

Skyline 2020

State of the Project
12 years after inception

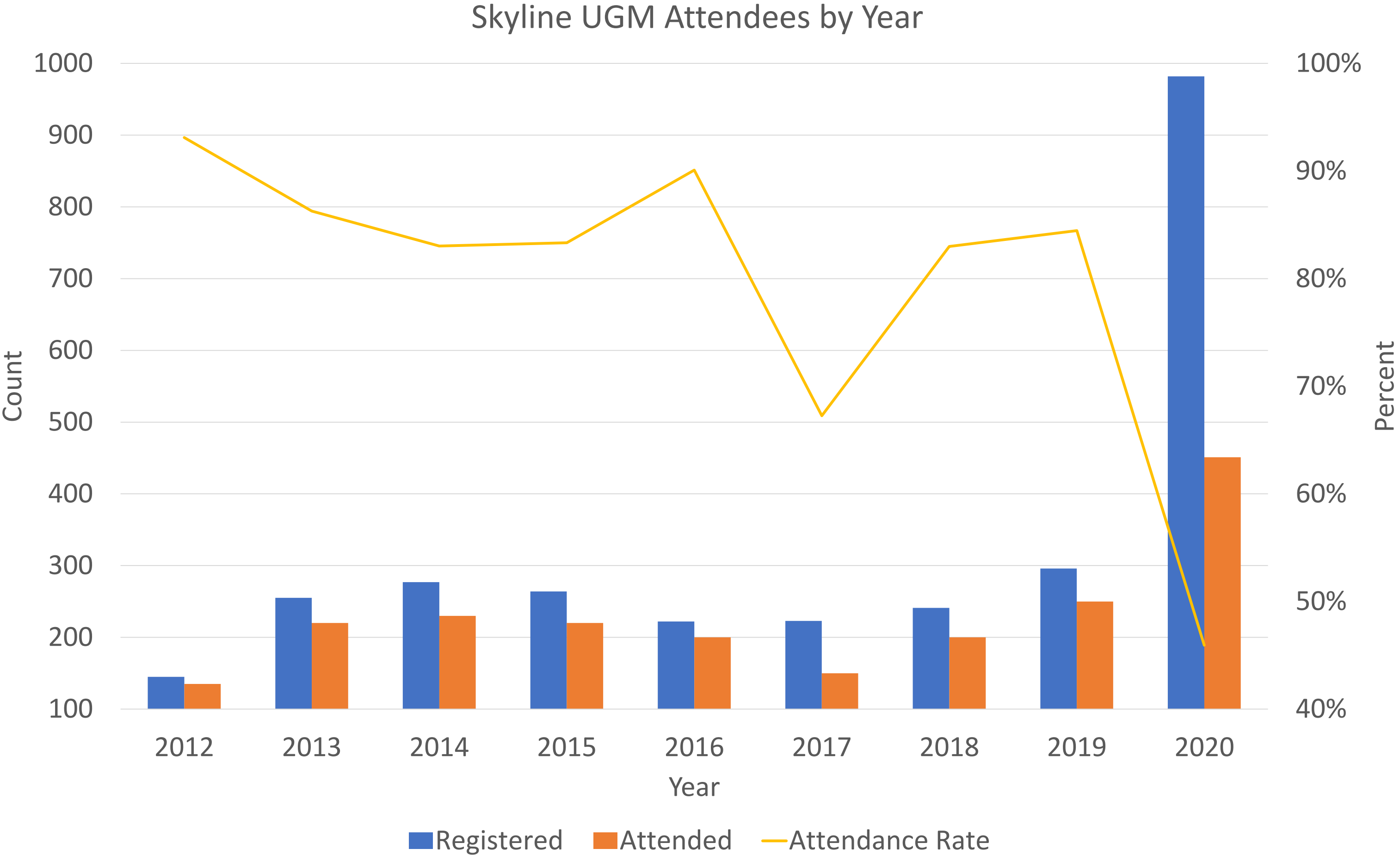
Brendan MacLean



UNIVERSITY *of*
WASHINGTON



Meeting Attendance by Year



Teaching Targeted Proteomics in 2018

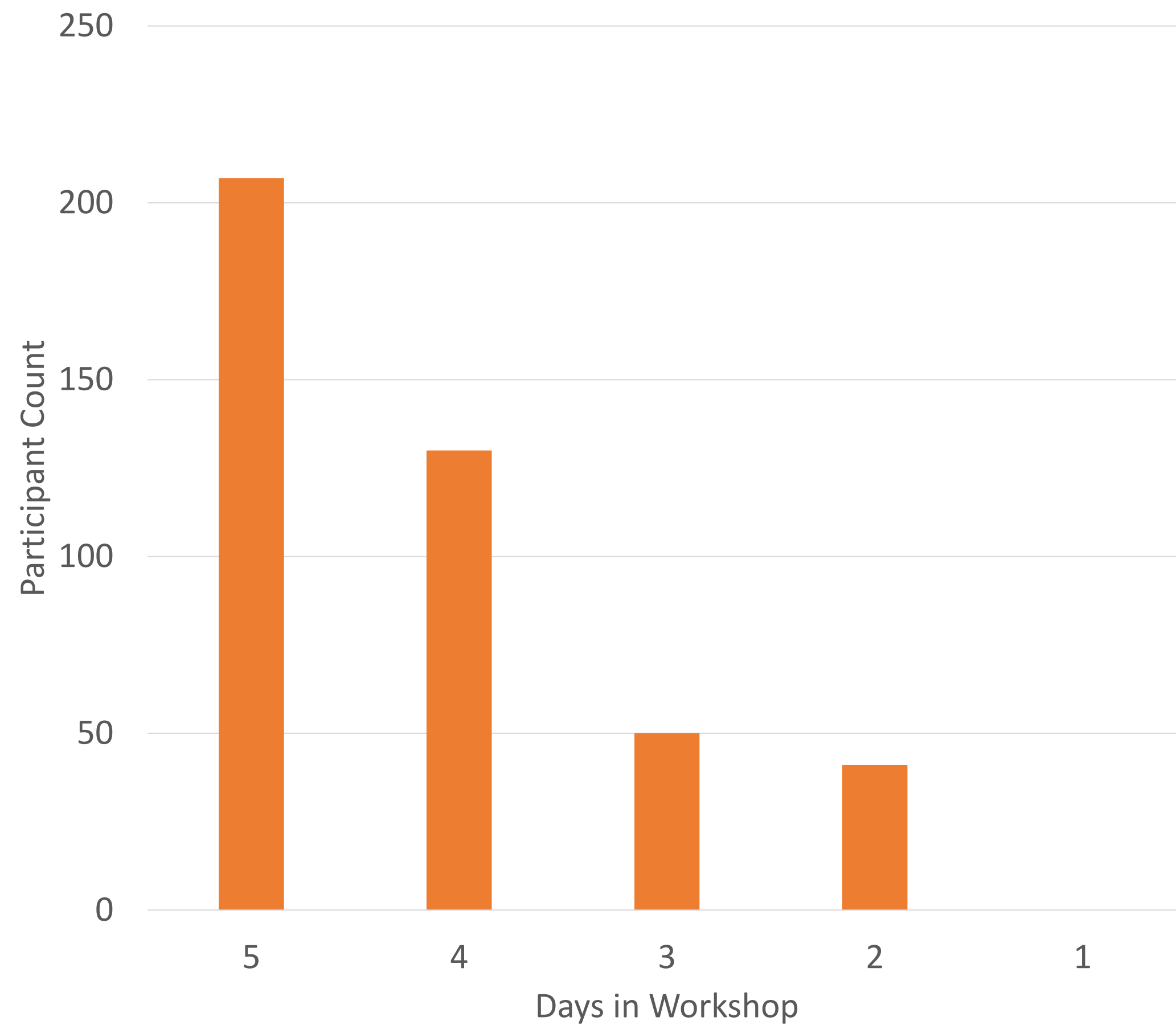
- 5-Buck Institute, Novato, CA – April 2-6 (32)
- 5-NEU, Boston + Statistics – April 30 – May 11(35)
- 5-ETH, Zurich – All DIA – July 2-6 (55)
- 5-U. of Wa., Seattle – July 30 – August 3 (28)
- 5-CRG, Barcelona – November 12-16 (25)
- 5-**New!** Duke, Durham, NC – December 10-14 (32)
- 4-**New!** Shanghai – 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 2019

