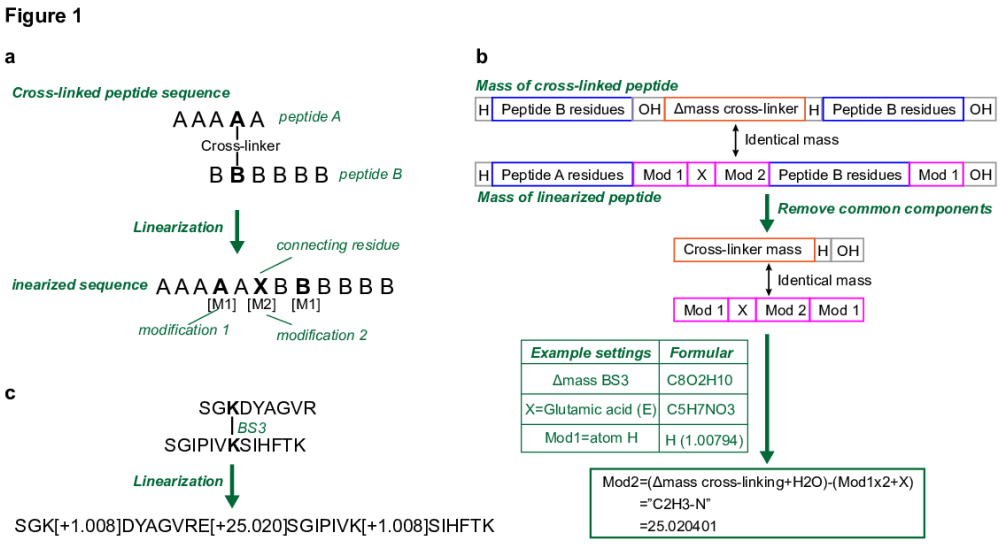

Figure 1

Linearization of cross-linked peptide sequences (*a*) A scheme for converting the sequence of a cross-linked peptide to a linear sequence with identical mass. (*b*) The scheme of mass calculation for the linearization of cross-linked peptide sequences. (*c*) An example for converting the sequence of a BS^3^ cross-linked peptide into the linear sequence for work in Skyline, applying the settings shown in (*b*).

Figure 2

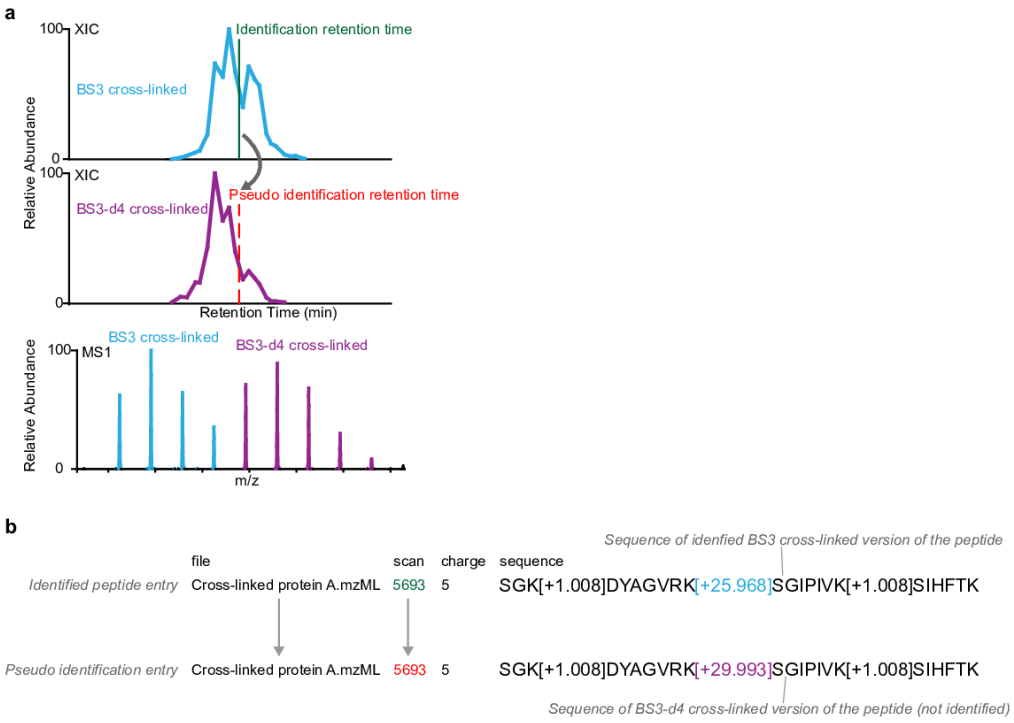

Figure 2

Schematic demonstration on how to generate pseudo identification entries in the .ssl file (*a*) The extracted ion chromatogram (XIC) of the BS^3^- (light) and the BS^3^-d4- (heavy) version of a cross-linked peptide (the MS1 spectrum is shown below). The overlapped elution window of the light and the heavy versions of the cross-linked peptide allowed for applying the retention time of the identified MS2 event of the light version as the retention time of the pseudo identification event for the heavy version

which not been actually identified. (*b*) An example on how to generate a pseudo identification entry of a BS^3^-d4 cross-linked peptide in an .ssl file, based on the MS2 information of the identified BS^3^ cross-linked version of this cross-linked peptide.

