



Tutorial Webinar #16

Small Molecule Research with Skyline

With

Brendan MacLean (Principal Developer, Skyline)
Brian Pratt (Lead Small Mol. Developer, Skyline)
Will Thompson (Asst. Director, Duke Proteomics and Metabolomics)

Agenda

- Welcome from the Skyline team!
- Small Molecule Research with Skyline
 - Introduction with Brendan MacLean
 - Skyline small molecule overview with Brian Pratt
 - Tutorial with Will Thompson
- Audience Q&A – submit questions to Google Form:

<https://skyline.ms/QA4Skyline.url>

It Began with Targeted Proteomics

- ASMS 2010 – Oral presentation... First question:
 - “When will Skyline support small molecules?”
- ASMS 2013 – Skyline User Group Meeting
 - Andy Hoofnagle presents lipidomics method in Skyline
 - Using amino acid modification trick
- Summer 2014 – Intern Max Horowitz-Gelb
 - Implemented charged loss fragments as custom ions
- March, 2015 – Skyline 3.1 released with first small molecule support
- September, 2016 – Skyline R01 (round 2) funds small molecules

Proteomics Roots

- “Peptide” instead of “molecule”
- “Protein” as the logical grouping for molecules
- “Charge” as a positive number (protonation)
- Isotopic labels as peptide modifications
- Molecular fragmentation calculated on the fly
- This is ideal for proteomics
- Not so great for generalized small molecules
- But as our users recognized it’s just a layer atop a body of code applicable to both regimes

Initial steps, circa 2015

- Introduced ability to define molecules with chemical formulas, and group them in molecule lists – but UI was largely still in terms of peptides and proteins
- Added support for negative ion mode data, but charge was still stored as a number rather than as an adduct description
- Ionization and isotopic labels had to be embedded in the molecule's chemical formula - so in reality we replaced “peptide” with an ion rather than a molecule
- Thus no concept of a neutral molecule with multiple ionizations and labels - a big handicap in quantification
- No spectral library support for small molecules

Current state of Skyline for small molecules

- Full support for adducts – de facto standards, and user-defined
- Isotopic labels are part of the adduct description
- This means Skyline finally understands the idea of a molecule with multiple ionizations and labels, enabling all of Skyline's quant capabilities
- Small molecule spectral libraries are supported, including NIST and various search pipelines, and can also be generated by Skyline
- We are currently adding fragment annotation capability to libraries (something not needed for peptides, where we can derive the fragmentation)
- UI is increasingly context aware, less peptide-centric as appropriate
- Ongoing ion mobility improvements, including support for Bruker TIMS data

Future directions for small molecule support in Skyline

- Chromatographic retention time is a major remaining built-in assumption in Skyline, and support for direct injection experiments is a priority
- Increasingly context-aware UI, and other usability improvements like smarter copy+paste from Excel to Skyline's targets window (bypassing the Edit|Insert|TransitionList window by understanding more column headers)
- Additional search pipeline result imports
- Populating transition lists from lists of InChiKey (or CAS, or HMDB etc) values
- Molecule and fragment visualization?
- What are your priorities? Take the survey at <https://goo.gl/forms/eo6iOXbwqGG6zs8r1> or email me at bspratt@proteinms.net

Learn More

- Webinar #17: TBD (coming soon)
- Weeklong Courses 2018
 - Buck Institute, Novato, CA – April 2-6
 - Northeastern University, Boston – April 30 – May 11
 - ETH, Zurich – July 2-6
 - University of Washington, Seattle – July 30 – August 3
- Workshops and Conferences 2017
 - Workshop at MSACL, Palm Springs – January 20&21
 - Workshop pre-Lorne, Melbourne – January 29-31
 - Workshop US HUPO, Minneapolis – March 10&11
 - Short Course at ASMS, San Diego – June 2&3
 - Skyline User Group Meeting at ASMS, San Diego – June 3

Listings updated in **Join Us** section of Skyline homepage:

<https://skyline.ms/Skyline.url>

Questions?

- Ask any questions at the following form:

<https://skyline.ms/QA4Skyline.url>

- Take the post-webinar survey:

<https://skyline.ms/survey4webinar.url>



Skyline Tutorial Webinar #16

This ends this Skyline Tutorial Webinar.

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

<https://skyline.ms/survey4webinar.url>

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.