Intact Protein Demo Workflows

Biologics Explorer Software Guidelines

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TPUB-CUST-16031-A

Intact Protein Demo Workflows

CONTENTS OF THIS GUIDE

Part A

Overview of the Intact Protein Demo Workflows

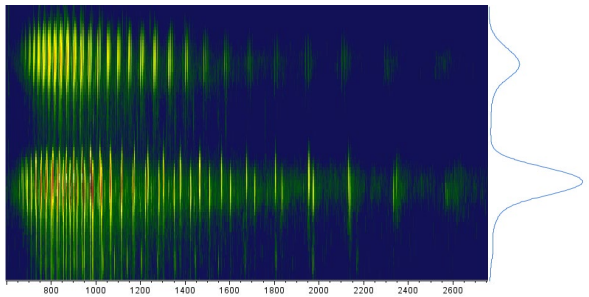
A

Overview of the Applications for Intact Protein Demo Workflows

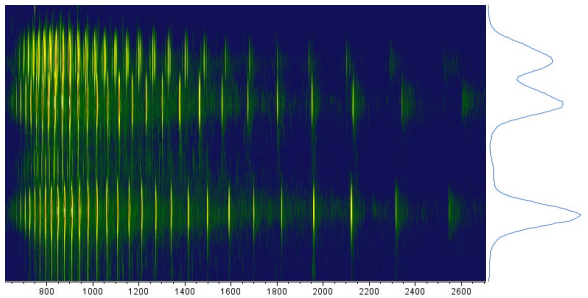
- The Intact Protein demo workflows contain examples of how to analyze intact, reduced, or partially cleaved biotherapeutics.
- **Spectral Deconvolution** is used to analyze species of interest that are chromatographically well resolved.
  - The RT ranges for deconvolution are selected in the TIC or UV data, or can be specified manually.
- **Time-Resolved (TR) Deconvolution** is used to analyze data that contains complex mixtures that are poorly resolved.
  - Each RT scan is deconvoluted to create an ion map of the deconvoluted data.

TR Deconvolution:

Complex Chromatography



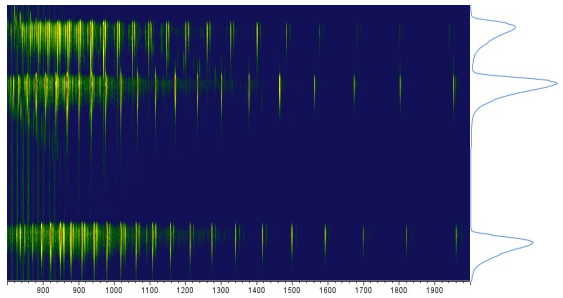
Fragments: unresolved



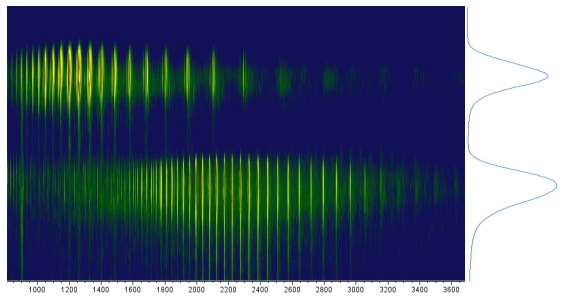
Fragments: overlapped

Spectral Deconvolution:

Simple Chromatography



Fragments: resolved



Subunits: resolved

Overview of the Intact Protein Demo Workflows

Intact_SpectralDeconvolution_MS_Demo:

- An Intact Protein analysis workflow with spectral deconvolution of each RT range that was identified in the TIC.

Intact_SpectralDeconvolution_MS+UV_Demo:

- An Intact Protein analysis workflow with spectral deconvolution of each RT range that was identified in the UV chromatogram.

Intact_TRDeconvolution_MS_Demo:

- An Intact Protein analysis workflow with time-resolved (TR) deconvolution.

Intact_TRDeconvolution_MS+UV_Demo:

- An Intact Protein analysis workflow with time-resolved (TR) deconvolution and UV peak detection.

Pepmap_ReviewSnapshots_Demo:

- A workflow to open or review saved results.

Intact_BatchProcessing_Demo:

- A version of the Intact_SpectralDeconvolution_MS+UV workflow that analyzes multiple data files, one sample at a time.

Part B

Information About Intact Protein Demo Workflows

B

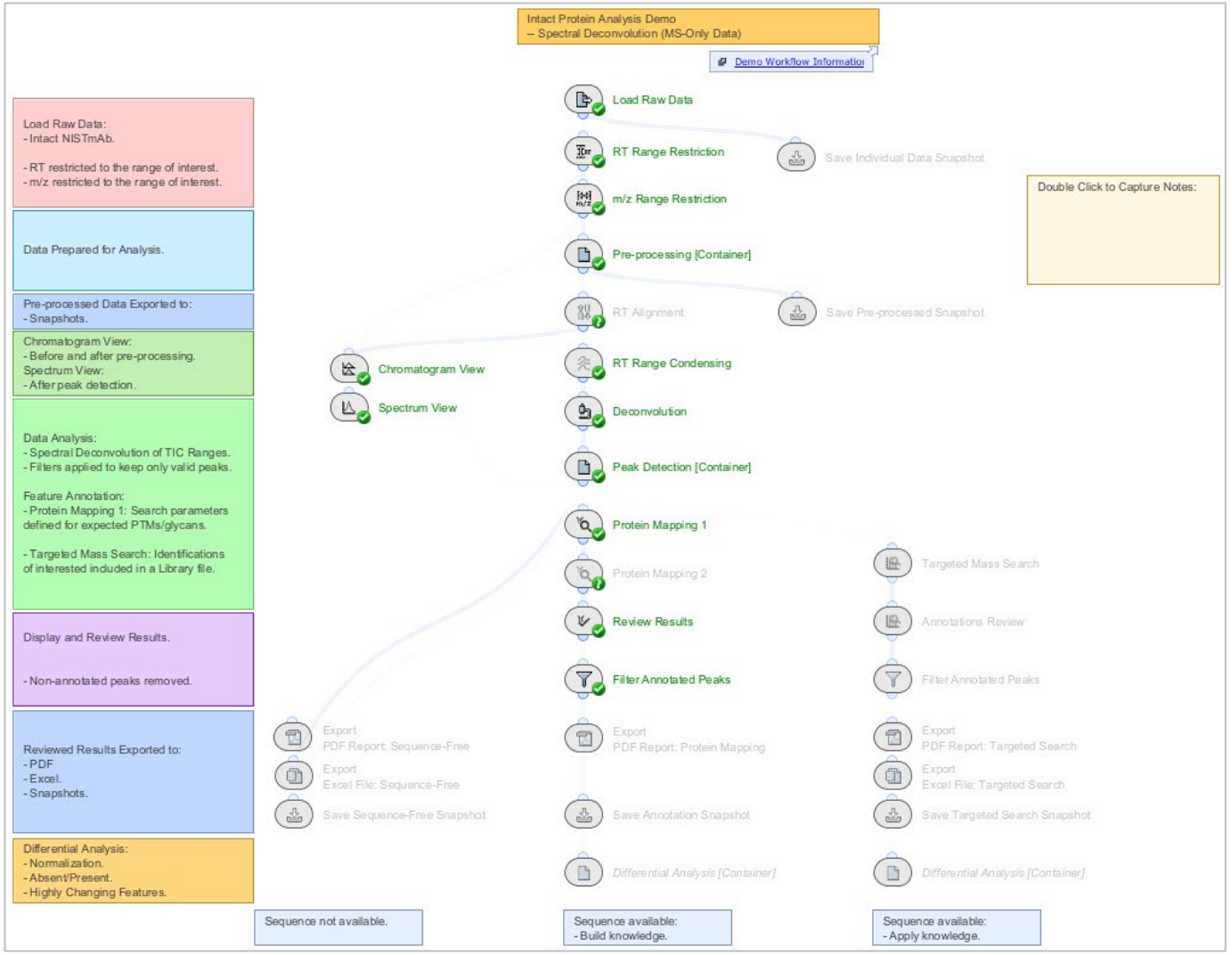
Spectral Deconvolution with MS Data

Demo Workflow Information

B1

Overview and Application: Intact_SpectralDeconvolution_MS_Demo

- This workflow uses data from an intact biotherapeutic molecule.
- This workflow uses the Total Ion Chromatogram (TIC) to select the RT ranges for deconvolution.
- The search parameters in the *Protein Mapping* activity node are optimized to identify the intact (**Fully Connected**) molecule, as well as all other possible combinations with **2 Additional Chains**.
- For more information about how to use Biologics Explorer software, refer to the *Biologics Explorer Quick Guide* and *Intact Protein Template Workflow Guidelines*.



