

Bruderer Data Settings

- Open reports\MSstats_annotations.sky
- New document – Settings > Default – No (New document again to clear Audit Log)
- Save as Bruderer.sky
- Peptide Filter – Length 6 to 55, Exclude 0

Peptide Settings

Digestion Prediction **Filter** Library Modifications Quantification

Min length: 6 Max length: 55

Exclude N-terminal AAs: Q

Exclude potential ragged ends

Exclude peptides containing:

- Cys
- Met
- His
- NXT/NXS
- RP/KP

Edit list...

Auto-select all matching peptides

OK Cancel

- Peptide Modifications – Carb (C), Oxy (M), Acetyl (N-term), Water Loss, Ammonia Loss

Peptide Settings

Digestion Prediction Filter Library **Modifications** Quantification

Structural modifications:

- Carbamidomethyl (C)
- Oxidation (M)
- Acetyl (N-term)
- Water Loss (D, E, S, T)
- Ammonia Loss (K, N, Q, R)

Edit list...

Max variable mods: 3 Max losses: 1

Isotope label type: heavy

Isotope modifications:

Edit list...

Internal standard type: heavy

OK Cancel

- Transition Filter – Pre charge: 1-7, Ion charge: 1-6, Ion types: y, b, p (1 through last)

Transition Settings

Prediction Filter Library Instrument Full-Scan Ion Mobility

Peptides

Precursor charges: 2, 3, 4, 5, 6, 7 Ion charges: 1, 2, 3, 4, 5, 6 Ion types: y, b, p

Product ion selection

From: ion 1 To: last ion

Special ions:

- N-terminal to Proline
- C-terminal to Glu or Asp
- iTRAQ-114
- iTRAQ-115
- iTRAQ-116
- iTRAQ-117

Edit List...

Precursor m/z exclusion window: m/z

Auto-select all matching transitions

OK Cancel

- Transition Library – Pick 6 with 6 min, charges and types

Transition Settings ✕

Prediction Filter **Library** Instrument Full-Scan Ion Mobility

Ion match tolerance:
 m/z

If a library spectrum is available, pick its most intense ions

Pick:
 product ions
 minimum product ions

From filtered ion charges and types
 From filtered ion charges and types plus filtered product ions
 From filtered product ions

- Transition Instrument – 300 to 1800 m/z

Transition Settings ×

Prediction Filter Library **Instrument** Full-Scan Ion Mobility

Min m/z: m/z Max m/z: m/z

Dynamic min product m/z

Method match tolerance m/z:
 m/z

Firmware transition limit: Firmware inclusion limit:

Min time: min Max time: min

Triggered chromatogram acquisition

- Transition Full-Scan – DIA Centroided 15 ppm (import scans/mzML file) – Predicted RT

Edit Isolation Scheme [X]

Name:

Use results data isolation targets

Isolation width: Deconvolution:

Prespecified isolation windows

Measurement

Start	End	Margin
400	430	1
428	459	1
457	483	1
481	506	1
504	531	1
529	554	1
552	576	1

Deconvolution: Specify Margin Specify CE Range

Windows per scan:

Transition Settings ✕

Prediction Filter Library Instrument Full-Scan Ion Mobility

MS1 filtering

Isotope peaks included: Precursor mass analyzer:
None

Peaks: Resolution:
 m/z

Isotope labeling enrichment:

MS/MS filtering

Acquisition method: Product mass analyzer:
DIA Centroided

Isolation scheme: Mass Accuracy:
Bruderer (19-variant) 15 ppm

Use high-selectivity extraction

Retention time filtering

Use only scans within 5 minutes of MS/MS IDs

Use only scans within 5 minutes of predicted RT

Include all matching scans

OK Cancel

- Import Assay Library “mcp.M114.044305-2-Profiling.csv” (330 lines of errors)

330 transitions contained errors. Skip these 330 transitions and import the rest?

Error	Line	Column
Precursor m/z 689.3396 does not match the closest possible value 681.3421 (delta = 7.9975), peptide MLGPEGGEGFVK. Che...	13,694	7
Precursor m/z 689.3396 does not match the closest possible value 681.3421 (delta = 7.9975), peptide MLGPEGGEGFVK. Che...	13,695	7
Precursor m/z 689.3396 does not match the closest possible value 681.3421 (delta = 7.9975), peptide MLGPEGGEGFVK. Che...	13,696	7
Precursor m/z 689.3396 does not match the closest possible value 681.3421 (delta = 7.9975), peptide MLGPEGGEGFVK. Che...	13,697	7
Precursor m/z 689.3396 does not match the closest possible value 681.3421 (delta = 7.9975), peptide MLGPEGGEGFVK. Che...	13,698	7
Precursor m/z 689.3396 does not match the closest possible value 681.3421 (delta = 7.9975), peptide MLGPEGGEGFVK. Che...	13,699	7
Precursor m/z 689.3396 does not match the closest possible value 681.3421 (delta = 7.9975), peptide MLGPEGGEGFVK. Che...	13,700	7
Precursor m/z 689.3396 does not match the closest possible value 681.3421 (delta = 7.9975), peptide MLGPEGGEGFVK. Che...	13,701	7
Precursor m/z 689.3396 does not match the closest possible value 681.3421 (delta = 7.9975), peptide MLGPEGGEGFVK. Che...	13,702	7
The precursor m/z 400.2242 of the peptide IHNFGLIQEK is outside the range covered by the DIA isolation scheme. Check the i...	14,815	7
The precursor m/z 400.2242 of the peptide IHNFGLIQEK is outside the range covered by the DIA isolation scheme. Check the i...	14,816	7
The precursor m/z 400.2242 of the peptide IHNFGLIQEK is outside the range covered by the DIA isolation scheme. Check the i...	14,817	7
The precursor m/z 400.2242 of the peptide IHNFGLIQEK is outside the range covered by the DIA isolation scheme. Check the i...	14,818	7