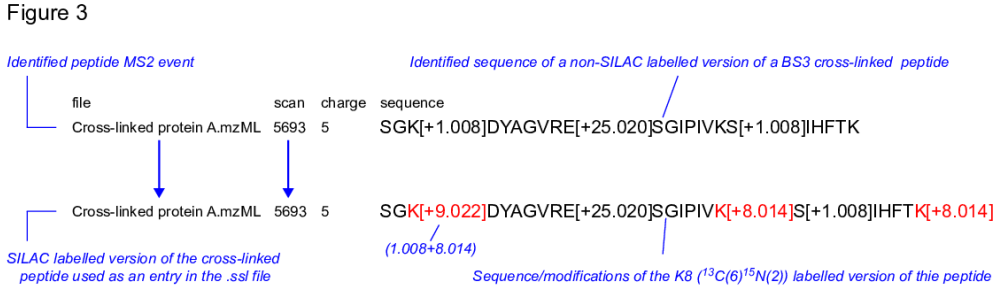

Figure 3

Schematic demonstration on how to define isotopic modifications in peptide sequence and generate SILAC labelled version of cross-linked peptide identification as entries in an .ssl file

Figure 4

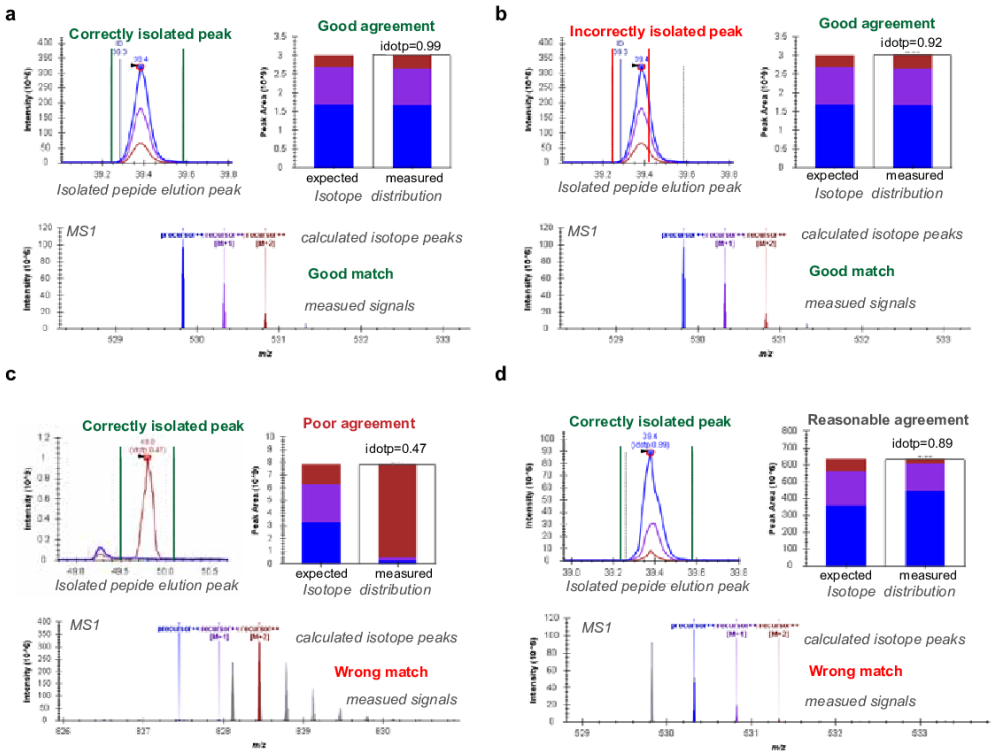

Figure 4

Common errors on peptide signal isolation in Skyline (*a*) An example of a correctly isolated chromatographic signal of a peptide. The elution peak is correctly isolated; the isotope distribution of the isolated signals is in good agreement (with the "idotp" close to 1) with the expected pattern calculated based on the sequence of the target peptide; the measured signals of isotope peaks are in good matches with the calculated m/z of isotope peaks of the target peptide. (*b*) One error on peptide signal picking is

incorrectly isolated elution peak of the target peptide. (*c*) Skyline might pick wrong signals for a target peptide, the error can be indicated by poor agreement on isotope distribution between the expected and the measured pattern, showing as low “idotp”. (*d*) The mistakenly selected signal may share the same charge as the target peptide which might resulted in an “idotp” value close to one. Such signal picking error would only be revealed when compare isotope peaks of the isolated signal in the MS1 spectra with the m/z of expected isotope peaks of the target peptide.