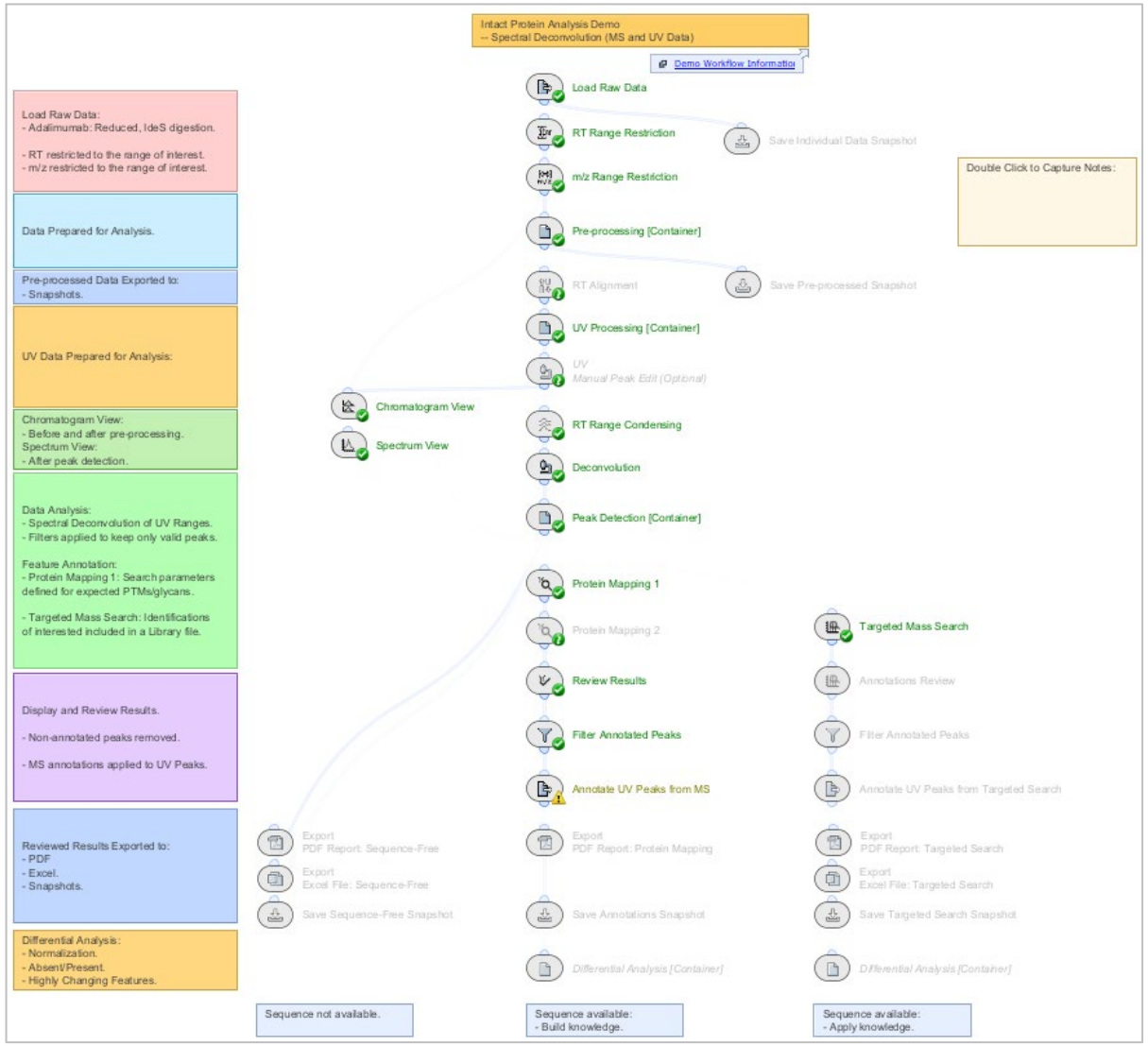
Spectral Deconvolution with MS+UV Data

Demo Workflow Information

B2

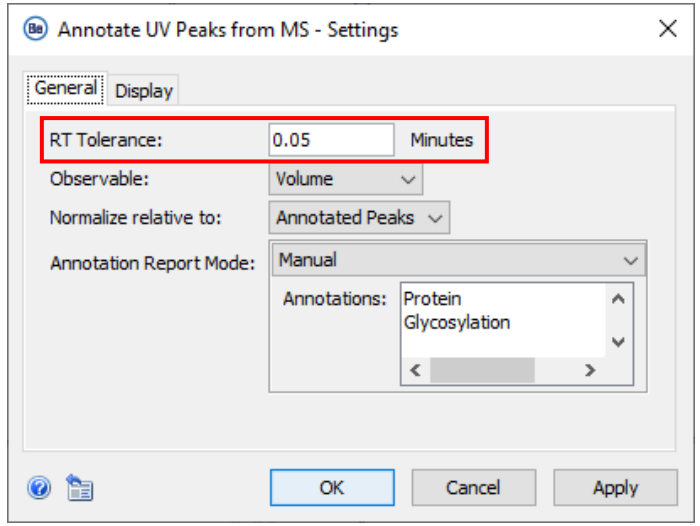
Overview and Application: Intact_SpectralDeconvolution_MS+UV_Demo

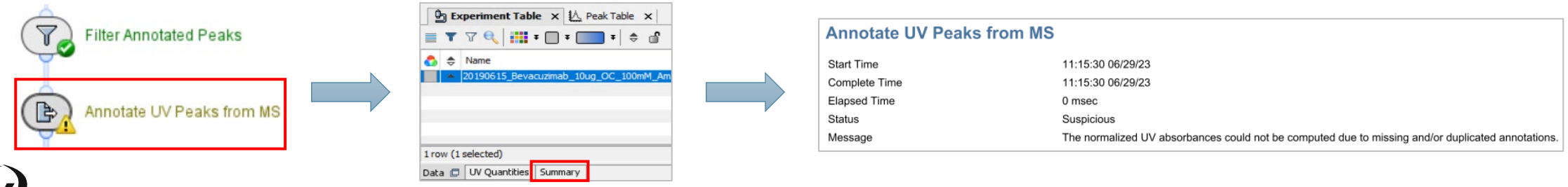
- This workflow uses data from a reduced biotherapeutic molecule after IdeS digestion.
- This workflow uses the UV trace to select the RT ranges for deconvolution.
- The search parameters in the *Protein Mapping* activity node are optimized to identify the **Fully Reduced** subunits (LC, Fd', Fc/2).
- For more information about how to use Biologics Explorer software, refer to the *Biologics Explorer Quick Guide* and *Intact Protein Template Workflow Guidelines*.



Annotate UV Peaks from MS

- This activity node uses MS peak information to annotate the peaks in the **UV Chromatogram**.
  - A related peak must elute in the specified **RT Tolerance**.
- The UV absorbance is used to calculate the relative ratio of the UV peaks.
  - A protein sequence is required to normalize UV absorbance values.
  - If the UV normalization factor cannot be calculated, then the activity node shows a **yellow warning**.
    - UV normalization is not available with *TR Deconvolution*.
    - UV normalization is not applicable for *Targeted Mass Search* because this activity node does not contain the protein sequence.