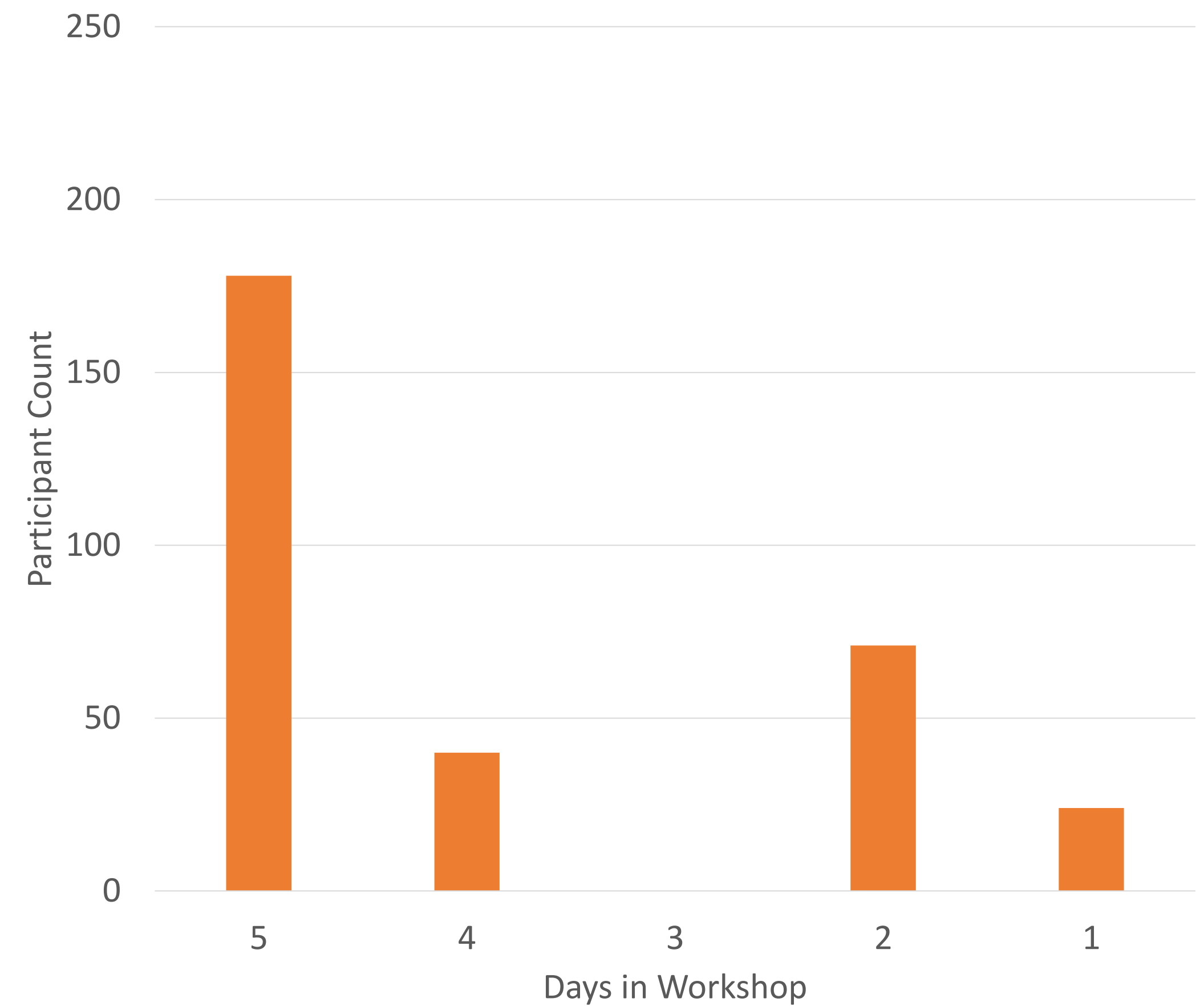
- 5-Buck Institute, Novato, CA – March 18-22 (35)
- 5-NEU, Boston – April 29 - May 10 (50)
- 5-U. of Wa., Seattle – July 22-26 (28)
- 5-Brisbane, Australia – September 9-13 (40)
- 5-CRG, Barcelona – November 11-15 (25)
- 4-Cape Town, SA – November 18-22 (40)
- 2-US HUPO, Washington DC – March 2&3 (28)
- 2-MSACL, Palm Springs – March 31-April 2 (18)
- 2-ASMS, Atlanta – June 1&2 (25)
- 1-MRM Proteomics, Montreal – May 17 (12)
- 1-EUPSS, Brixen, Italy – July 29-31 (12)

