



Skyline

Targeted Mass Spec Environment

10th Birthday!

Brendan MacLean



Panorama

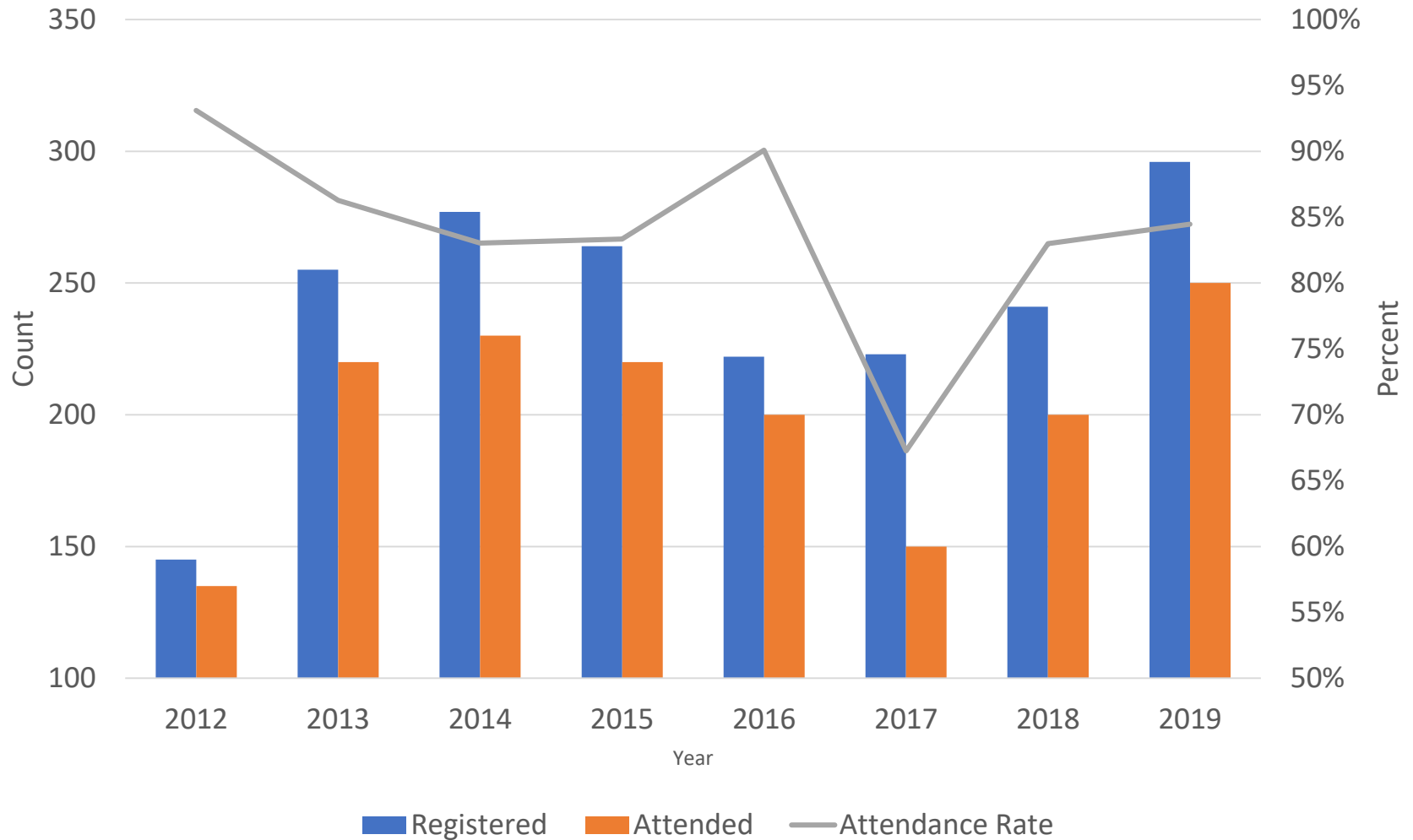
6 years

Skyline Poster at ASMS 2009

- ▶ **Skyline 0.5-preview** release, free, and open-source
- ▶ 10-year anniversary this year!! (2019)
- ▶ Skyline hoodies in Atlanta!
- ▶ Still free
- ▶ Still open-source
- ▶ **Skyline 19.1 - SOON**



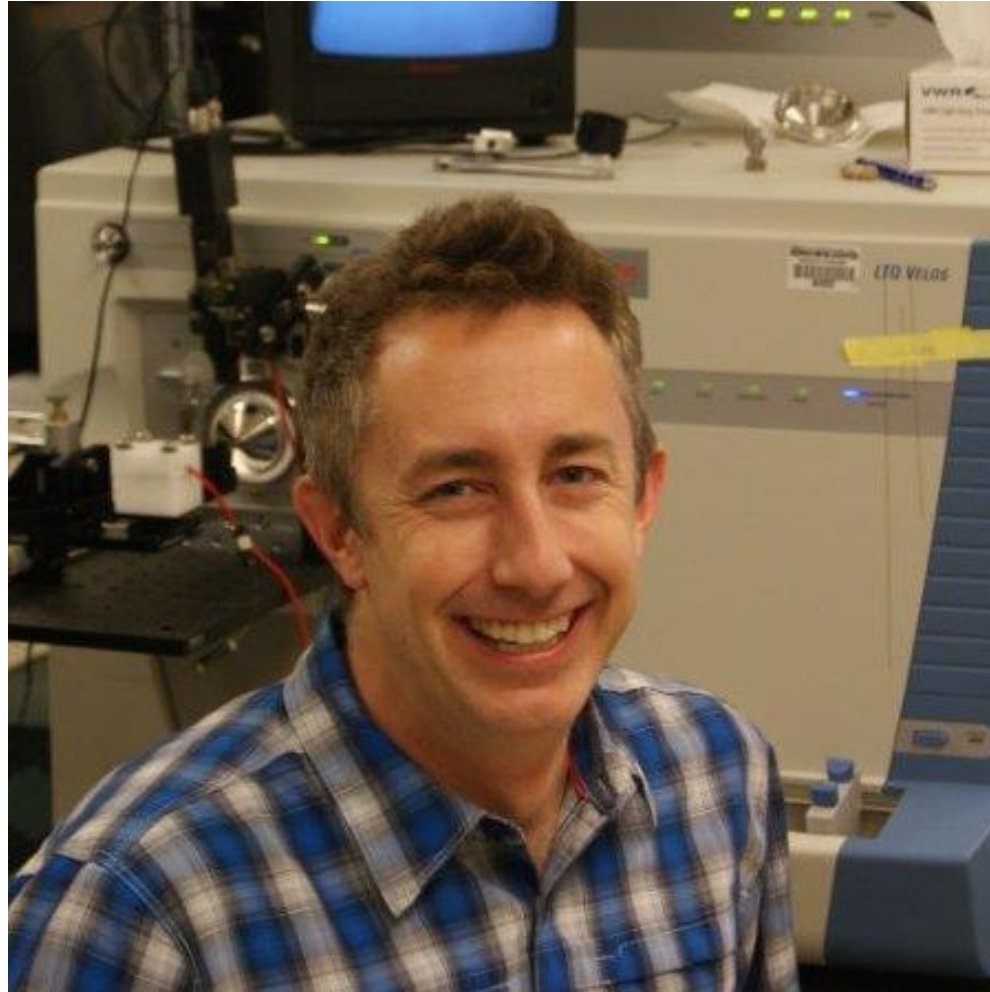
Meeting Attendance by Year



Thanks!