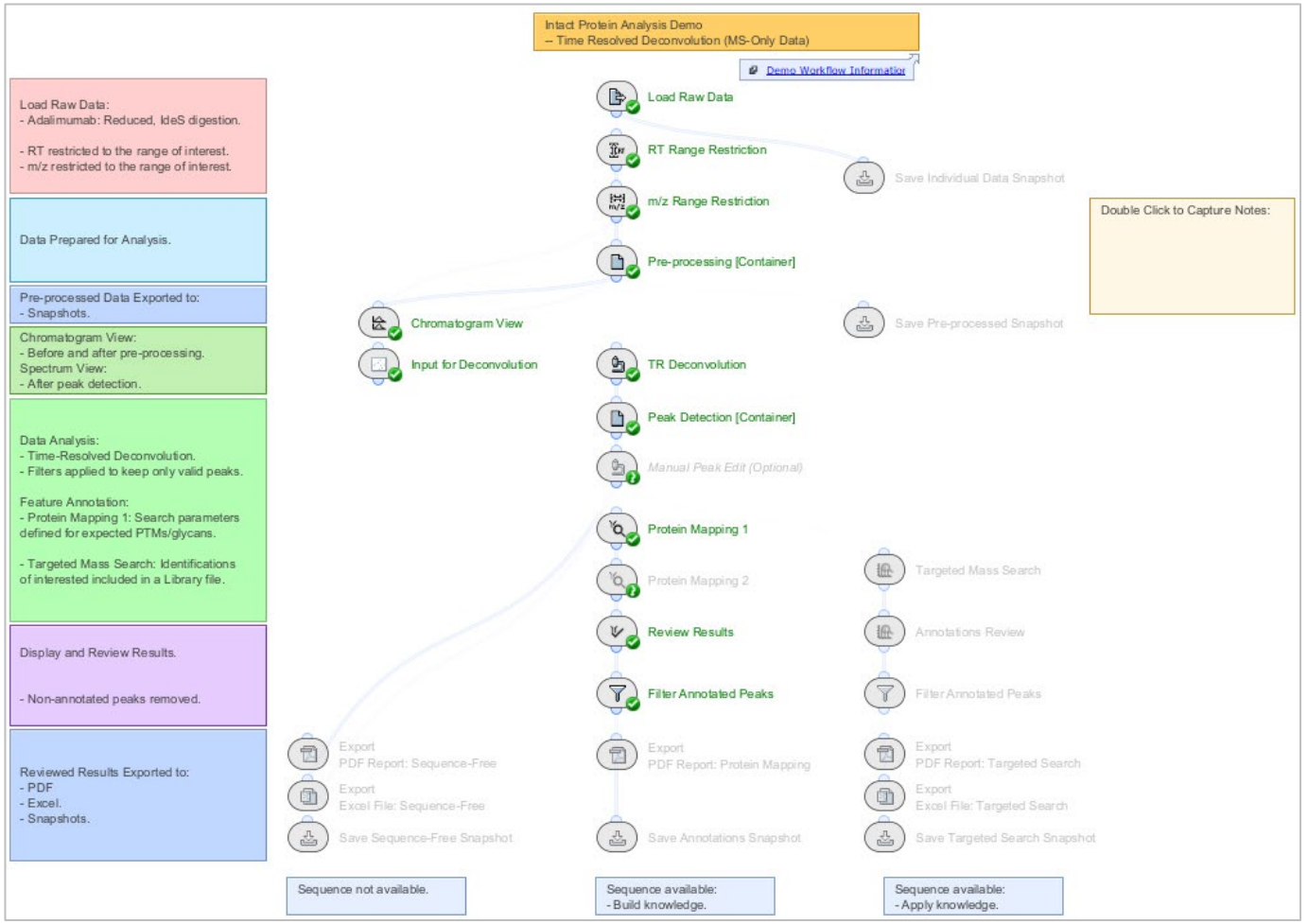
TR Deconvolution with MS-Only Data

Demo Workflow Information

B3

Overview and Application: Intact_TRDeconvolution_MS_Demo

- This workflow uses data from a reduced biotherapeutic molecule after IdeS digestion.
- Each RT scan is deconvoluted create an ion map of the deconvoluted proteins.
- The search parameters in the *Protein Mapping* activity node are optimized to identify the **Fully Reduced** subunits (LC, Fd', Fc/2).
- For more information about how to use Biologics Explorer software, refer to the *Biologics Explorer Quick Guide* and *Intact Protein Template Workflow Guidelines*.



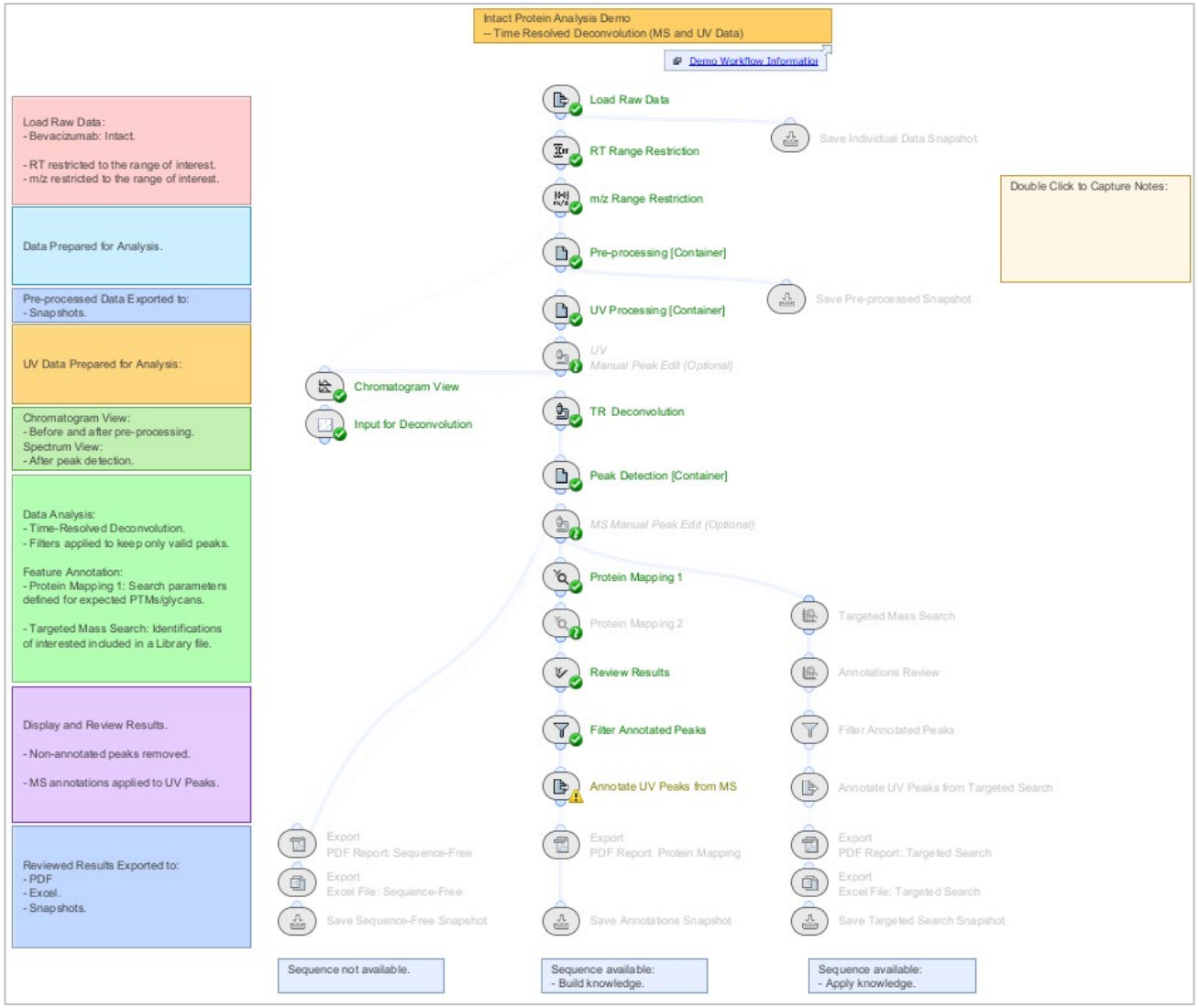
TR Deconvolution with MS+UV Data

Demo Workflow Information

B4

Overview and Application: Intact_TRDeconvolution_MS+UV_Demo

- This workflow uses data from an intact biotherapeutic molecule.
- Each RT scan is deconvoluted create an ion map of the deconvoluted proteins.
- The search parameters in the *Protein Mapping* activity node are optimized to identify the intact (**Fully Connected**) molecule.
- For more information about how to use Biologics Explorer software, refer to the *Biologics Explorer Quick Guide* and *Intact Protein Template Workflow Guidelines*.