Figure 5

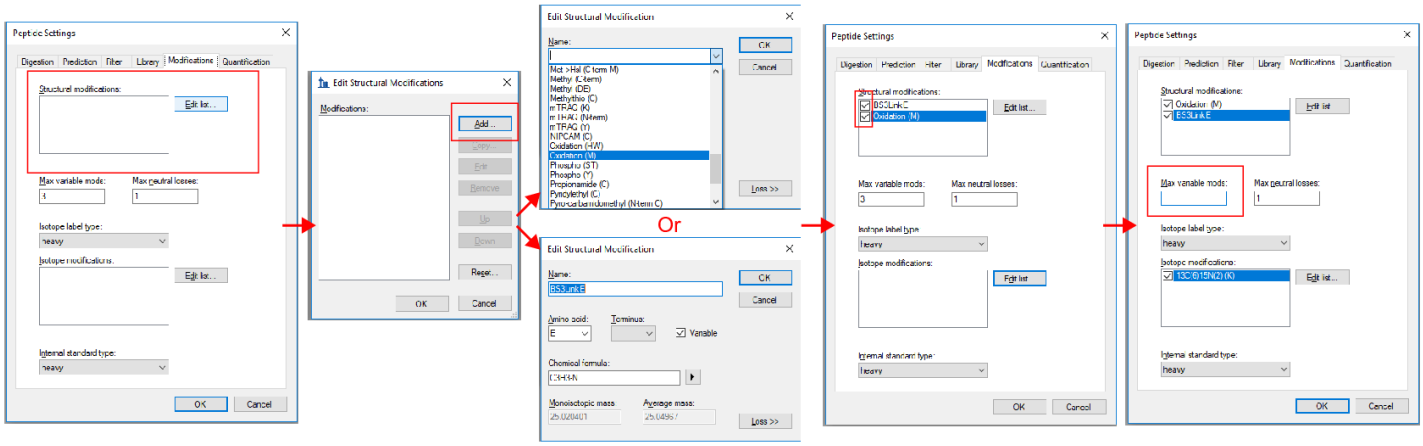

Figure 5

Demonstration on how to define modifications in Skyline

Figure 6

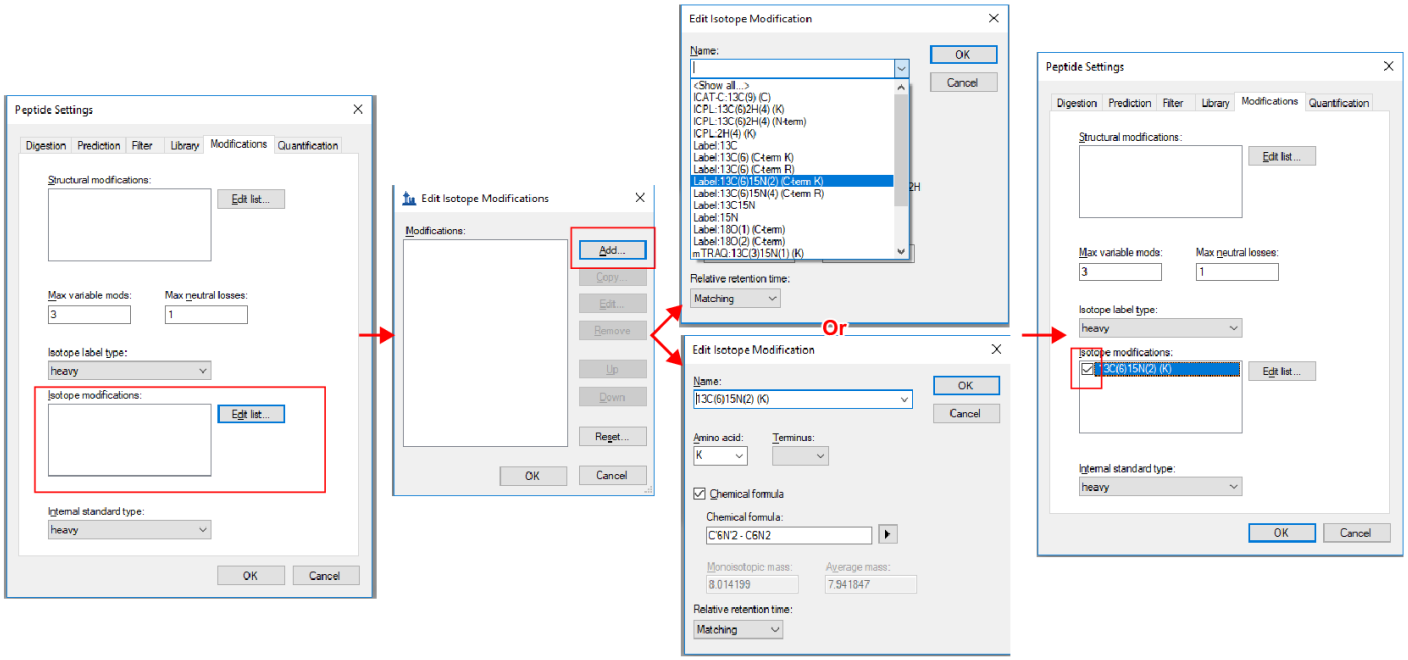

Figure 6

Demonstration on how to define isotopic modifications in Skyline

Figure 7

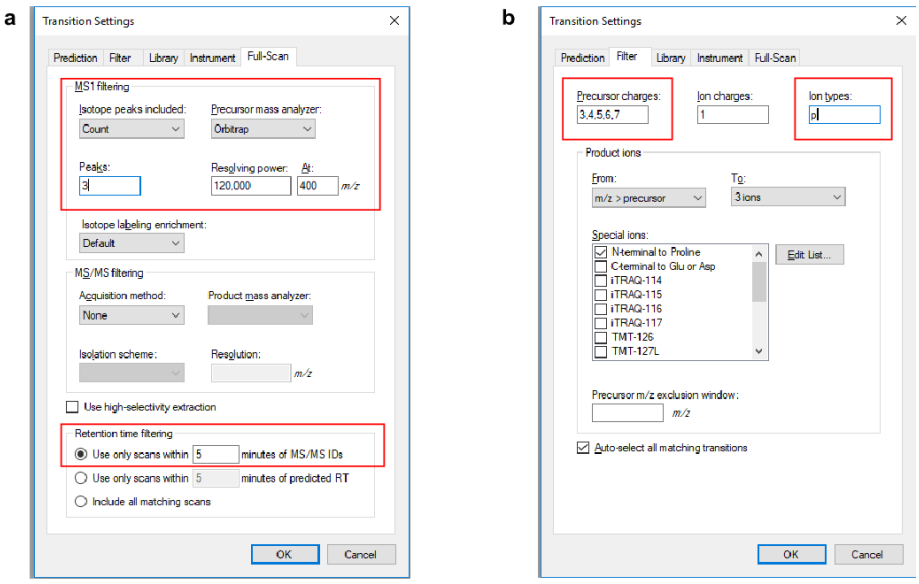

Figure 7

Transition settings that need to be defined when quantifying (*a*) For "Full Scan" settings and (*b*) for transition filter