Mike MacCoss



Skyline Team

▶ Nick Shulman



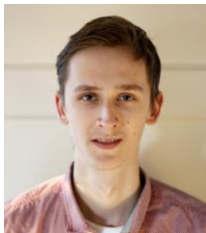
▶ Brian Pratt



▶ Matt Chambers



▶ Tobi Rohde



▶ Nat Brace



▶ Vagisha Sharma



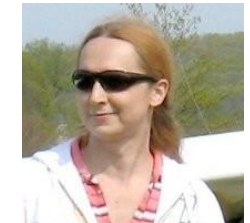
▶ Brian Connolly



▶ Josh Aldrich

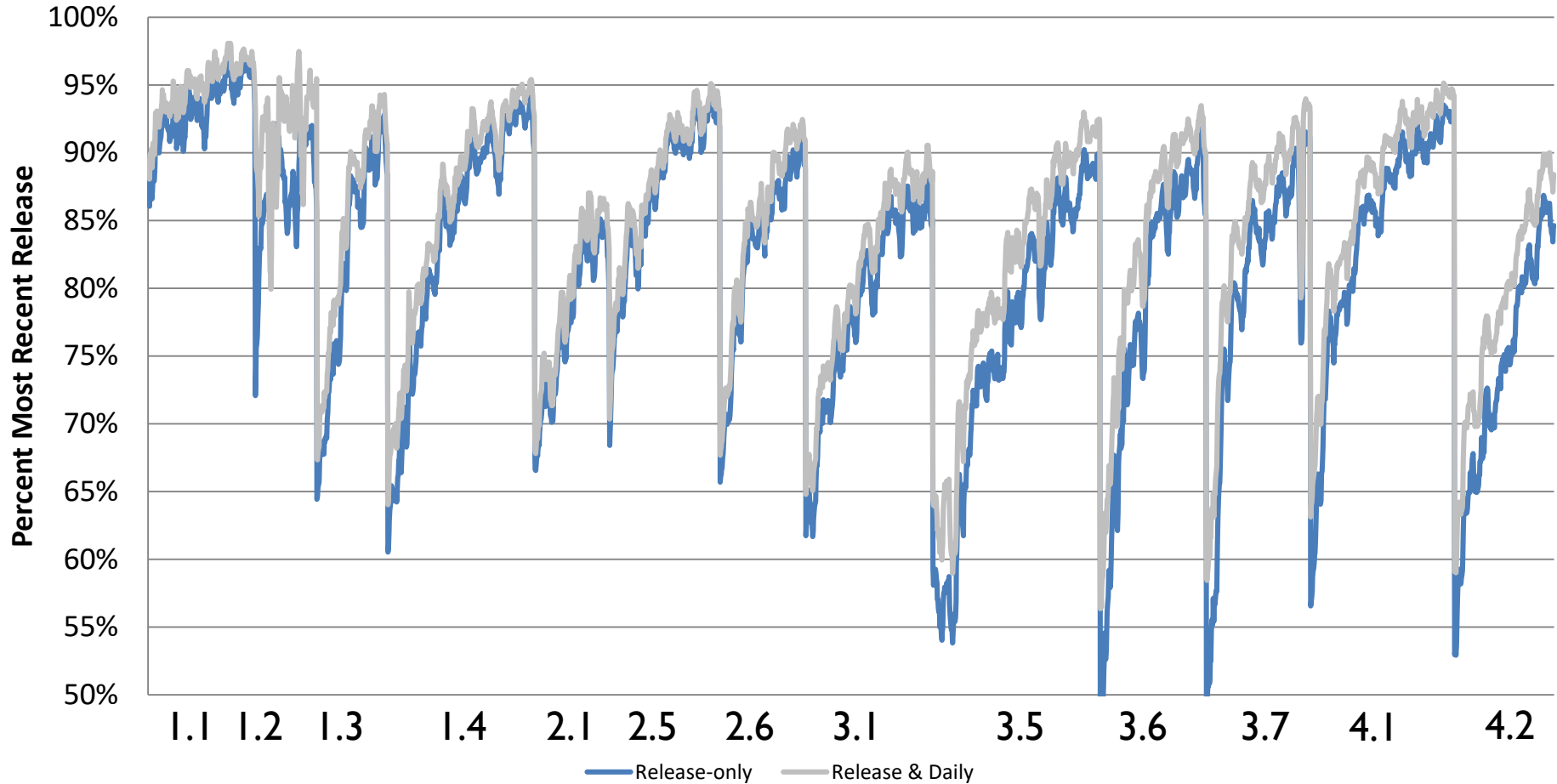