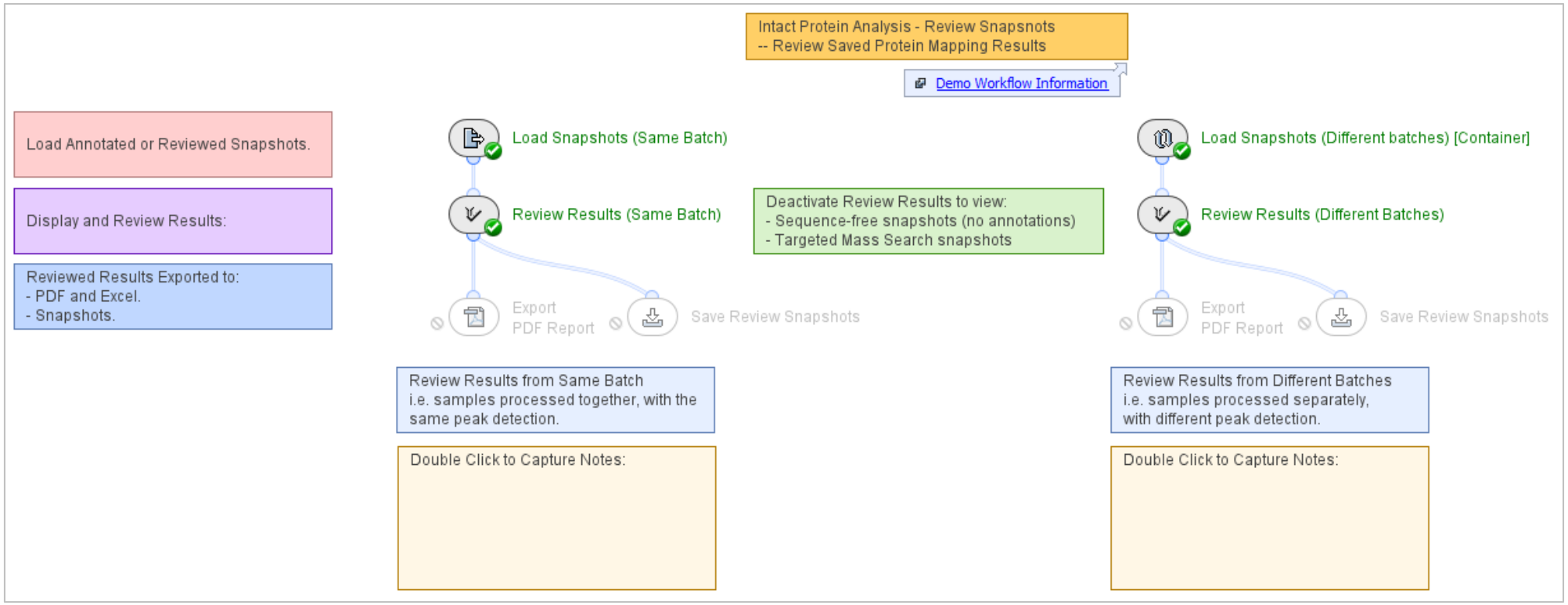


Review Snapshots

Demo Workflow Information

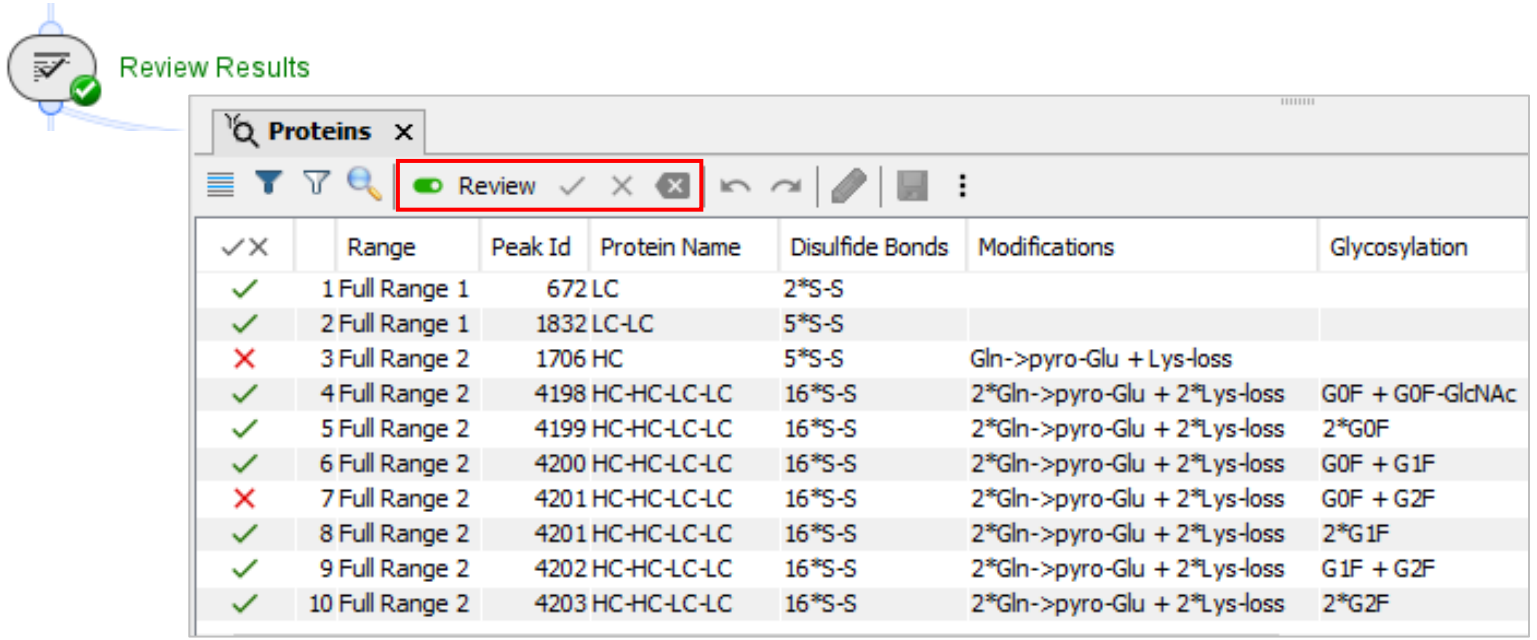
B5

Overview and Application: IntactProtein_ReviewSnapshots_Demo



- This workflow uses saved Snapshots to show results that have protein annotations.
- For more information about how to use Biologics Explorer software, refer to the *Biologics Explorer Quick Guide* and *Intact Protein Template Workflow Guidelines*.