Figure 8

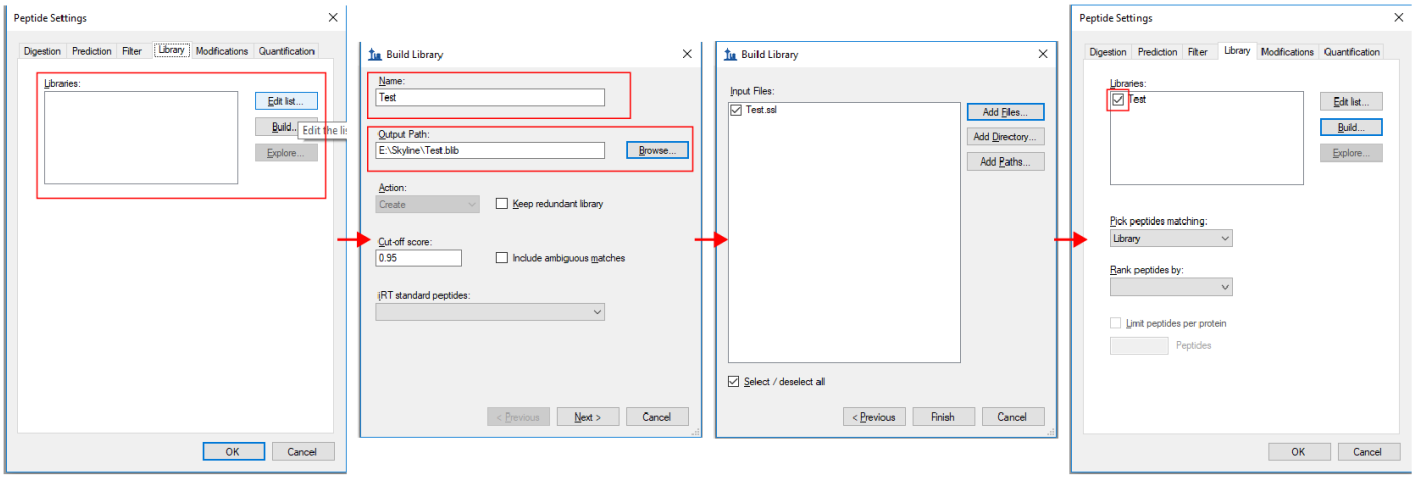

Figure 8

Demonstration on how to build a library in Skyline

Figure 1

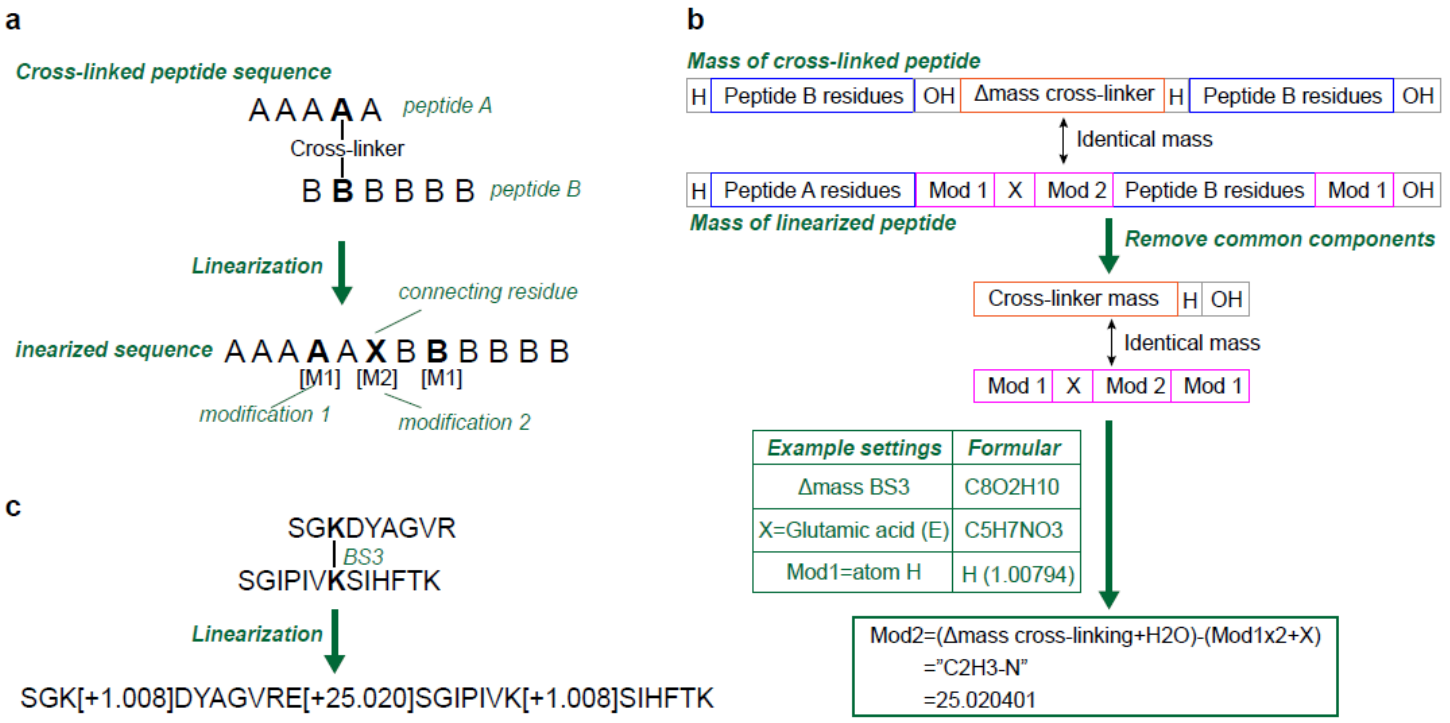

Figure 9

Figure 1 Linearization of cross-linked peptide sequences (a) A scheme for converting the sequence of a cross-linked peptide to a linear sequence with identical mass. (b) The scheme of mass calculation for the linearization of cross-linked peptide sequences. (c) An example for converting the sequence of a BS3 cross-linked peptide into the linear sequence for work in Skyline, applying the settings shown in (b).