▶ Rita Chupalov

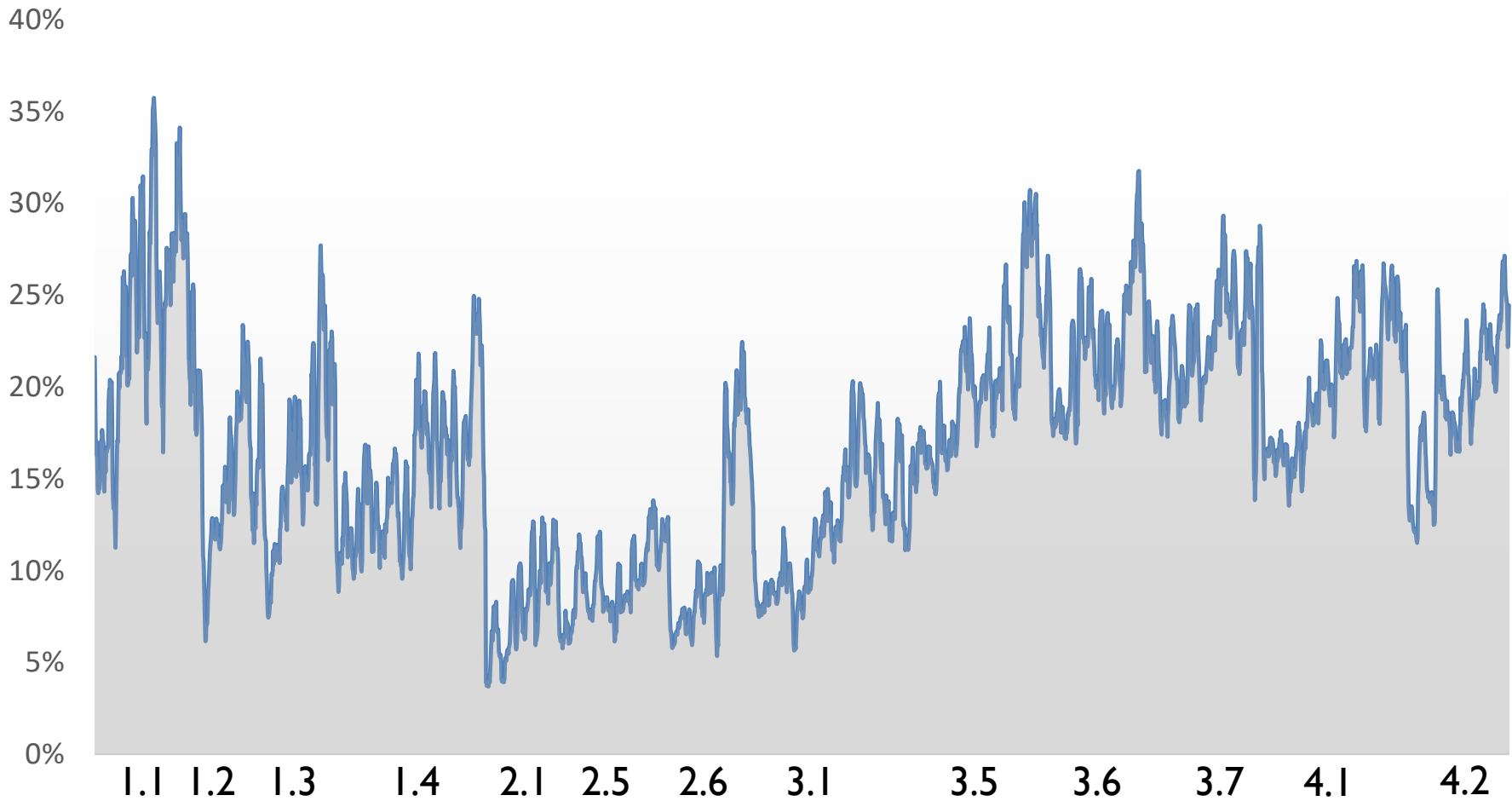


New Version Adoption

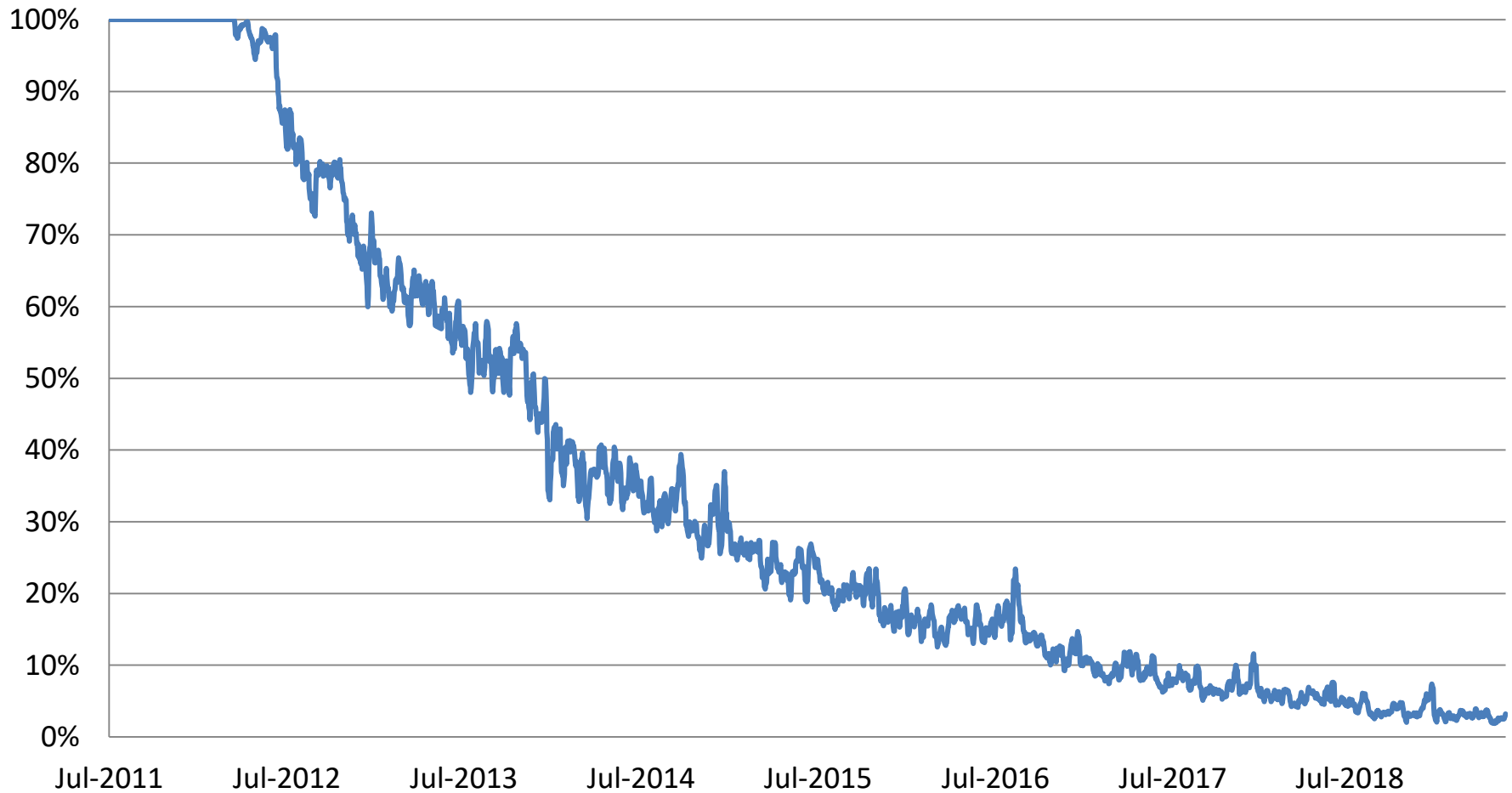
Percentage of Trailing 7-day Start-Ups Using Most Recent Release



New Version Adoption



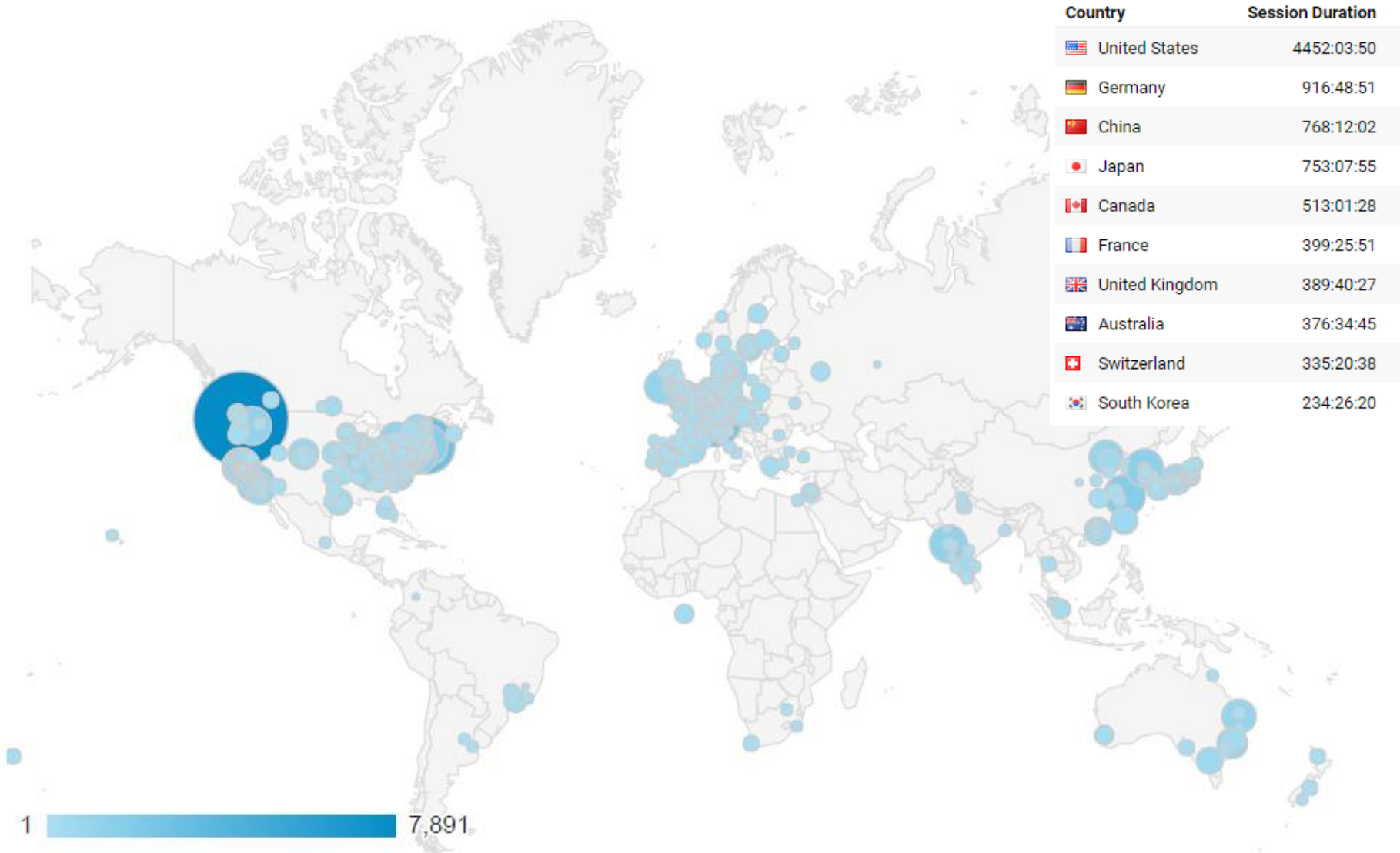
Decline in 32-bit Use (2-3%)