Review Saved Results



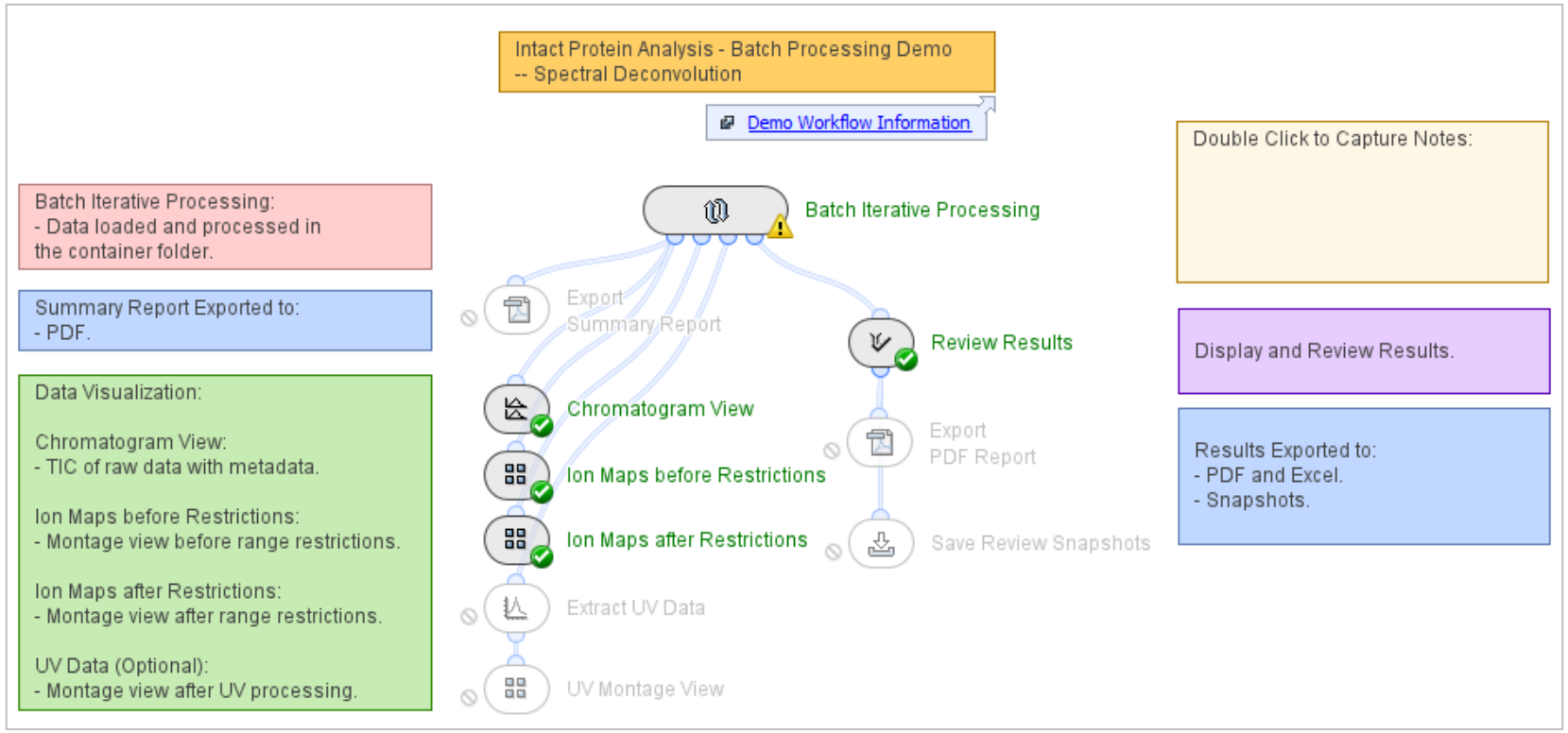
✓✗		Range	Peak Id	Protein Name	Disulfide Bonds	Modifications	Glycosylation
✓	1	Full Range 1	672	LC	2*S-S		
✓	2	Full Range 1	1832	LC-LC	5*S-S		
✗	3	Full Range 2	1706	HC	5*S-S	Gln->pyro-Glu + Lys-loss	
✓	4	Full Range 2	4198	HC-HC-LC-LC	16*S-S	2*Gln->pyro-Glu + 2*Lys-loss	G0F + G0F-GlcNAc
✓	5	Full Range 2	4199	HC-HC-LC-LC	16*S-S	2*Gln->pyro-Glu + 2*Lys-loss	2*G0F
✓	6	Full Range 2	4200	HC-HC-LC-LC	16*S-S	2*Gln->pyro-Glu + 2*Lys-loss	G0F + G1F
✗	7	Full Range 2	4201	HC-HC-LC-LC	16*S-S	2*Gln->pyro-Glu + 2*Lys-loss	G0F + G2F
✓	8	Full Range 2	4201	HC-HC-LC-LC	16*S-S	2*Gln->pyro-Glu + 2*Lys-loss	2*G1F
✓	9	Full Range 2	4202	HC-HC-LC-LC	16*S-S	2*Gln->pyro-Glu + 2*Lys-loss	G1F + G2F
✓	10	Full Range 2	4203	HC-HC-LC-LC	16*S-S	2*Gln->pyro-Glu + 2*Lys-loss	2*G2F

- The *Review Results* activity node opens a copy of the previous analysis.
  - Any previously accepted or rejected proteins have the applicable entry in the **Flags** column.
  - Another stage of review is then possible.
  - The reviewed sbf files and a new report can be saved.

Intact Protein Batch Processing
Demo Workflow Information

B6

Overview and Application: IntactProtein_BatchProcessing_Demo



- This workflow uses sequence information from metadata that was imported into the workflow to analyze multiple samples independently.
- For more information about how to use Biologics Explorer software, refer to the *Biologics Explorer Quick Guide* and *Intact Protein Template Workflow Guidelines*.

How to Use the Batch Processing Workflow

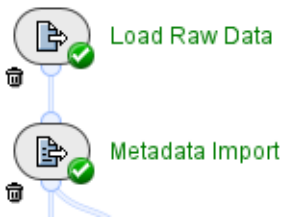
Use this workflow to analyze multiple samples independently.

- The samples do not require consistent chromatography.
- The samples can have different protein sequences.

All samples and their associated metadata are analyzed in the *Batch Iterative Processing* container.

- Intermediate results for each sample are not saved when the *Batch Iterative Processing* container is used for data analysis.
- To optimize workflow parameters, deactivate the **Trash** icon for activity nodes in the *Batch Iterative Processing* container, and use a single representative sample.
- To save memory when large numbers of samples are analyzed, activate the **Trash** icon for activity nodes in the *Batch Iterative Processing* container.

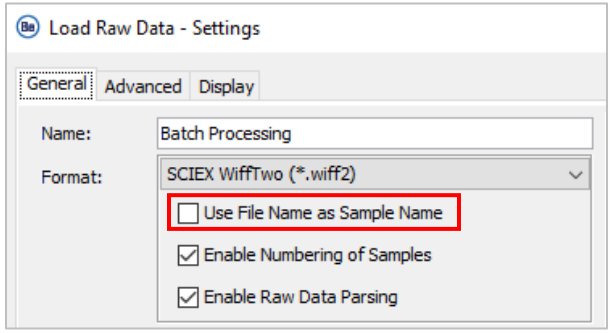
Load Raw Data: Experiment Names and Metadata