Figure 2

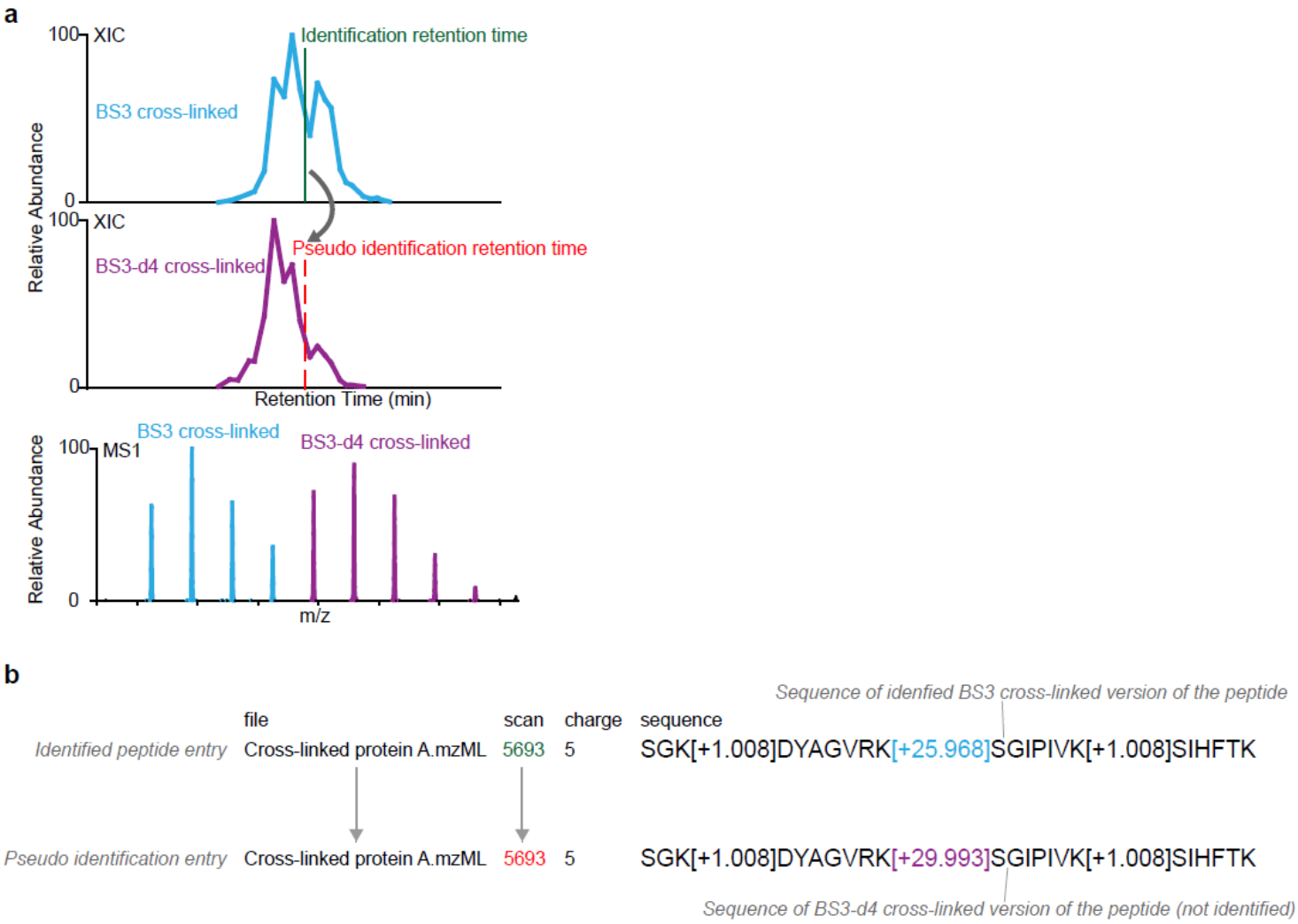

Figure 10

Figure 2 Schematic demonstration on how to generate pseudo identification entries in the .ssl file (a) The extracted ion chromatogram (XIC) of the BS3- (light) and the BS3-d4- (heavy) version of a cross-linked peptide (the MS1 spectrum is shown below). The overlapped elution window of the light and the heavy versions of the cross-linked peptide allowed for applying the retention time of the identified MS2 event of the light version as the retention time of the pseudo identification event for the heavy version which not been actually identified. (b) An example on how to generate a pseudo identification entry of a BS3-d4 cross-linked peptide in an .ssl file, based on the MS2 information of the identified BS3 cross-linked version of this cross-linked peptide.

Figure 3

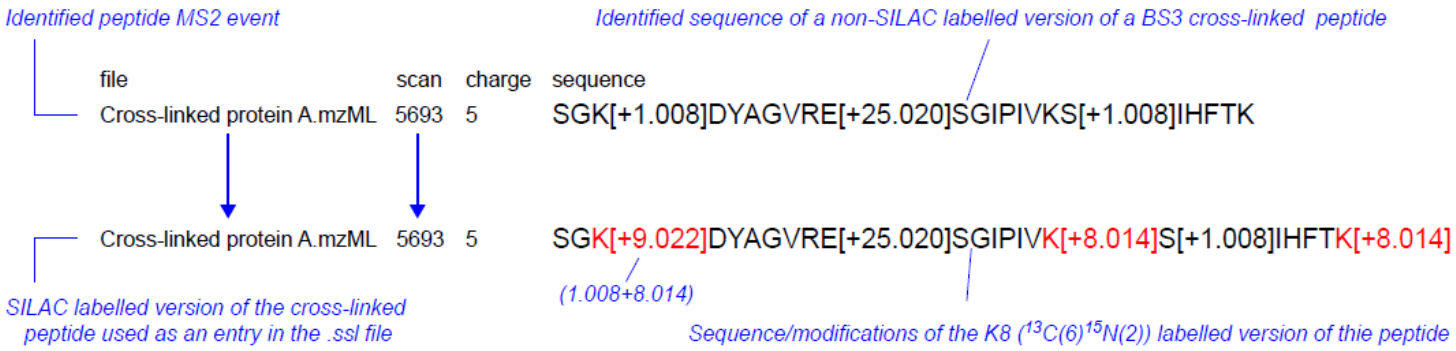

Figure 11

Figure 3 Schematic demonstration on how to define isotopic modifications in peptide sequence and generate SILAC labelled version of cross-linked peptide identification as entries in an .ssl file