Multiple Instrument Vendors



Skyline Web Site Visits (past 6 months)



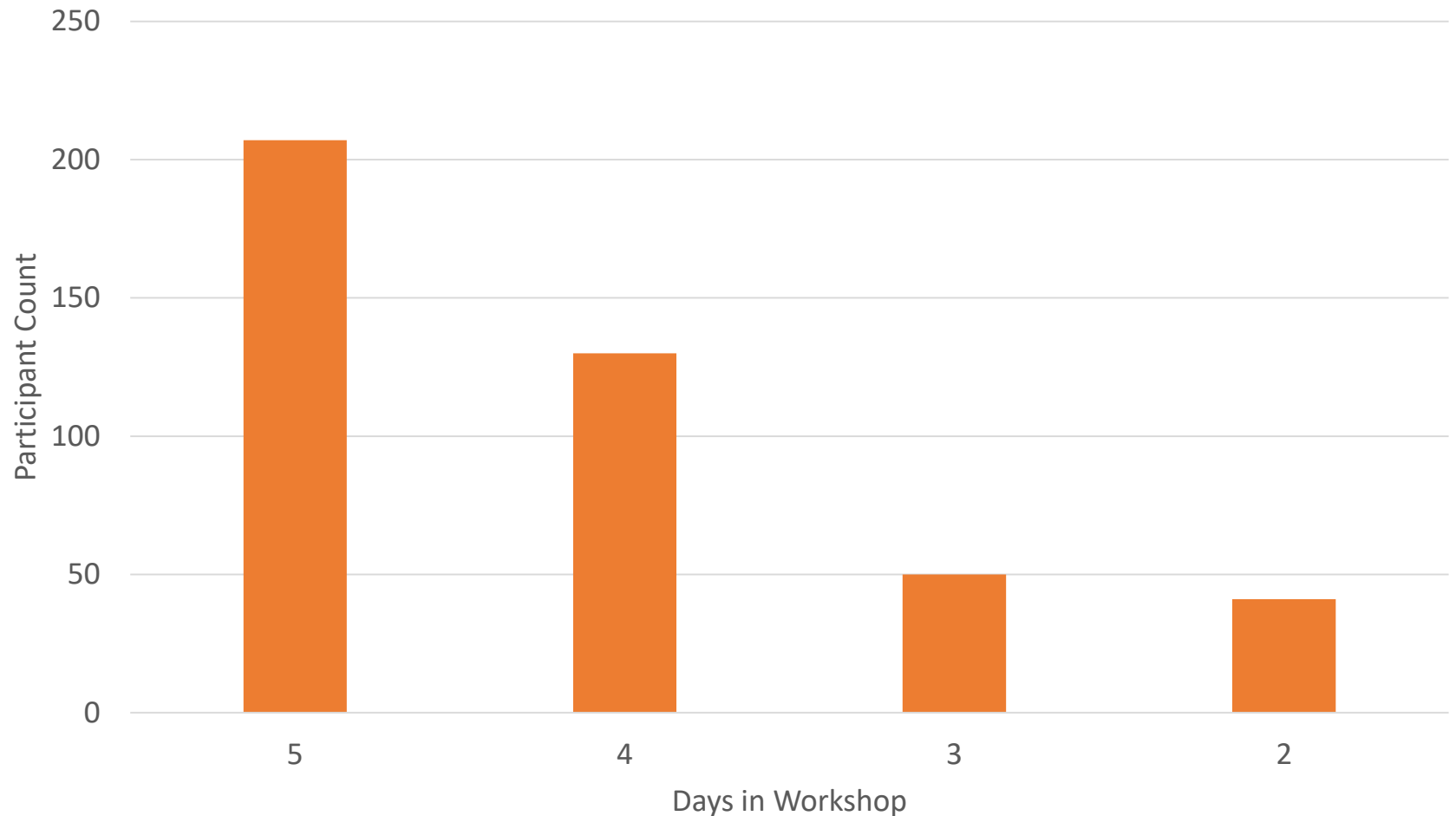
Teaching Targeted Proteomics in 2018

- 5-Buck Institute, Novato, CA – April 2-6 (32)
 - 5-NEU, Boston + Statistics – April 30 – May 11 (35)
 - 5-ETH, Zurich – All DIA – July 2-6 (55)
 - 5-U. of Wa., Seattle – July 30 – August 3 (28)
 - 5-CRG, Barcelona – November 12-16 (25)
 - 5-**New!** Duke, Durham, NC – December 10-14 (32)
 - 4-**New!** Shanghai – October 22-26 (50)
 - 4-IIT Bombay, Mumbai – February (40)
 - 4-CNPEM, Campinas, Brazil – November 7-9 (50)
 - 3-pre-Lorne, Melbourne – January 29-31 (50)
 - 2-MSACL, Palm Springs – January 20&21 (8)
 - 2-US HUPO, Minneapolis – March 10&11 (15)
 - 2-ASMS, San Diego – June 2&3 (18)
-



Teaching Targeted Proteomics in 2018

2018 Instruction Participants by Days in Workshop (Total of 428)



Aggregating and Publishing



Vagisha Sharma



Josh Eckels

- ▶ Upload Skyline documents to private project
- ▶ Publish to **Panorama Public**
 - ▶ 124 public data sets (50 more in review)

Abbatiello, et al. MCP Guidelines, 2017
Sharma, et al. MCP 2018

- ▶ Longitudinal instrument QC data
- ▶ Quant support
- ▶ Build chromatogram libraries
- ▶ Biopharmaceuticals QC – in progress

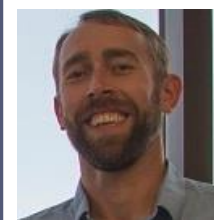


Panorama

- ▶ Free hosted version (<http://panoramaweb.org>)
 - ▶ >376 separate projects so far (CPTAC, LINCS & ABRF sPRG)
 - ▶ >6700 data sets uploaded (>10,000 QC docs)
 - ▶ User controlled security

