

# Recent Advances in Skyline: Further Improvements in Small Molecule Targets and Ion Mobility

Brian Pratt<sup>1</sup>, J. Will Thompson<sup>2</sup>, Erin Baker<sup>3</sup>, Michael J. MacCoss<sup>1</sup>, Brendan MacLean<sup>1</sup>

<sup>1</sup>University of Washington, Seattle WA, <sup>2</sup>Duke University School of Medicine, Durham NC, <sup>3</sup>Pacific Northwest National Laboratory, Richland WA



<https://skyline.ms>

## Overview:

The Skyline Targeted Proteomics Environment has distinguished itself as a useful and reliable tool for chromatography-based quantitative proteomics. From its initial focus on selected reaction monitoring (SRM) to its current support for full-scan methods including MS1 filtering, parallel reaction monitoring (PRM) and data independent acquisition (DIA – including the approach popularized as SWATH), Skyline has continuously evolved to meet the changing needs of proteomics researchers, as well as adding support for generalized small molecules.

Recent advances in this freely available and open source tool include spectral libraries for small molecules, collisional cross section values for ion mobility prediction, ion mobility libraries built from training data sets, and enhanced support for automated workflows and external tools integration.

## Introduction:

### Skyline History

The Skyline project began in 2008 as an effort to create a completely new instrument-vendor-neutral software tool designed specifically for targeted proteomics. Most other tools in this area had been vendor-specific and adapted from small molecule quantitative software.

With the generous support of many mass spectrometer vendors and with the help of the large and active Skyline user community, Skyline has undergone continuous development and become a sophisticated tool that directly interacts with equipment from all major mass spectrometer vendors for rapid and convenient targeted proteomics method creation and refinement.

### History of Extending Skyline for Other “Omics”

As proteomics researchers have increasingly embraced “multi-omics” in their work, Skyline has evolved to work with non-proteomic molecules. Early adopters led the way by artfully constructing peptide modifications that resulted in the chemical formula of interest [1,2]. The Skyline team responded by enabling the direct specification of molecules by ion formula, though due to Skyline’s proteomic roots there was a built in assumption of positive-only ionization. Today, Skyline supports a full range of ionization adducts (e.g. “[M+Na]”, “[2M+Hac-H]”) and can track molecules using standard identifiers such as InChiKey, CAS, HMDB, etc.

### Targeted Mass Spectrometry Basics

The process typically begins with a large list of likely precursors and fragments of interest (the “targets”) which Skyline then helps iteratively refine to produce an optimal method or transition list. The predictable nature of peptide ionization, fragmentation and chromatography allows Skyline to provide excellent automation for creation of initial methods from peptide search results.

Figure 1: Targeted mass spectrometry method refinement cycle.

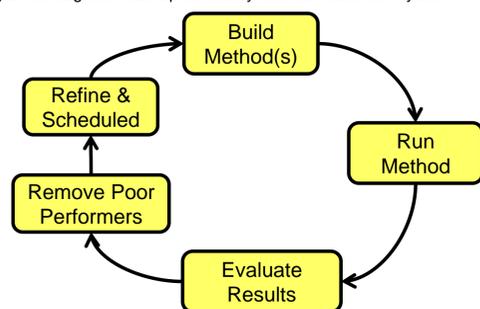
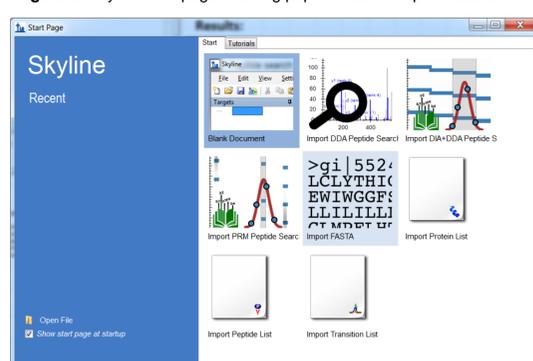


Figure 2: Skyline start page showing peptide search import wizards.