Figure 4

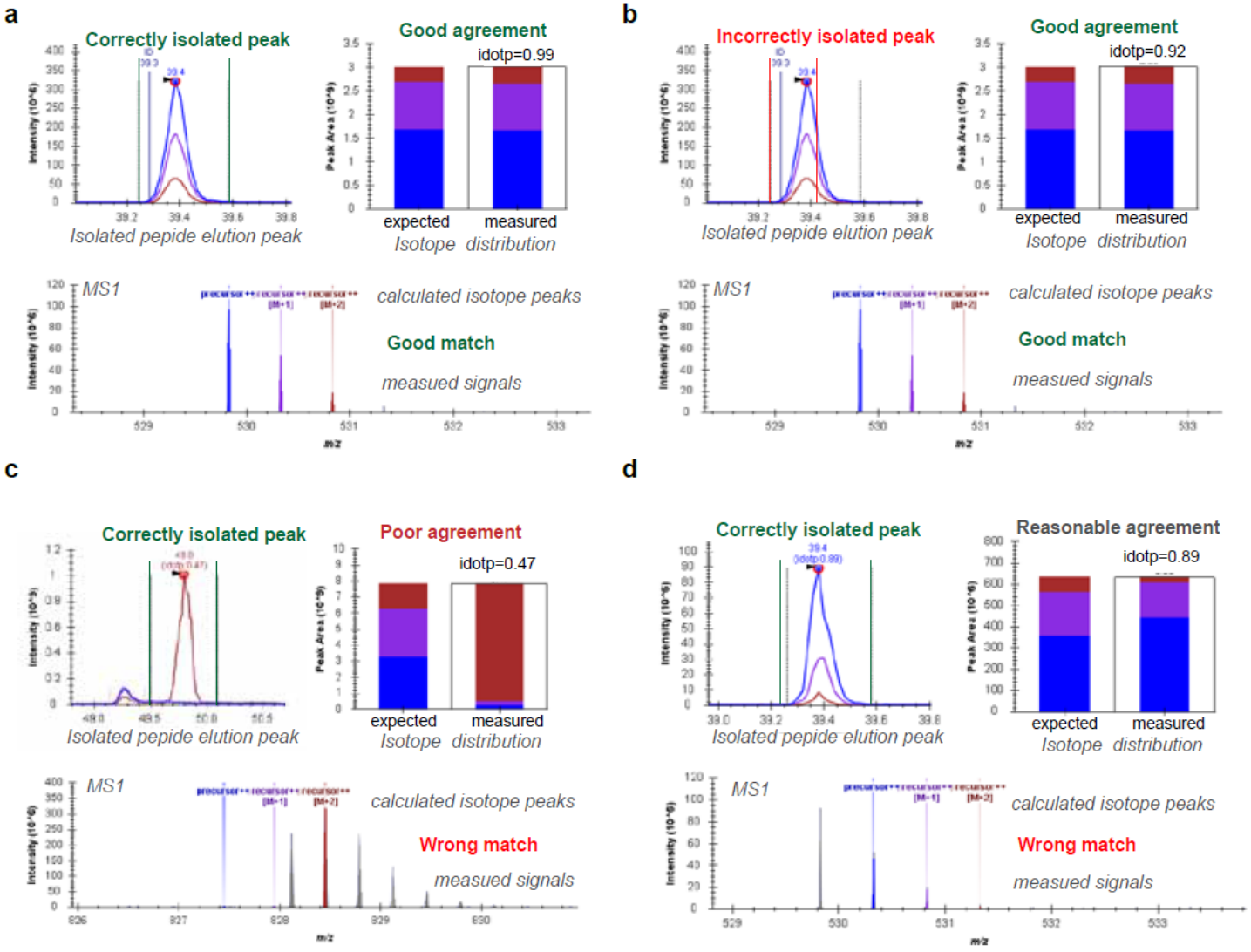

Figure 12

Figure 4 Common errors on peptide signal isolation in Skyline (a) An example of a correctly isolated chromatographic signal of a peptide. The elution peak is correctly isolated; the isotope distribution of the isolated signals is in good agreement (with the “idotp” close to 1) with the expected pattern calculated based on the sequence of the target peptide; the measured signals of isotope peaks are in good matches with the calculated m/z of isotope peaks of the target peptide. (b) One error on peptide signal picking is incorrectly isolated elution peak of the target peptide. (c) Skyline might pick wrong signals for a target peptide, the error can be indicated by poor agreement on isotope distribution between the expected and the measured pattern, showing as low “idotp”. (d) The mistakenly selected signal may share the same charge as the target peptide which might resulted in an “idotp” value close to one. Such signal picking error would only be revealed when compare isotope peaks of the isolated signal in the MS1 spectra with the m/z of expected isotope peaks of the target peptide.