▶ **New**

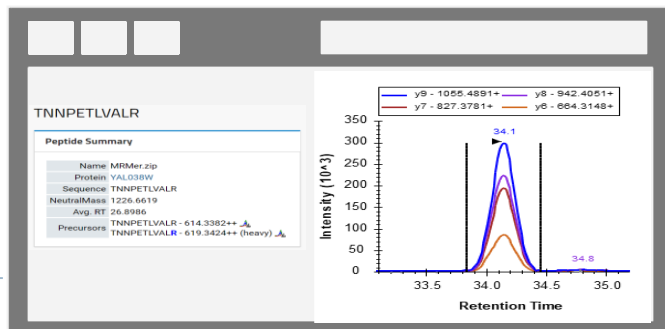
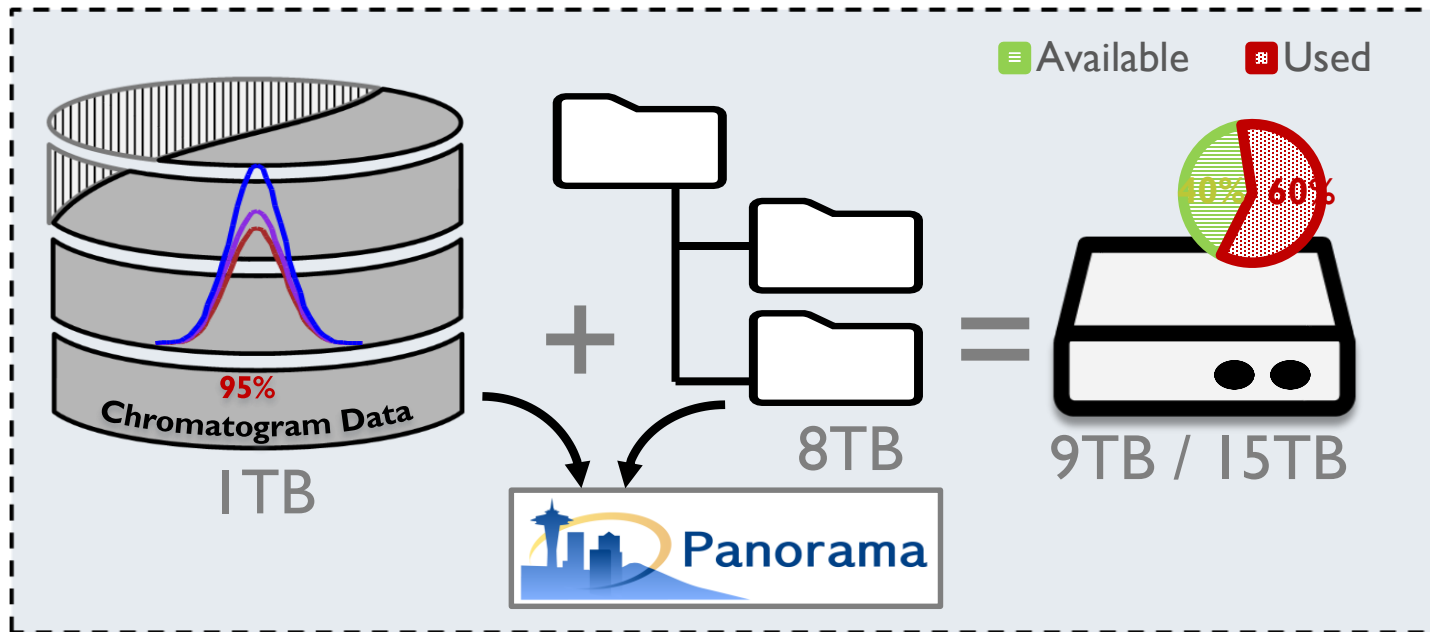
- ▶ Locally
- ▶ Rock



Josh Eckels – MP 430

Customizable quality control metrics and notifications in Panorama

PanoramaWeb After 6 Years



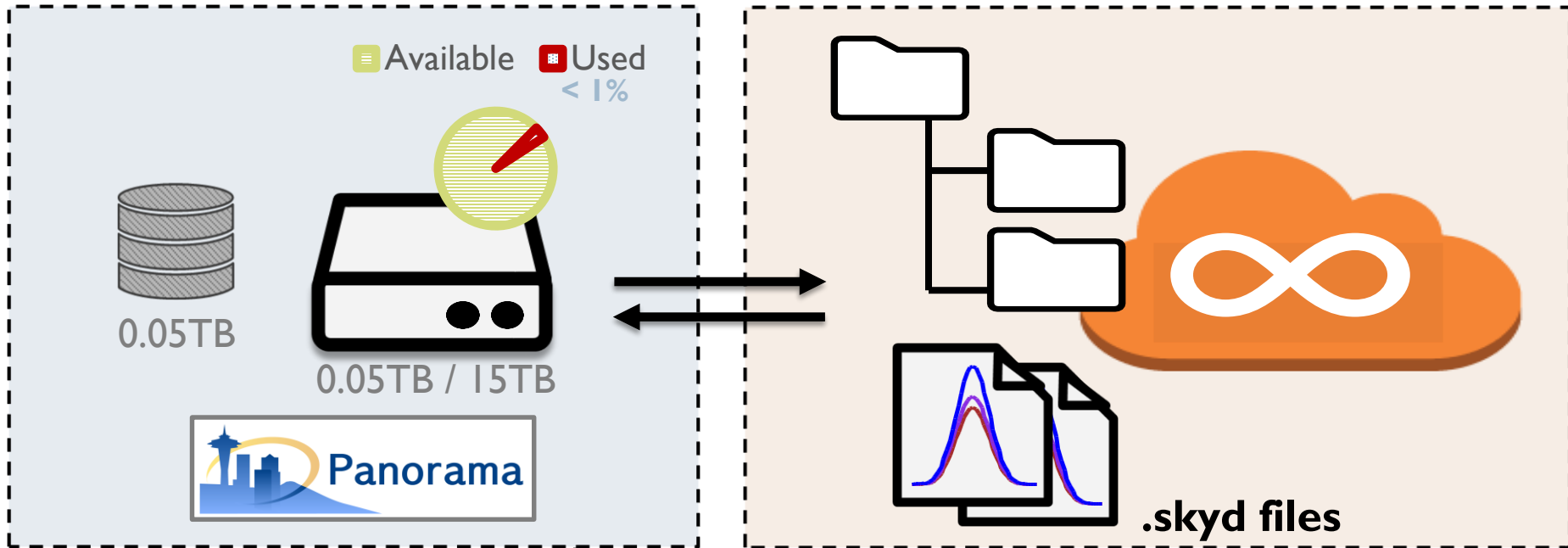
Panorama Public

Panorama Public Datasets

Files

Name	Last Modified	Size	Cre...	Descript...	Usa...
BSA_Frag_100nM_18May15_Fir_15-04-02.d.zip	2018-03-08	945 MB			
Yeast_Opt1ug_a_BSA_0pM_18May15_Fir_15-04-01.d.zip	2018-03-08	3.5 GB			
Yeast_Opt1ug_BSA_100nM_18May15_Fir_15-04-01.d.zip	2018-03-08	3.5 GB			
Yeast_Opt1ug_BSA_100pM_18May15_Fir_15-04-01.d.zip	2018-03-08	3.6 GB			
Yeast_Opt1ug_BSA_10nM_18May15_Fir_15-04-01.d.zip	2018-03-08	3.5 GB			
Yeast_Opt1ug_BSA_1uM_18May15_Fir_15-04-01.d.zip	2018-03-08	3.5 GB			
Yeast_Opt1ug_BSA_500nM_19May15_Fir_15-04-01.d.zip	2018-03-08	3.5 GB			
Yeast_Opt1ug_BSA_50nM_18May15_Fir_15-04-01.d.zip	2018-03-08	3.5 GB			
Yeast_Opt1ug_BSA_5pM_18May15_Fir_15-04-01.d.zip	2018-03-08	3.5 GB			

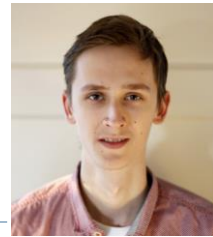
PanoramaWeb With Room To Grow



The screenshot shows the 'Panorama Public' web interface. It features a search bar at the top, a 'Sign In' button, and a 'Peptide Summary' section on the left. The main content area displays a peptide sequence 'TNNPETLVAI' and a mass spectrometry plot with peaks at 'y0 - 1055.4891+' and 'y0 - 942.4051+'. A profile picture of Vagisha Sharma is shown next to her name and affiliation.