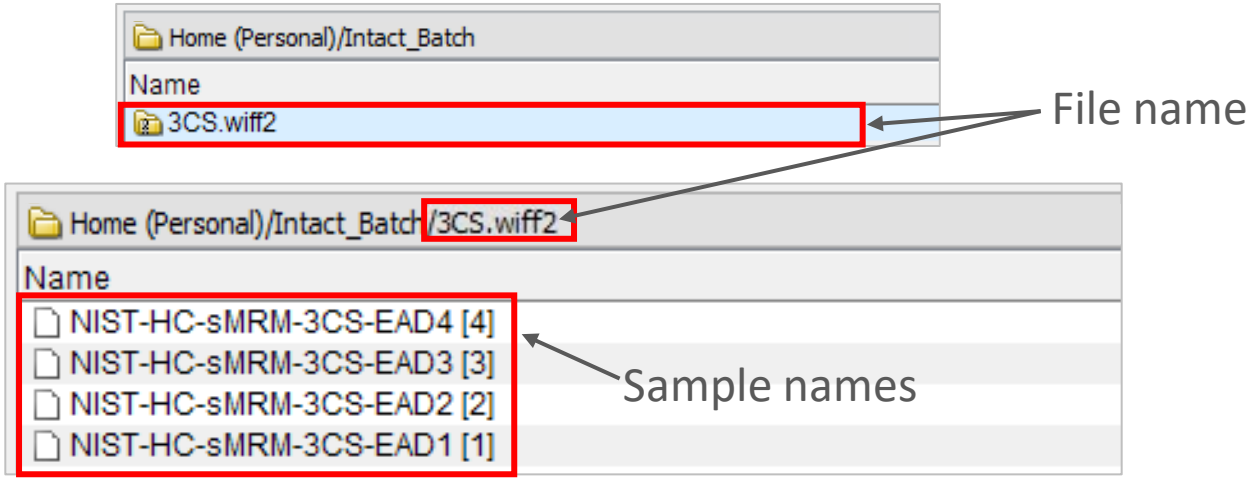


To analyze replicate samples from different acquisition files:

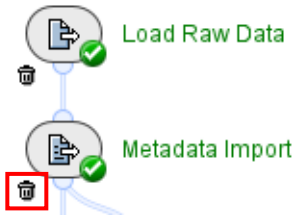
1. Select **Use File Name as Sample Name** in *Load Raw Data*.
2. Use the **File Name** (name of the wiff or wiff2 container file) in the **Experiment** column of the txt file for *Metadata Import*.

- To analyze multiple samples from a single acquisition file:
  1. <u>Do not</u> select **Use File Name as Sample Name** in *Load Raw Data*.
  2. Use the **Sample Name** in the **Experiment** column of the txt file for *Metadata Import*.





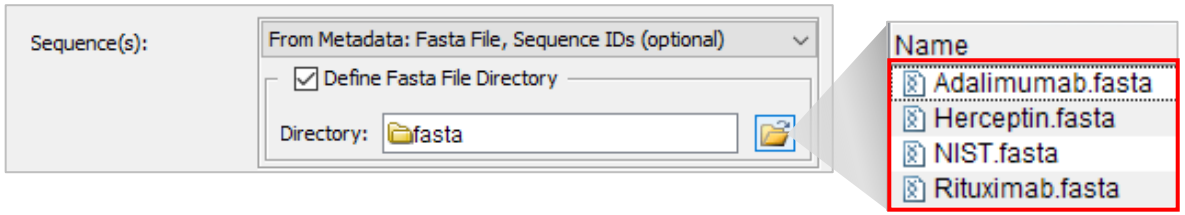
Metadata Import



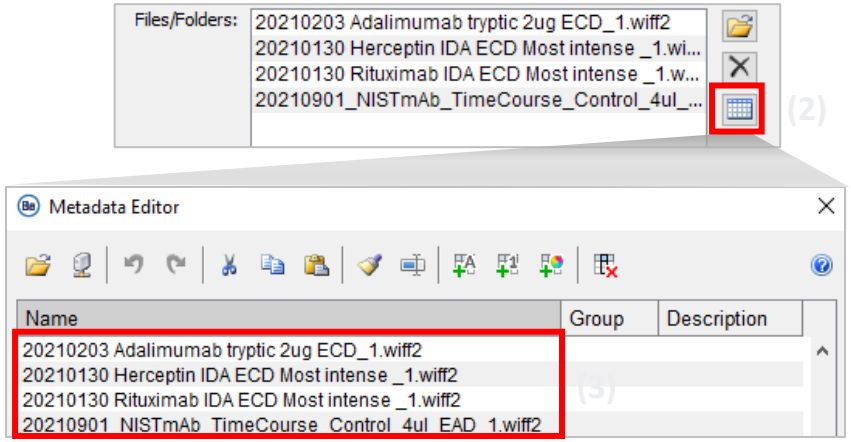
- To analyze multiple samples with the same sequence:
  - Deactivate the **Trash** icon, and then activate the **Bypass** icon for *Metadata Import*.
  - On the **Sequences** tab in *Protein Mapping*, select **From Text** or **From Fasta File**.
- To analyze multiple samples with different sequences:
  - Use *Metadata Import* to select the FASTA file (protein sequence) that will be used for identification in the *Protein Mapping* activity nodes.

- Upload a txt file with *Metadata Import* that links each sample to the correct FASTA file.
  - The name in the **Experiment** column must be the same as in the **Experiment** table in *Load Raw Data*.
  - The name in the **Fasta File** column must be the same as the name of the FASTA file that is in the specified **Fasta File Directory**, including the file extension (fasta or txt).

	A	B
1	Experiment	Fasta File
2	20210203 Adalimumab tryptic 2ug ECD_1	Adalimumab.fasta
3	20210130 Herceptin IDA ECD Most intense _1	Herceptin.fasta
4	20210130 Rituximab IDA ECD Most intense _1	Rituximab.fasta
5	20210901_NISTmAb_TimeCourse_Control_4ul_EAD_1	NIST.fasta



Metadata Import: Create the Metadata File



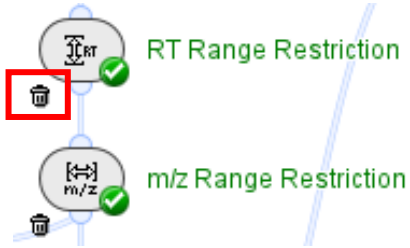
Note: Any metadata added in the *Load Raw Data* **Metadata Editor** table must be completed for all rows (all samples).

Experiment	Fasta File
20210203 Adalimumab tryptic 2ug ECD_1	Adalimumab.fasta
20210130 Herceptin IDA ECD Most intense _1	Herceptin.fasta
20210130 Rituximab IDA ECD Most intense _1	Rituximab.fasta
20210901_NISTmAb_TimeCourse_Control_4ul_EAD_1	NIST.fasta

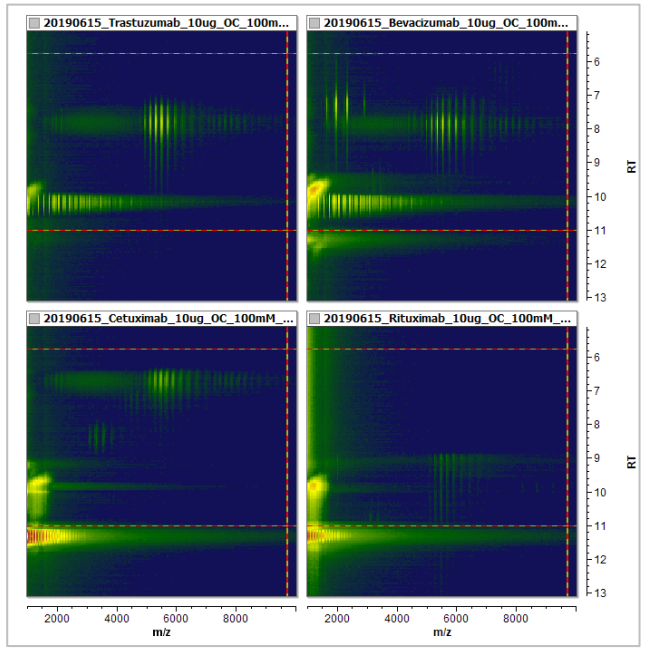
(4)

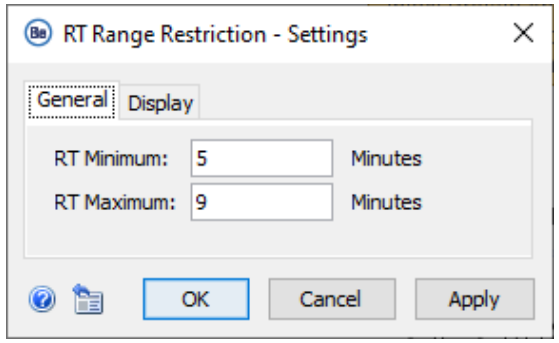
- To create the metadata file in Excel or Notepad:
  1. Select the samples for batch processing in *Load Raw Data*.
  2. Open the **Metadata Editor** table.
  3. Select all of the entries in the **Metadata Editor** table, and then select copy.
  4. Paste the entries into the **Experiment** column of the metadata txt file.
  5. Delete ".wiff" or ".wiff2" from the end of each name. (Tip: Use the Replace command in Excel or Notepad.)
  6. Type the applicable FASTA file name in each row in the **Fasta File** column.
  7. Save the file in txt format, and then upload the file in the *Metadata Import* activity node.