Figure 5

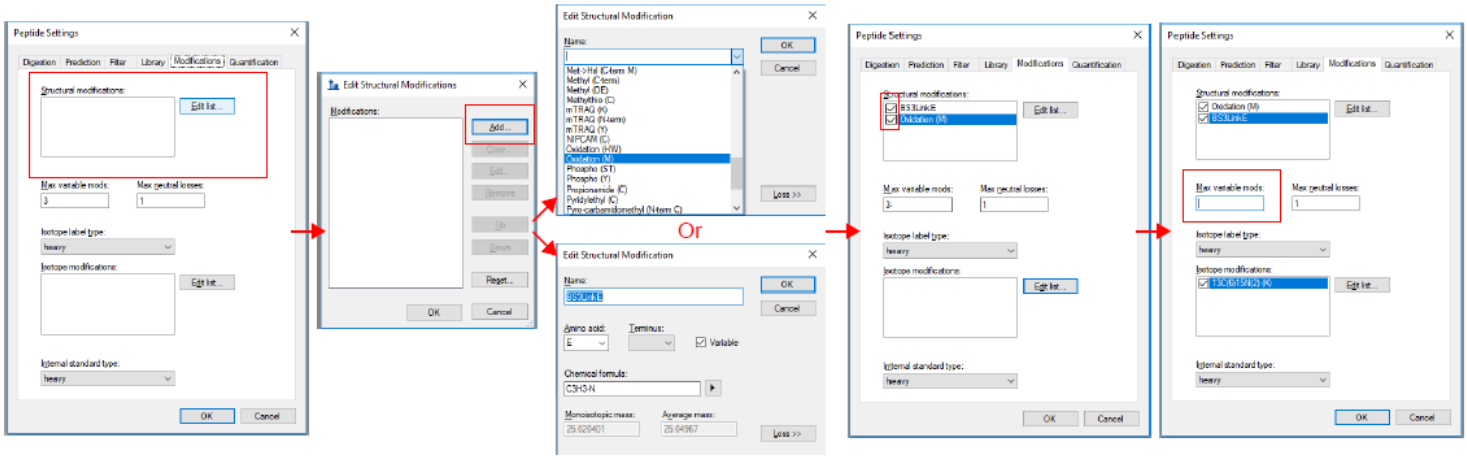

Figure 13

Figure 5 Demonstration on how to define modifications in Skyline

Figure 6

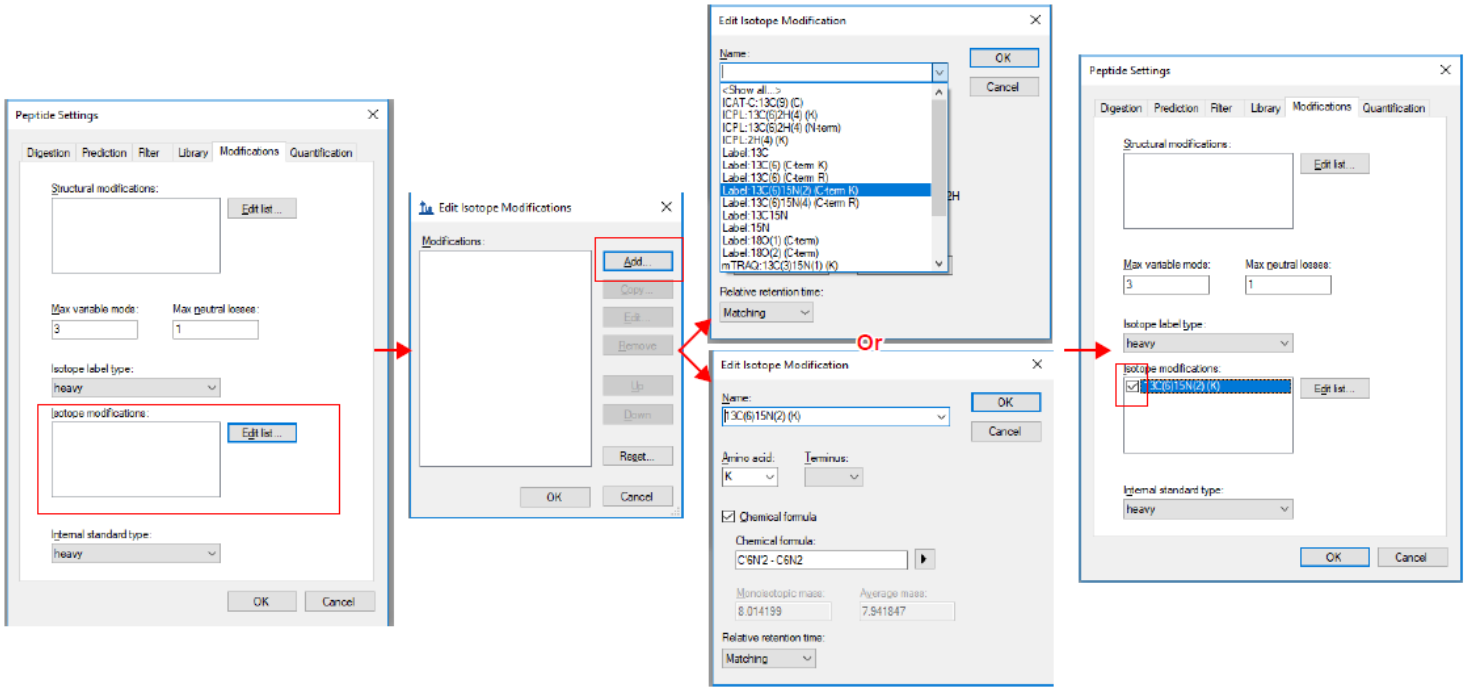

Figure 14

Figure 6 Demonstration on how to define isotopic modifications in Skyline

Figure 7

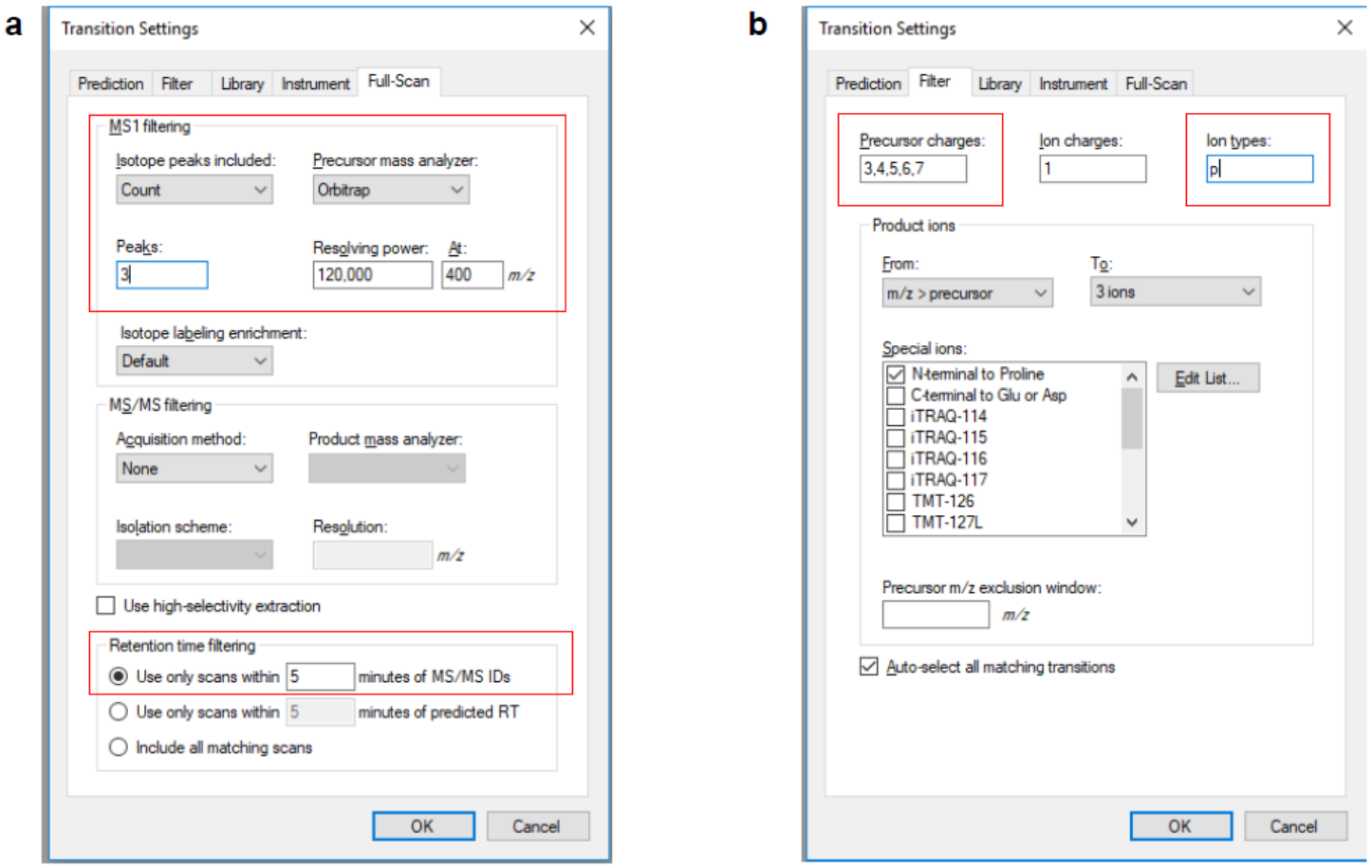

Figure 15