Vagisha Sharma – MP 44 I
Panorama Public: ProteomeXchange and Cloud Storage Integration

Improving Reproducibility



- ▶ Audit logging
- ▶ **Soon!** In Panorama

Document Grid: All Info

Views ▾ | 1 of 8 | Export... | Find: | Clear log | Enable audit logging

Time Stamp	All Info Message
6/3/2018 10:33:...	Audit logging has been enabled
6/3/2018 10:34:...	Settings > Peptide Settings -- Prediction > Retention time predictor > Calculator changed
6/3/2018 10:34:...	Settings > Peptide Settings -- Quantification > Regression fit changed from "none" to "linear"
6/3/2018 10:34:...	Settings > Peptide Settings -- C
6/3/2018 10:34:...	Settings > Peptide Settings -- C
6/3/2018 10:34:...	Settings > Peptide Settings -- C
6/3/2018 10:34:...	Settings > Peptide Settings -- C
6/3/2018 10:34:...	Settings > Peptide Settings -- C

Customize View

View Name: Detailed

Columns Filter

<input checked="" type="checkbox"/> Ab Time Stamp	Time Stamp
<input type="checkbox"/> Ab Undo Redo Message	Skyline Version
<input type="checkbox"/> Ab Summary Message	User
<input checked="" type="checkbox"/> Ab Skyline Version	All Info Message
<input type="checkbox"/> # Document Format Version	Reason
<input checked="" type="checkbox"/> Ab User	

OK Cancel

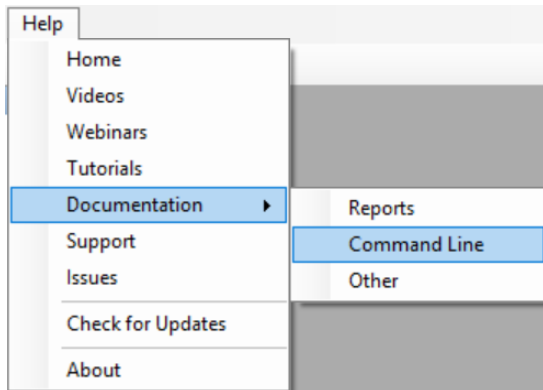
Rita Chupalov – MP 43 I

Audit logs to enforce document integrity in Panorama and Skyline

Improving Command-Line Interface



- ▶ Now on Linux (Docker Container on Wine)
- ▶ Improved argument help



Documentation Viewer

To access the command-line interface for Skyline you can use either SkylineRunner.exe or SkylineCmd.exe.

SkylineRunner.exe is a tiny shim executable less than 10 KB in size. It requires a Skyline installation, performed with the self-updating web installers, on the computer on which SkylineRunner is run. SkylineRunner simply starts a separate Skyline process running without any user interface, pipes the parameter options from the command-line to the running Skyline instance, and prints output from its Skyline instance to the command console. You can still run multiple instances of SkylineRunner and it will start multiple instances of Skyline. The Skyline instance started by SkylineRunner is independent of any other instances that may already be running on the same machine. It is not necessary to have a visible instance of Skyline running on your computer for SkylineRunner to work.

SkylineCmd.exe is a much simpler executable which is always installed beside Skyline.exe. It uses the Skyline.exe module as a DLL providing a much cleaner command-line implementation than SkylineRunner.exe, but you must know where SkylineCmd.exe lives on disk to run it, which is usually not the case with the web installer. Usually, you will use either the "Unplugged" installer or the Administrator installer (or recently the Wine Docker Container for Linux systems) if you want to use SkylineCmd.exe. The "Unplugged" installer comes in a ZIP file and you can just unzip it, find SkylineCmd.exe and run it in-place. The Administrator installer puts SkylineCmd.exe in C:\Program Files\Skyline.

The Skyline command-line interface is intended for automating tasks such as quality control, scheduling and refinement, during acquisition. With the Skyline command-line interface, you can open a Skyline document, import a newly acquired data file, and export a report or new method. You can also run large-scale chromatogram extraction and peak picking for proteomewide DIA and DDA experiments faster and consuming less memory than using the full graphic user interface. (See Webinar 14: <https://skyline.ms/webinar14.url>)

General input/output

Argument	Description
--in=path/to/file.sky	Open a Skyline file
--save	Saves any changes to the file
--out=path/to/file.sky	Same as save except writes to the specified file
--share-zip[=path/to/file.sky.zip]	Saves the entire project (document, results, libraries, etc.) to a ZIP archive. Defaults to a minimal archive.
--share-type= <minimal complete>	Specifies the type, 'minimal' or 'complete', of archive to share when --share-zip is specified. Minimal is the default attempting to reduce the size of the archive to the minimum necessary to represent the targets in the document while 'complete' maintains full fidelity of all project files.

Improving Command-Line Interface

- ▶ Now on Linux (Docker Container on Wine)
- ▶ Improved argument help



Command Prompt

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General input/output

Argument	Description
--in=path/to/file.sky	Open a Skyline file
--save	Saves any changes to the file
--out=path/to/file.sky	Same as save except writes to the specified file
--share-zip[archive.
--share-type	--share-zip the archive 'complete'



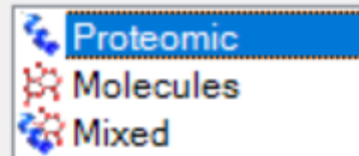
Josh Aldrich – ThP 225
Providing local and cloud pipeline support for data analysis with Skyline in Panorama using Uber Cadence

Proteomics and Molecules UI Modes

Select a Default User Interface

Skyline supports both proteomic and non-proteomic workflows, and adjusts its user interface according to a document's contents.

Please select a default user interface style for use with newly created Skyline documents.