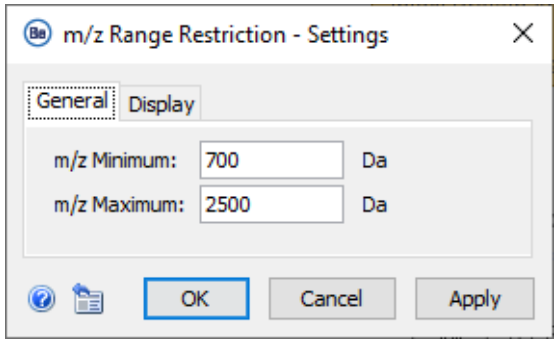
Restriction of RT and *m/z* Ranges



- To optimize settings and select applicable ranges, deactivate the **Trash** icon.
- To identify the RT ranges where there is meaningful data, open (double-click) *Load Raw Data* after the data is loaded.
  - Unless minor components are of interest, limit the ranges to the target protein.
  - Make sure that the selected ranges are wide enough to include all of the samples.
  - If the molecules require very different ranges, then activate the **Bypass** icon on the *Range Restriction* activity nodes

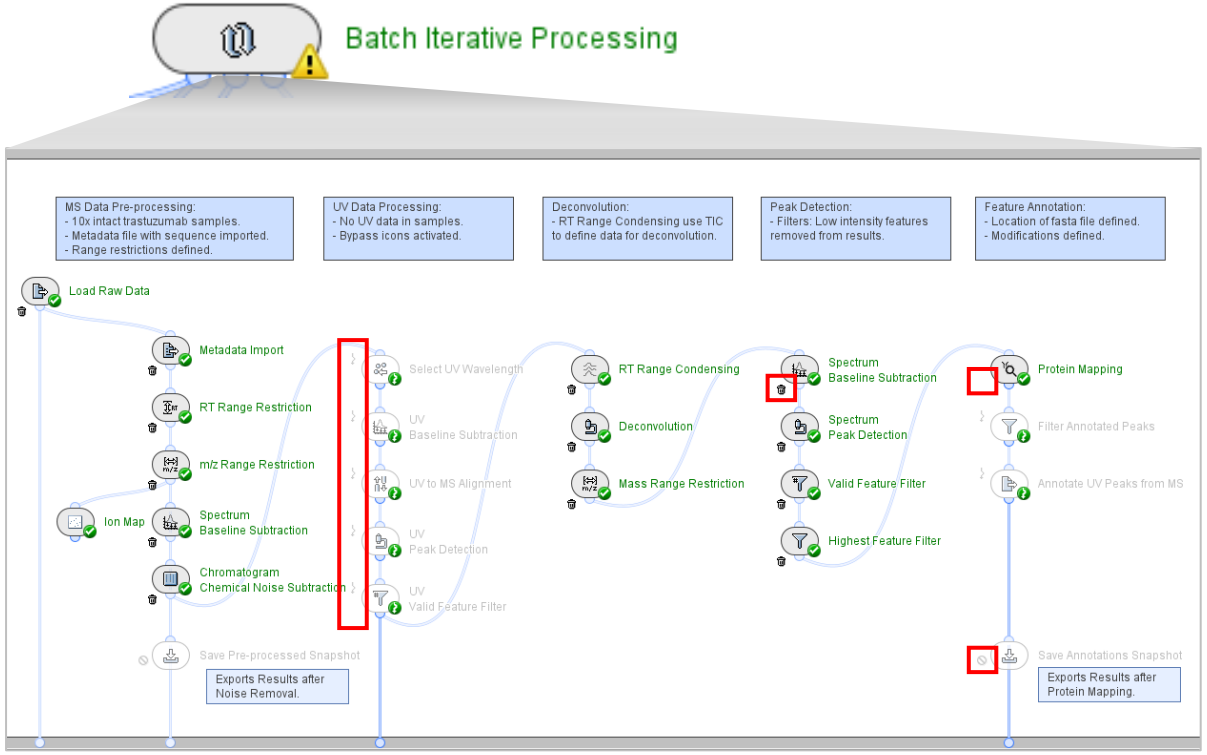






Batch Iterative Processing Container

- The *Batch Iterative Processing* container is not the same as other Biologics Explorer software containers.
  - Only intermediate results from the last sample to be processed can be opened from the activity nodes in the *Batch Iterative Processing* container.



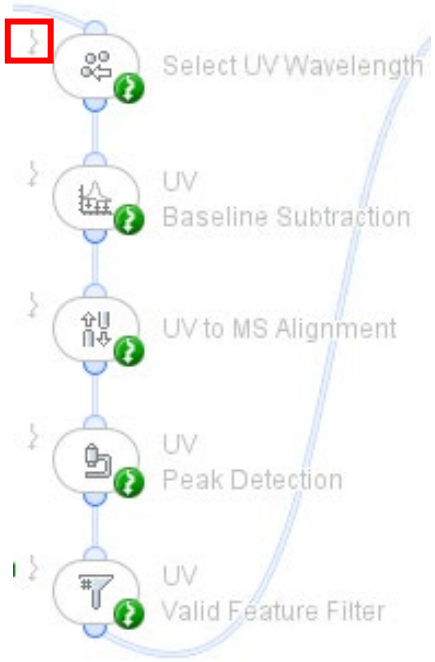
Note: If activity nodes in the container have the **Bypass** icon activated, then the container shows a yellow warning symbol.

- To open the intermediate results of an activity node, deactivate the **Trash** icon before the workflow is started.
- Do not activate the **Trash** icon for activities that are used in the PDF Report.

Activity nodes in the *Batch Iterative Processing* container do not have a **Run** or **Reset** icon.

- Activity nodes in the *Batch Iterative Processing* container cannot be run individually.
  - To use a *Save Snapshot* or *Export* activity node in the *Batch Iterative Processing* container, deactivate the **Block** icon before the workflow is started.

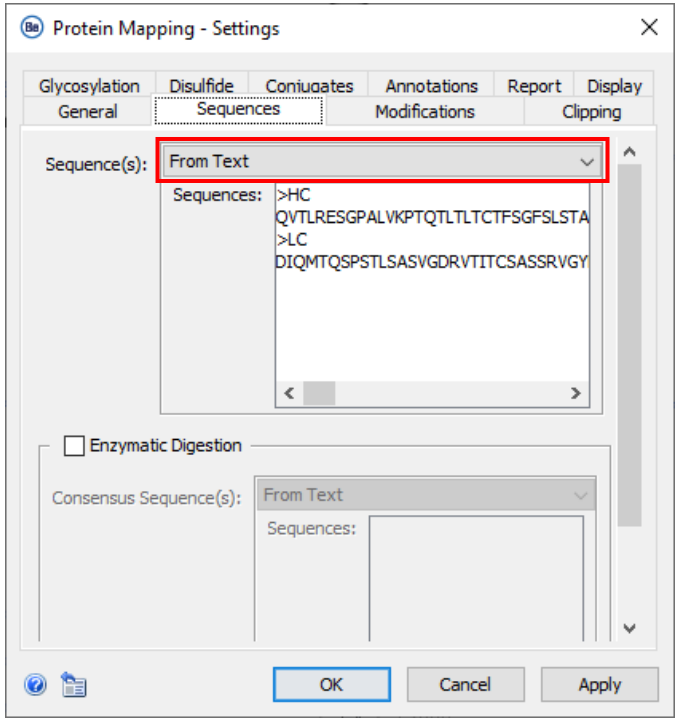
UV Data Processing



- All activity nodes for UV data have the **Bypass** icon activated in the template workflow.
- To use UV peaks in the data to identify the RT ranges for deconvolution:
  1. Deactivate the **Bypass** icon and then activate the **Trash** icon (to save memory) for all of the activity nodes with the prefix UV and *Annotate UV Peaks from MS*.