Generalized “omics” (metabolomics, lipidomics, glycomics, etc.) presents a broader and less predictable range of molecules, and Skyline’s ability to interact with external expert systems and workflows is critical in its ability to be part of a targeted multi-omics mass spectrometry solution. Skyline has great flexibility in dealing with a diverse range of target description formats, and provides excellent support for user-developed external tools for directly generating targets and spectral libraries.

### Advances in Ion Mobility

Ion mobility separation (IMS) technology provides an additional degree of separation that is useful for reducing peak interference. This can be especially helpful in lipidomics and glycomics, where the mass range of many precursor targets is relatively narrow. IMS technology continues to mature and we see increasing use of collisional cross section (CCS) as a molecular property that can be used to predict ion mobility. Skyline can both use and derive IMS library data, including CCS, when dealing with suitably equipped mass spectrometers.

## Methods:

### Automation and Openness in Skyline

Skyline is generously licensed open source software that anyone can integrate with, extend, or contribute to.

Perhaps more importantly, though, Skyline presents a rich set of extensibility and integration features that do not require changing the underlying source code to create new functionality. Enabling domain experts to extend Skyline without necessarily being expert software developers is a key factor in Skyline’s ability to serve a broader targeted mass spec community beyond proteomics.

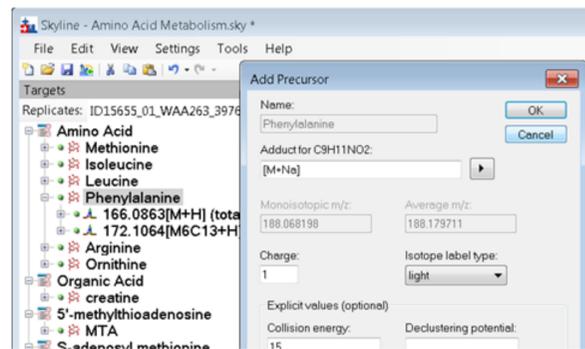
Users can integrate and customize Skyline by means of its command line interface or the External Tools API. This includes the ability to interact directly with the Skyline user interface as well as the “headless” operation required by automated workflows.

### Improved Small Molecule Model in Skyline

Skyline’s initial implementation of small molecule support, while welcome and highly useful, was hastily implemented in the interest of serving immediate user needs. There was no distinction made between a molecule and a precursor ion – the molecule was described by its ion formula.

A more complete implementation is now in place, which more closely agrees with the peptide molecule/ion model in Skyline. A small molecule is now described by its neutral chemical formula (and optionally, by a standard identifier such as CAS, InChiKey, HMDB etc.) to which one or more precursor adducts can be applied. This enables more of the automated handling of targets that is native to Skyline for peptide precursor ions, including charge state filtering, quantitative analysis when multiple ionizing adducts are present, and the use of spectral libraries.

Figure 4: Editing a small molecule target list in Skyline.



## Results:

Given the diversity in molecules of interest in the world of multi-omics, making it easy for the community to add expert domain knowledge to Skyline’s existing capabilities is a key development activity of the Skyline team.

The ISAS Lipidomics Group’s LipidCreator[3] is a prime example of using Skyline’s external tools integration capability to create and import small molecule targets and spectral libraries.

Figure 5: LipidCreator, an independently developed lipidomics front end for Skyline.

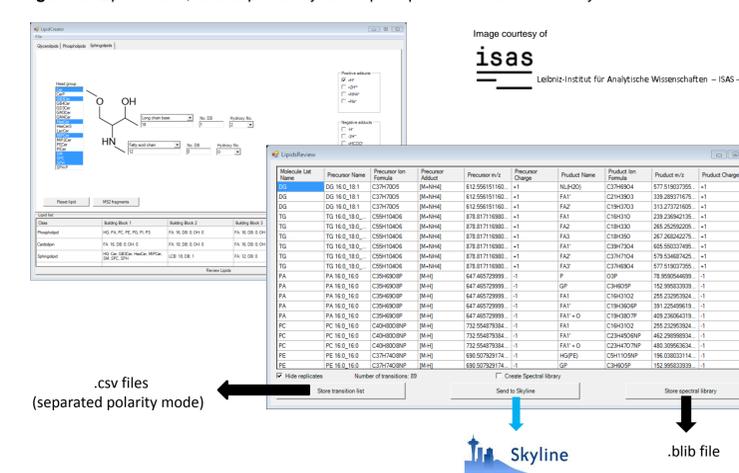


Figure 6: Targeted metabolomics method development in Skyline.