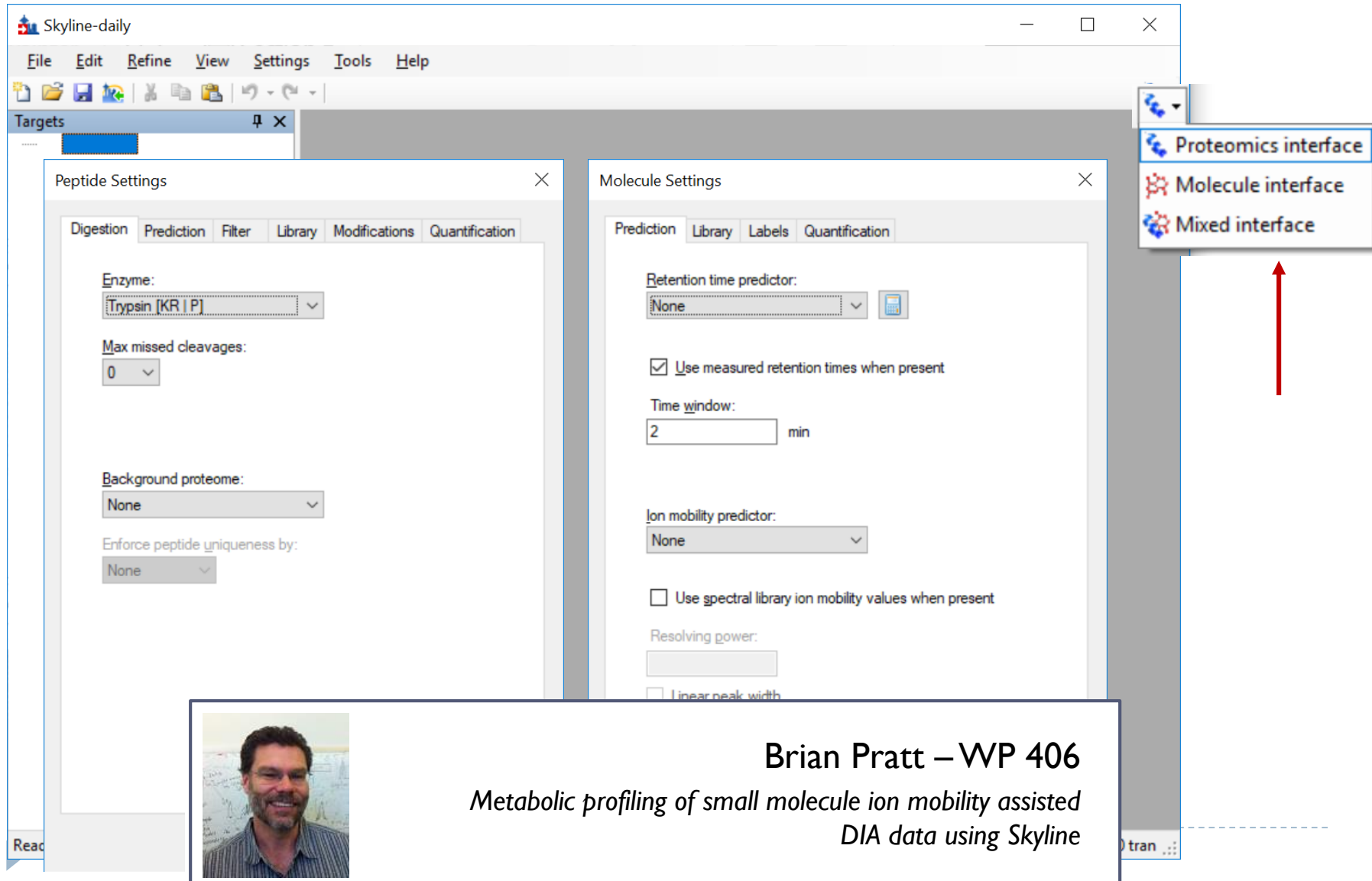


You can change this selection at any time using the control in the upper right corner of the Skyline main window.

OK



Proteomics and Molecules UI Modes



The screenshot displays the Skyline-daily software interface. The main window has a menu bar with 'File', 'Edit', 'Refine', 'View', 'Settings', 'Tools', and 'Help'. Below the menu is a toolbar with various icons. A 'Targets' panel is visible on the left. Two settings panels are open: 'Peptide Settings' and 'Molecule Settings'. The 'Peptide Settings' panel has tabs for 'Digestion', 'Prediction', 'Filter', 'Library', 'Modifications', and 'Quantification'. It includes fields for 'Enzyme' (set to 'Trypsin [KR|P]'), 'Max missed cleavages' (set to '0'), 'Background proteome' (set to 'None'), and 'Enforce peptide uniqueness by:' (set to 'None'). The 'Molecule Settings' panel has tabs for 'Prediction', 'Library', 'Labels', and 'Quantification'. It includes fields for 'Retention time predictor:' (set to 'None'), a checked checkbox for 'Use measured retention times when present', 'Time window:' (set to '2 min'), 'Ion mobility predictor:' (set to 'None'), an unchecked checkbox for 'Use spectral library ion mobility values when present', and 'Resolving power:' (set to an empty field). A dropdown menu on the right side of the interface lists three options: 'Proteomics interface' (with a blue gear icon), 'Molecule interface' (with a red gear icon), and 'Mixed interface' (with a blue and red gear icon). A red arrow points upwards to the 'Mixed interface' option.

Proteomics interface
Molecule interface
Mixed interface

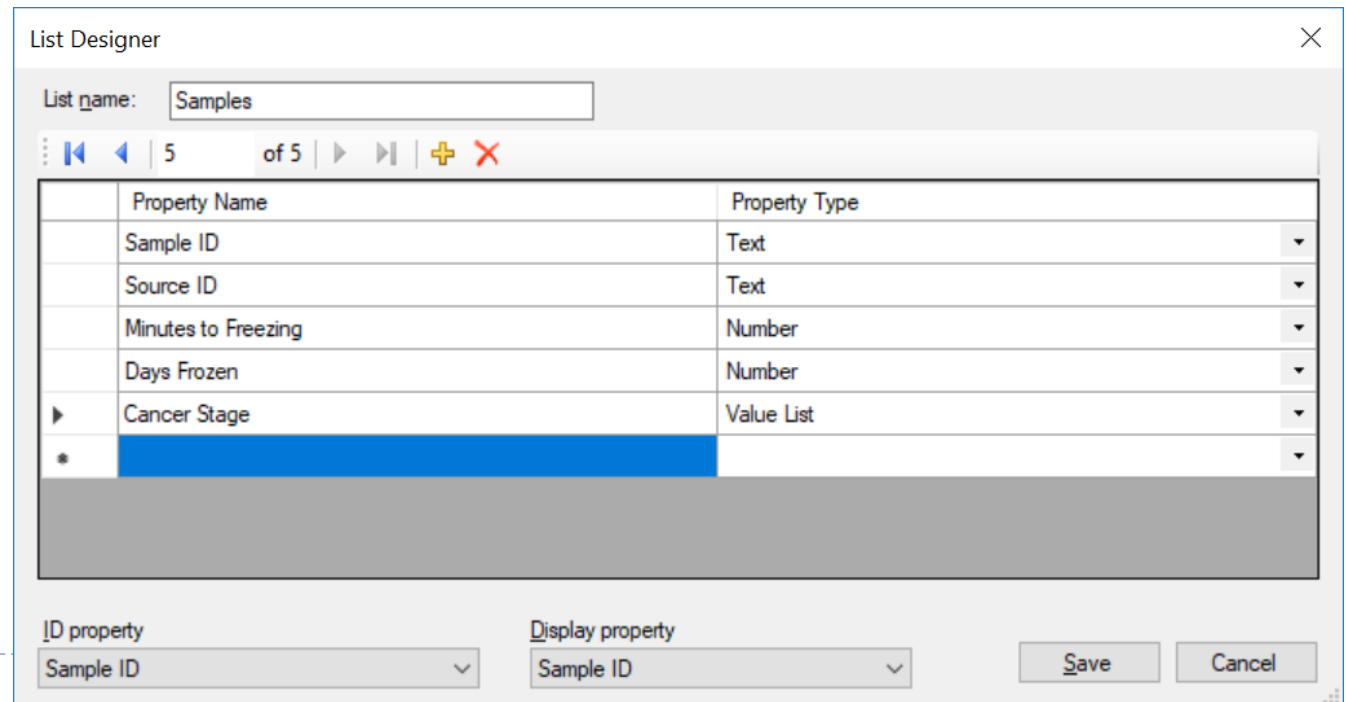
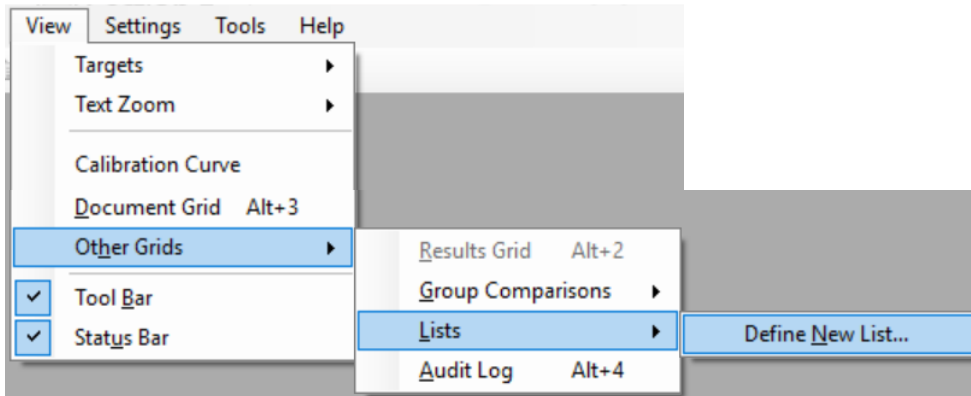
Peptide Settings
Enzyme: Trypsin [KR|P]
Max missed cleavages: 0
Background proteome: None
Enforce peptide uniqueness by: None