Figure 7 Transition settings that need to be defined when quantifying (a) For “Full Scan” settings and (b) for transition filter

Figure 8

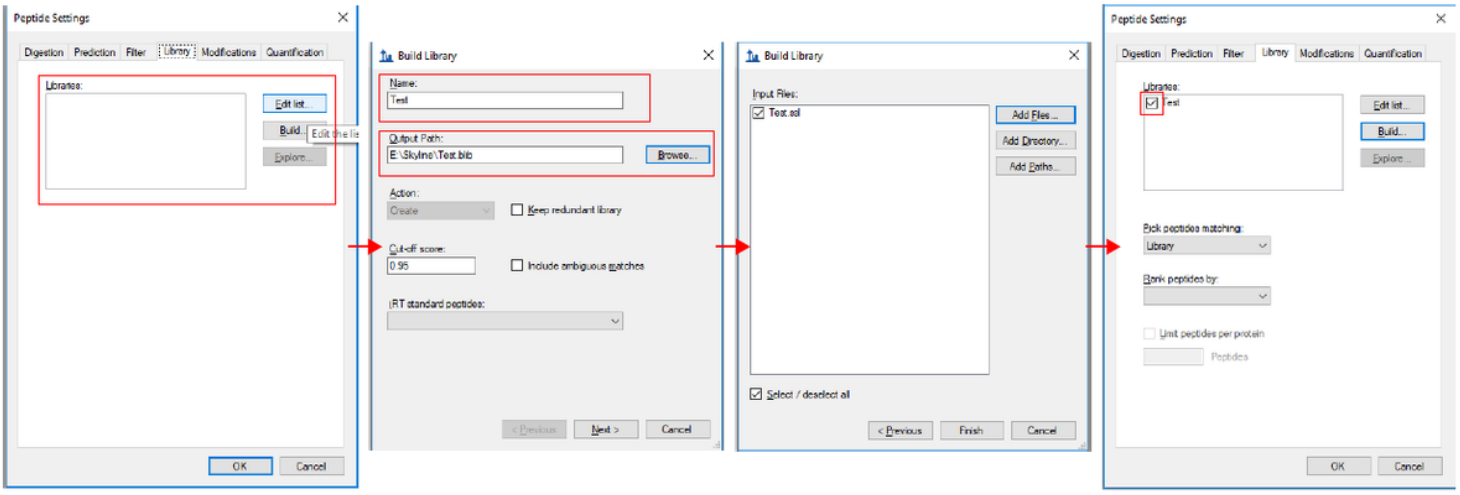

Figure 16

Figure 8 Demonstration on how to build a library in Skyline

Supplementary Files

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- supplement0.pdf
- supplement0.pdf