Teaching Targeted Proteomics

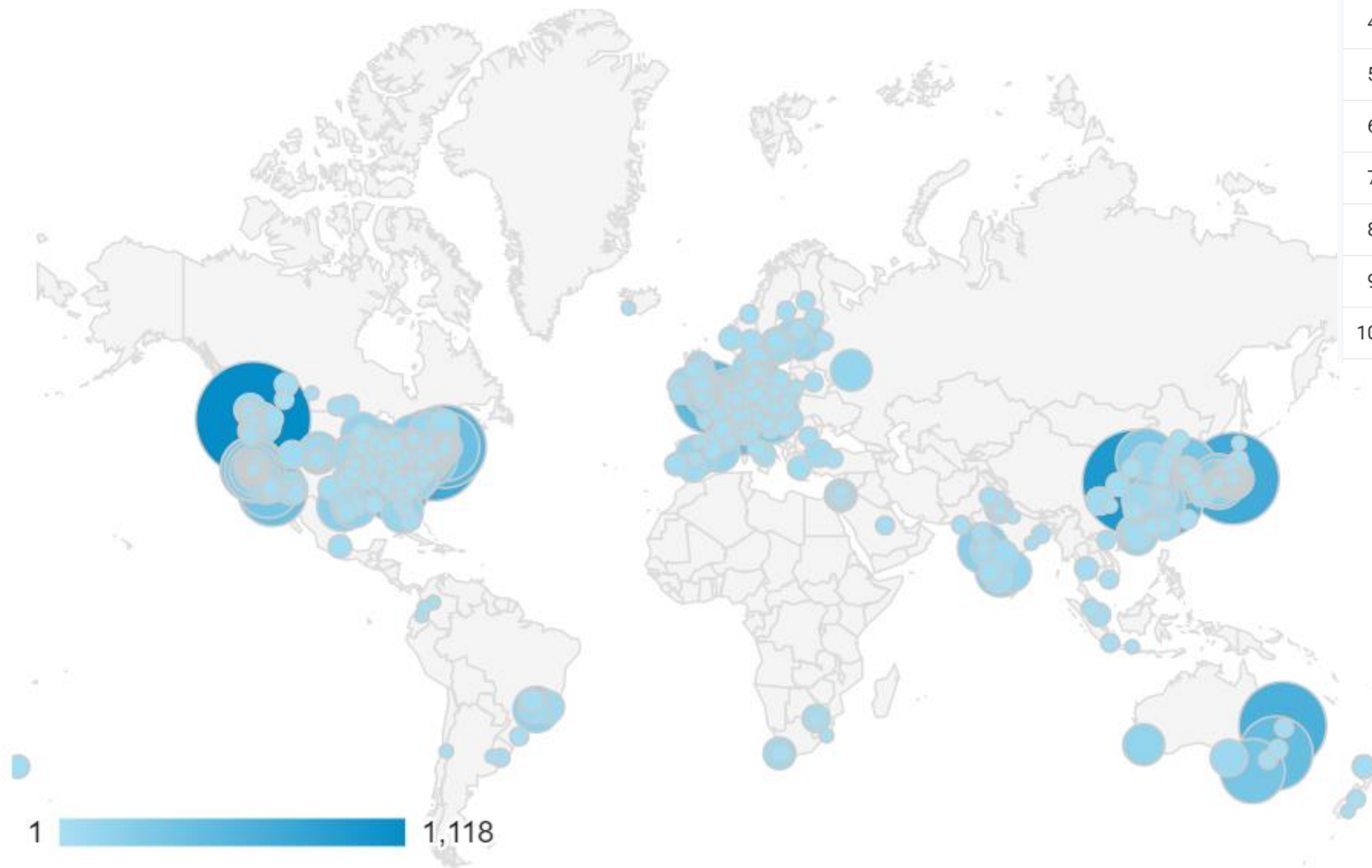
2018 Instruction Participants by Days in Workshop
(Total of 428)