Show / hide line text

OK Cancel

- Explain precursors between 400 and 401 and adjust Transition Settings
- Import Assay Library (89 lines of errors – explain Oxi (M) and Acetyl (N-term) and move on)

89 transitions contained errors. Skip these 89 transitions and import the rest?

Error	Line	Column
Precursor m/z 689.3396 does not match the closest possible value 681.3421 (delta = 7.9975), peptide MLGPEGGEGFVK. Che...	13,694	7
Precursor m/z 689.3396 does not match the closest possible value 681.3421 (delta = 7.9975), peptide MLGPEGGEGFVK. Che...	13,695	7
Precursor m/z 689.3396 does not match the closest possible value 681.3421 (delta = 7.9975), peptide MLGPEGGEGFVK. Che...	13,696	7
Precursor m/z 689.3396 does not match the closest possible value 681.3421 (delta = 7.9975), peptide MLGPEGGEGFVK. Che...	13,697	7
Precursor m/z 689.3396 does not match the closest possible value 681.3421 (delta = 7.9975), peptide MLGPEGGEGFVK. Che...	13,698	7
Precursor m/z 689.3396 does not match the closest possible value 681.3421 (delta = 7.9975), peptide MLGPEGGEGFVK. Che...	13,699	7
Precursor m/z 689.3396 does not match the closest possible value 681.3421 (delta = 7.9975), peptide MLGPEGGEGFVK. Che...	13,700	7
Precursor m/z 689.3396 does not match the closest possible value 681.3421 (delta = 7.9975), peptide MLGPEGGEGFVK. Che...	13,701	7
Precursor m/z 689.3396 does not match the closest possible value 681.3421 (delta = 7.9975), peptide MLGPEGGEGFVK. Che...	13,702	7
Product m/z value 973.5102 in peptide LQGEFQLR has no matching product ion.	21,895	13
Product m/z value 513.278 in peptide WSLQK has no matching product ion.	24,863	13
Product m/z value 385.2194 in peptide WSLQK has no matching product ion.	24,864	13
Product m/z value 750.8883 in peptide VLENVEHYQELK has no matching product ion.	33,865	13

Show / hide line text

OK Cancel

- Separate transition list “Biognosys_iRT.csv”

Choose iRT Standard Peptides ✕

Existing iRT standard:
Biognosys-10 (iRT-C18) ▼

Protein name:
▼

Separate transition list:

- Refine > Advanced – min trans 6, auto-select trans

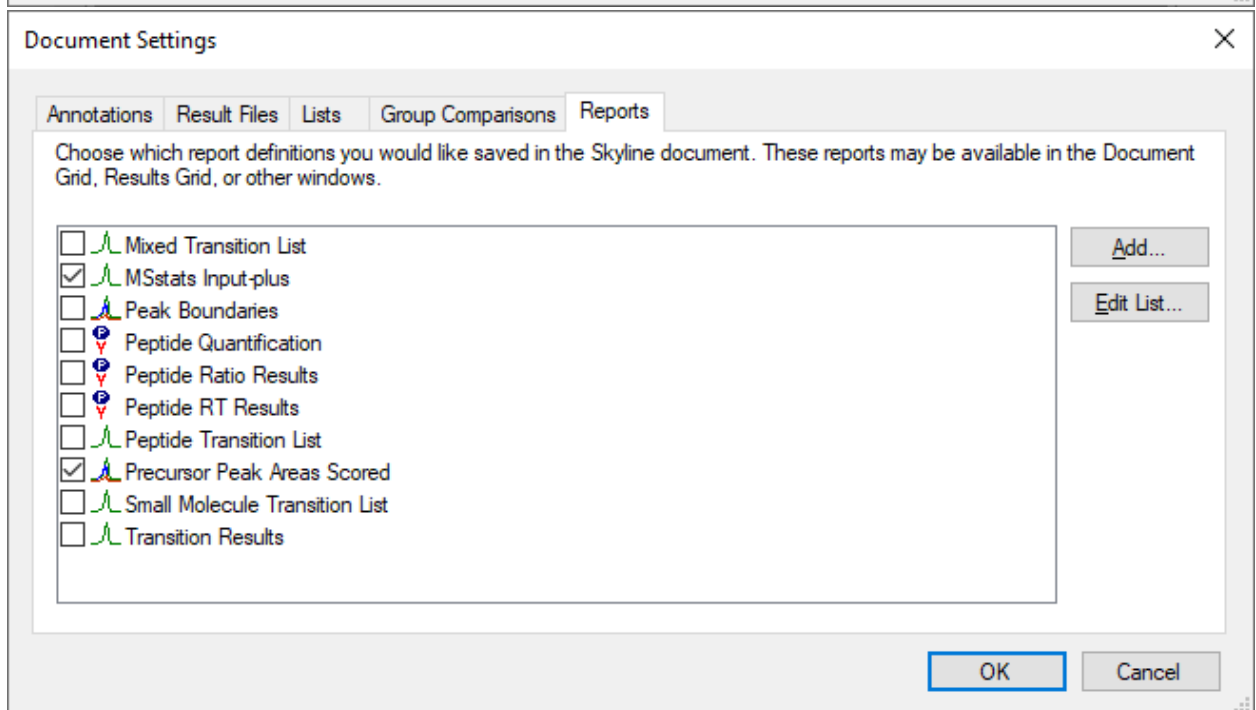
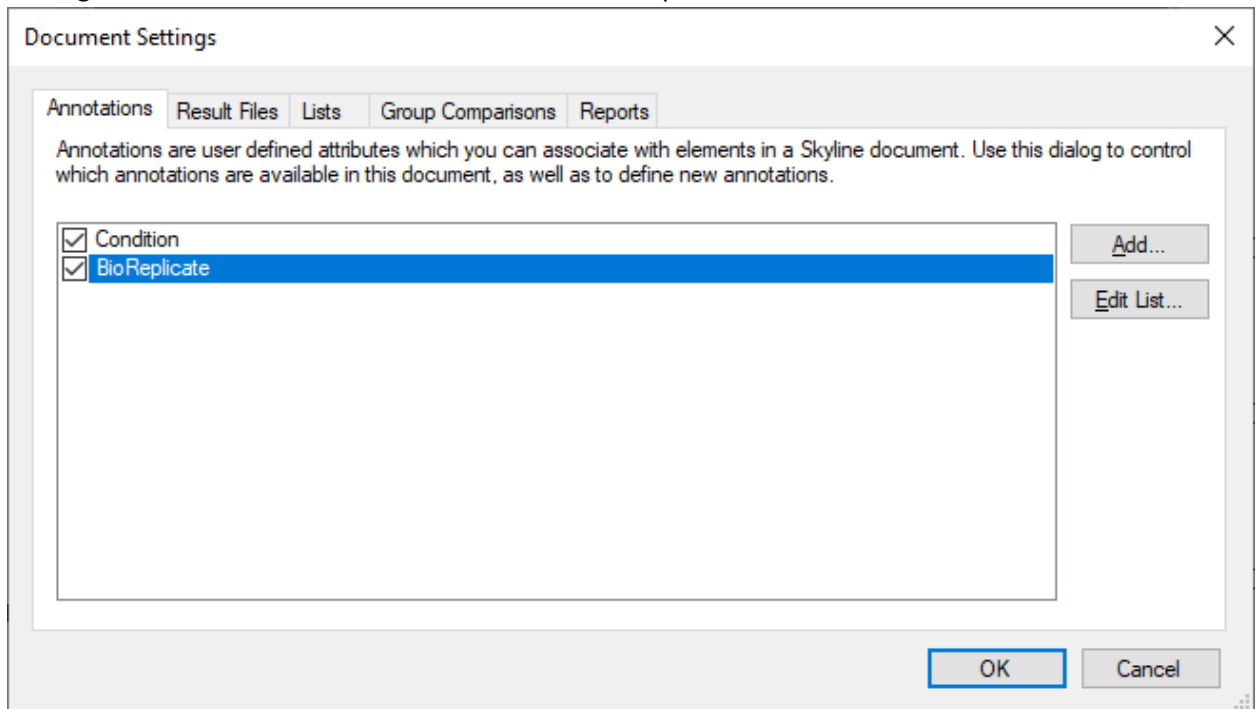
The screenshot shows a dialog box titled "Refine" with a close button (X) in the top right corner. It has two tabs: "Document" and "Group Comparison", with "Group Comparison" selected. The dialog contains several settings:

- Min peptides per protein:** An empty text input field.
- Remove repeated peptides:** An unchecked checkbox.
- Remove duplicate peptides:** An unchecked checkbox.
- Remove peptides missing library match:** An unchecked checkbox.
- Min transitions per precursor:** A text input field containing the number "6".
- Remove label type:** A dropdown menu with a downward arrow and an unchecked checkbox labeled "Add".
- Auto-select all:** A group of three checkboxes:
 - Peptides:** Unchecked.
 - Precursors:** Unchecked.
 - Transitions:** Checked.

At the bottom right of the dialog are "OK" and "Cancel" buttons.

1/3,866 prot 1/25,311 pep 1/29,238 prec 1/175,395 tran

- Settings > Document – add MSstats annotations and reports



- Save and exit