



Skyline Tutorial Webinar #10

Working with Modifications in Skyline

With
Brendan MacLean (Principal Developer)

Agenda

- ▶ Welcome from the Skyline team!
- ▶ **Working with Modifications in Skyline**
- ▶ Introduction and overview with Brendan MacLean
 - ▶ Setting up and importing PTMs and neutral losses
 - ▶ Adding peptides with isotope labeling
 - ▶ Importing large assay libraries
- ▶ Tutorial

- ▶ Audience Q&A – submit questions to Google Form:
<https://skyline.gs.washington.edu/labkey/qa4skyline.url>



Overview

- ▶ **Control Center – Peptide Settings – Modifications tab**
 - ▶ Defining modifications and neutral losses
- ▶ **Modified sequence formats**
- ▶ **Skyline guesses modifications**
 - ▶ Import > Peptide Search
 - ▶ View > Spectral Libraries
 - ▶ Insert > Peptides
 - ▶ Insert > Transition List
- ▶ **Tips and tricks**
 - ▶ Filtering for modifications
 - ▶ Targeting charged losses
 - ▶ Accepting modified peptide states
 - ▶ Modified sequences in reports



Peptide Settings - Modifications

The screenshot shows the 'Peptide Settings' dialog box with the 'Modifications' tab selected. The dialog has a title bar with a close button (X) and a tabbed interface with 'Digestion', 'Prediction', 'Filter', 'Library', and 'Modifications' tabs. The 'Modifications' tab contains several sections: 'Structural modifications' with a list of checkboxes (Carbamidomethyl (C) checked, Oxidation (M), Phospho (ST), Phospho (Y)) and an 'Edit list...' button; 'Max variable mods' (3) and 'Max neutral losses' (1) input fields; 'Isotope label type' (heavy) dropdown; 'Isotope modifications' with a list of checkboxes (Label: 13C(6)15N(2) (C-term K), Label: 13C(6)15N(4) (C-term R), Label: 15N) and an 'Edit list...' button; and 'Internal standard type' (heavy) dropdown. At the bottom are 'OK' and 'Cancel' buttons. Two red arrows point from the right side of the image to the 'Edit list...' buttons for structural and isotope modifications.

Peptide Settings

Digestion Prediction Filter Library Modifications

Structural modifications:

- Carbamidomethyl (C)
- Oxidation (M)
- Phospho (ST)
- Phospho (Y)

Edit list...

Max variable mods: 3

Max neutral losses: 1

Isotope label type: heavy

Isotope modifications:

- Label: 13C(6)15N(2) (C-term K)
- Label: 13C(6)15N(4) (C-term R)
- Label: 15N

Edit list...

Internal standard type: heavy

OK Cancel

Structural
Modifications

Isotope
Modifications

Modified Sequence Formats

- ▶ Skyline Internal – delta-mass to 1 decimal place
 - ▶ PEPC[+57.0]IDER
 - ▶ Spectral libraries
 - ▶ iRT calculators
 - ▶ Optimization libraries
 - ▶ Ion mobility libraries
- ▶ Skyline Display – delta-mass short
 - ▶ PEPC[+57]IDER
 - ▶ P[+1]E[+1]P[+1]C[+58]I[+1]D[+1]E[+1]R[+4]
- ▶ Explicit isotope labeling
 - ▶ PEPC[+57]IDER{+10}



Modified Sequence Formats (continued)

- ▶ **Named**

- ▶ PEPC[Carbomidomethyl (C)]IDER[Label:|3C(6)|5N(4) (C-term R)]

- ▶ **Short names (SCIEX)**

- ▶ PEPC[CAM]IDER

- ▶ PES[Pho]T[Pho]IC[CAM]IDER

- ▶ **Unimod**

- ▶ PEPC(unimod: 4)IDER

- ▶ PES(unimod: 2|)T(unimod: 2|)IC(unimod: 4)IDER



Tutorial



Learn More

- ▶ **Webinar #11: Panorama AutoQC and Panorama Public**
 - ▶ Tuesday, October 20th
 - ▶ **Webinar #12: Isotope Labeled Standards in Skyline**
 - ▶ Tuesday, December 1st
 - ▶ **Workshop in Tokyo, October 13**
 - ▶ **Workshop in Puerto Vallarta, November 8 & 9**
 - ▶ **Weeklong Course at IIT-Bombay**
 - ▶ December 10-14
 - ▶ **Weeklong Courses 2016**
 - ▶ ETH, Zurich – February 8-12
 - ▶ Buck Institute, San Francisco – March 7-11
 - ▶ Northeastern, Boston – May 2-6
 - ▶ University of Washington, Seattle – July 18-22
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Questions?

- ▶ Ask any questions you have on modifications in Skyline at the following form:

<http://tinyurl.com/QA4Skyline>

- ▶ Take the post-webinar survey:

<http://tinyurl.com/Survey4Webinar>





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This ends this Skyline Tutorial Webinar.

Please give us feedback on the webinar at the following survey:

<http://tinyurl.com/Survey4Webinar>

A recording of today's meeting will be available shortly at the Skyline website.

We look forward to seeing you at a future Skyline Tutorial Webinar.