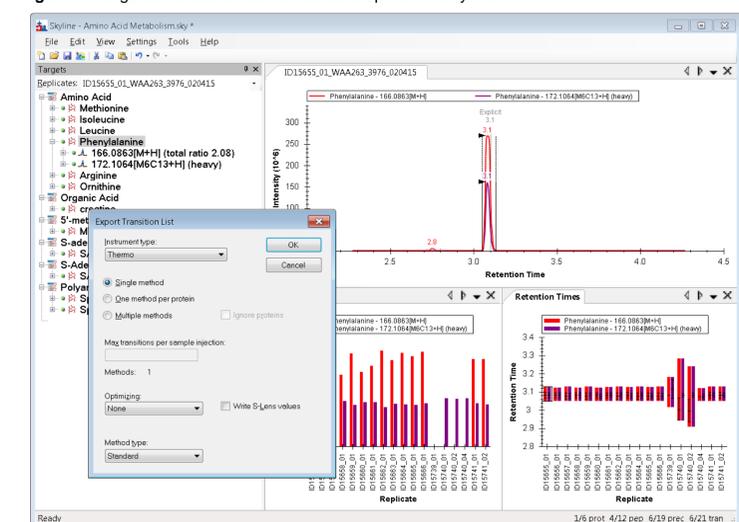


Figure 7: A small molecule spectral library, imported to Skyline from Shimadzu .MLB format.

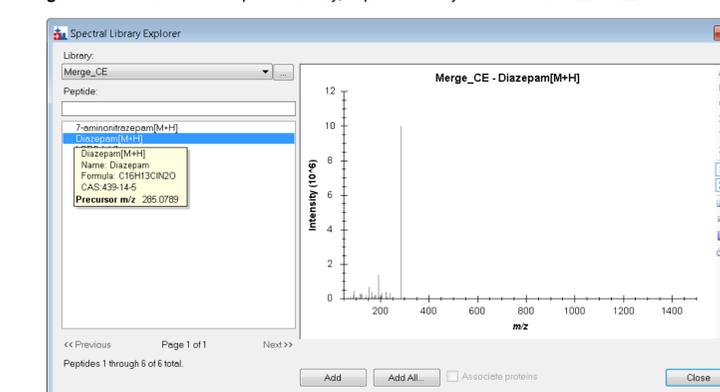
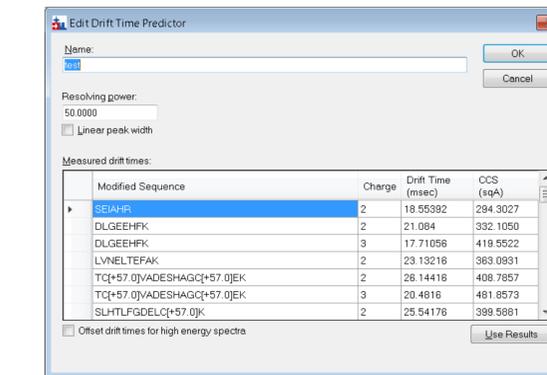


Figure 8: Collisional Cross Section values derived by Skyline from results of a training data set.



## Conclusions:

- Skyline’s support for generalized small molecules continues to grow.
- Skyline’s ability to integrate with automated workflows and its rich API for external tool building allow small molecule domain experts to implement a targeted mass spectrometry environment with minimal effort.
- Skyline supports the use of Collisional Cross Section as a molecular property for predicting ion mobility, leading to reduced peak interference.

Future work includes:

- Integration with more small molecule search tools and spectral library formats.
- Less protein oriented user interface language when used for small molecules.

References:  
[1] Hooftagle, A., Skyline Users Group Meeting at ASMS 2013 [2] Liu, S. et al, Proteomics 14: 169–80.  
[3] B. Peng, D. Kopczyński, R. Ahrens, LipidCreator: a tool to strengthen high-throughput targeted lipidomics. In preparation.

This work was supported in part by a grant from the National Institutes of Health (R01 GM103551).