Molecule Settings
Retention time predictor: None
 Use measured retention times when present
Time window: 2 min
Ion mobility predictor: None
 Use spectral library ion mobility values when present
Resolving power: [empty field]
 Linear peak width

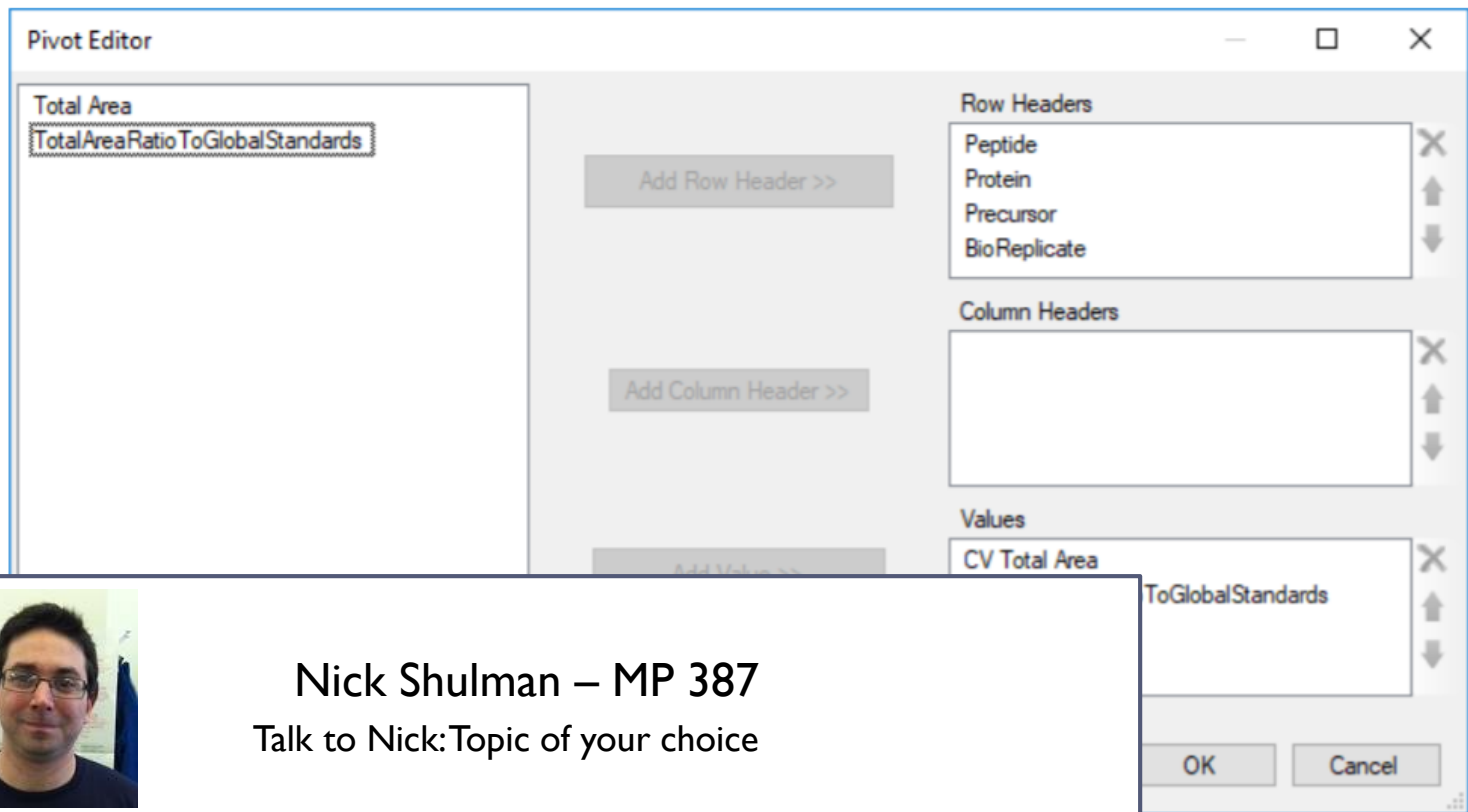
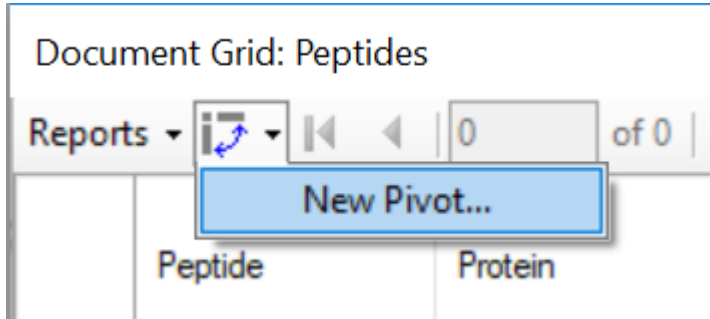
Brian Pratt – WP 406
Metabolic profiling of small molecule ion mobility assisted DIA data using Skyline



Skyline Relational Data and Grids



Skyline Relational Data Pivoted



Nick Shulman – MP 387
Talk to Nick: Topic of your choice

Future

- ▶ 10 more years of everything you love about Skyline



What You Can Do to Help

- ▶ Keep showing up!
- ▶ Tell your instrument vendors
- ▶ Convince your company to join Panorama Partners
- ▶ Write a letter of support for our grant
- ▶ Train other people
- ▶ Keep learning
- ▶ Keep giving us feedback



Instrument Vendor Collaborators

▶ Agilent Technologies

- ▶ John Fjeldsted
- ▶ Christine Miller
- ▶ Joe Roark



▶ Bruker

- ▶ Markus Lubeck
- ▶ Stephanie Kaspar
- ▶ Gary Kruppa
- ▶ Pierre-Olivier Schmit



▶ AB Sciex

- ▶ Mark Cafazzo
- ▶ David Cox
- ▶ Christie Hunter
- ▶ Arianna Jones



▶ Shimadzu

- ▶ Junko Iida
- ▶ Yuji Katsuyama
- ▶ Norio Makai
- ▶ Toshiya Matsubara



▶ Thermo-Scientific

- ▶ Susan Abbatiello
- ▶ Mary Blackburn
- ▶ Vlad Zabrouskov



▶ Waters

- ▶ James Langridge
- ▶ Martin Lunt
- ▶ Keith Richards
- ▶ David Varley
- ▶ Hans Vissers