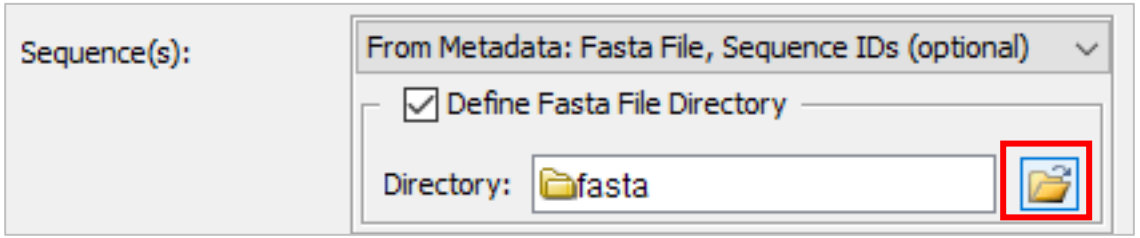
  - *Annotate UV Peaks from MS* uses MS peak identifications to add annotations to the peaks in the UV chromatogram. The peak must elute in the specified **RT Tolerance**.

Protein Mapping: Sequences



Sequences tab:

- **Sequence(s)**:
  - If all samples have the same sequence, then select **From Text** and type the sequence, or **From Fasta File** and select the applicable file.
  - If different samples require different sequences, then select **From Metadata: Fasta File, Sequence IDs (optional)**, and then browse to the location of the folder that contains all of the applicable FASTA files.



- For more information, refer to the next page: *Review Results*: Protein Name in FASTA Files.

Review Results: Protein Name in FASTA Files

- If the protein sequence names <u>are unique</u> across the FASTA files used for identification:

*Bevacizumab.fasta - Notepad

File Edit Format View Help

```
>HC(Bevacizumab)
EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY
>LC(Bevacizumab)
DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS
```

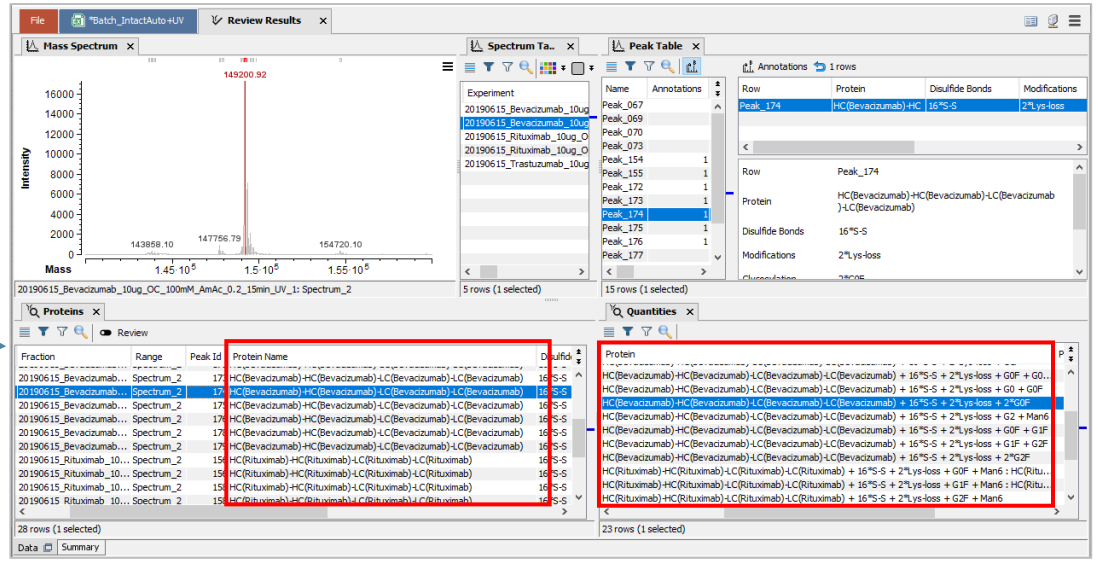
*Rituximab.fasta - Notepad

File Edit Format View Help

```
>HC(Rituximab)
QVQLQQPGAELVKPGASVKMSCKASGYTFTSYNMHWVKQTPGRGLEWIGAIYPGNGDTSY
>LC(Rituximab)
QIVLSQSPAILSASPGEKVTMTCRASSSVSYIHWFQQKPGSSPKPWIYATSNLASGVPVR
```

  - The protein sequence name in *Review Results* is the same as the name in the FASTA file.

Note: If the names are too long, then some table columns in the PDF report might be missing.



- If the protein sequence names <u>are not unique</u> across the FASTA files used for identification:

*Bevacizumab.fasta - Notepad

File Edit Format View Help

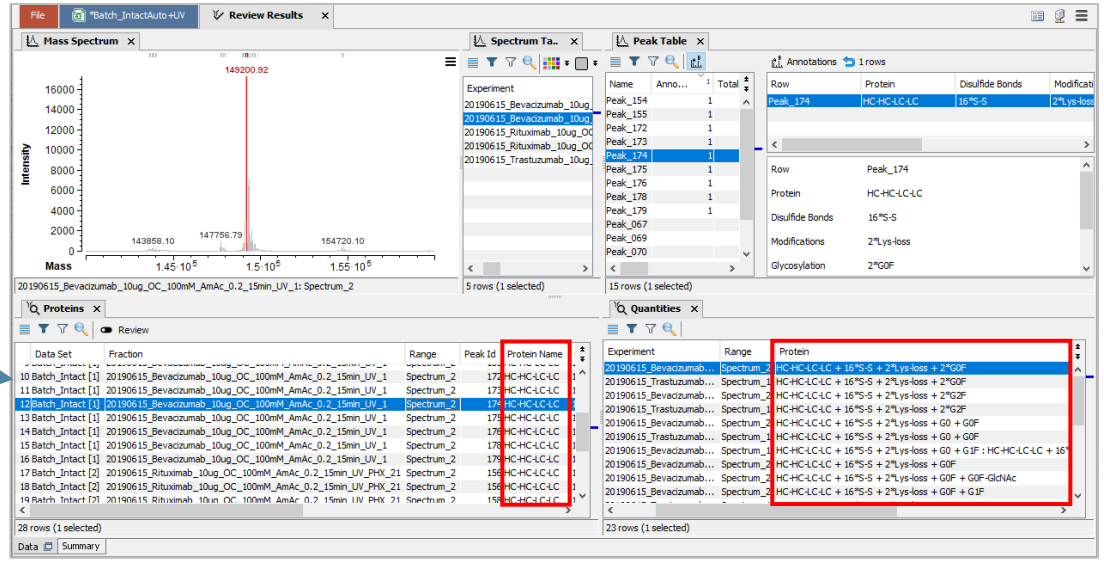
```
>HC
EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY
>LC
DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS
```

*Rituximab.fasta - Notepad

File Edit Format View Help

```
>HC
QVQLQQPGAELVKPGASVKMSCKASGYTFTSYNMHWVKQTPGRGLEWIGAIYPGNGDTSY
>LC
QIVLSQSPAILSASPGEKVTMTCRASSSVSYIHWFQQKPGSSPKPWIYATSNLASGVPVR
```

  - The protein sequence name in *Review Results* includes the FASTA file name.



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