2019 Instruction Participants by Days in Workshop
(Total of 313)

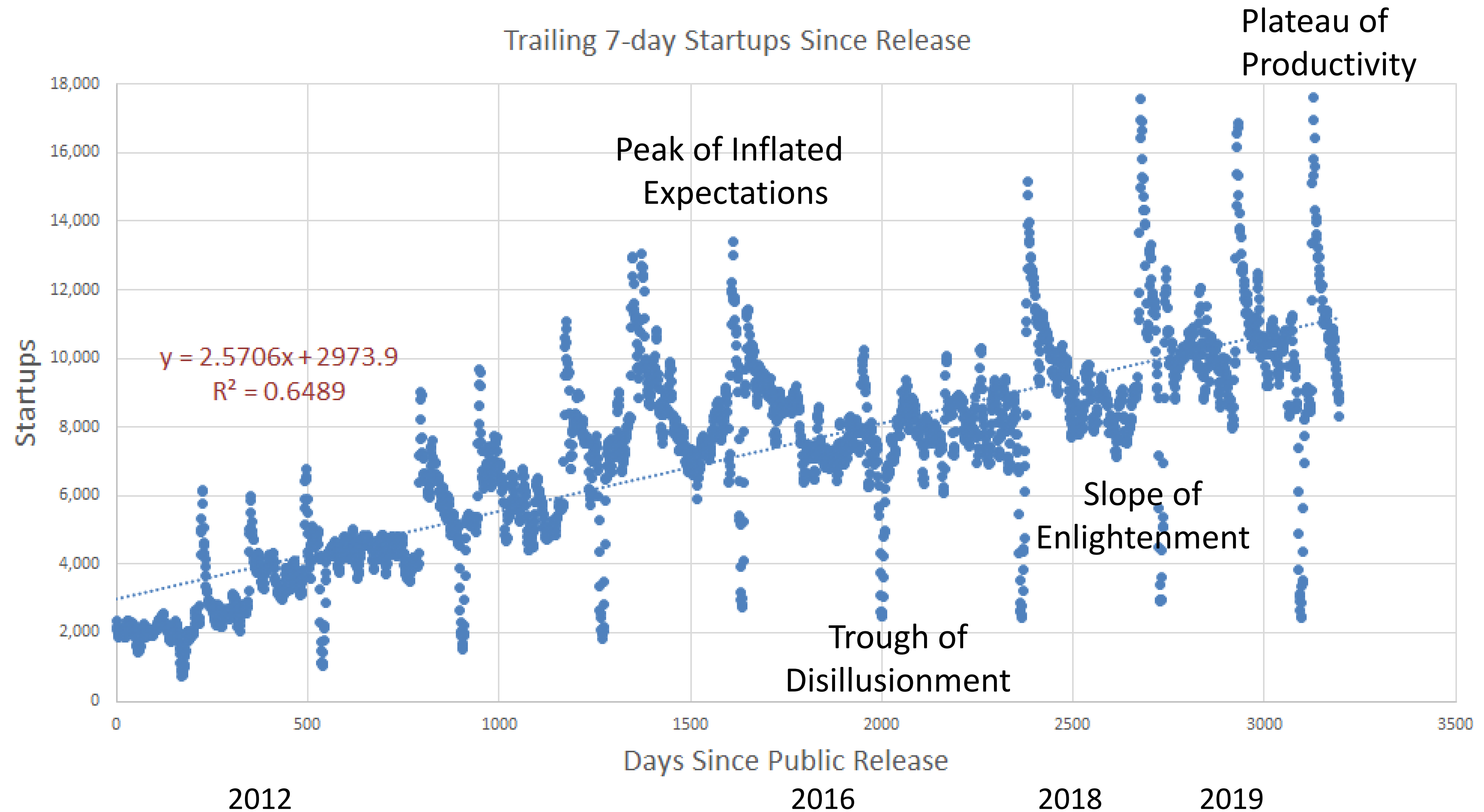


Is Skyline a Pandemic?

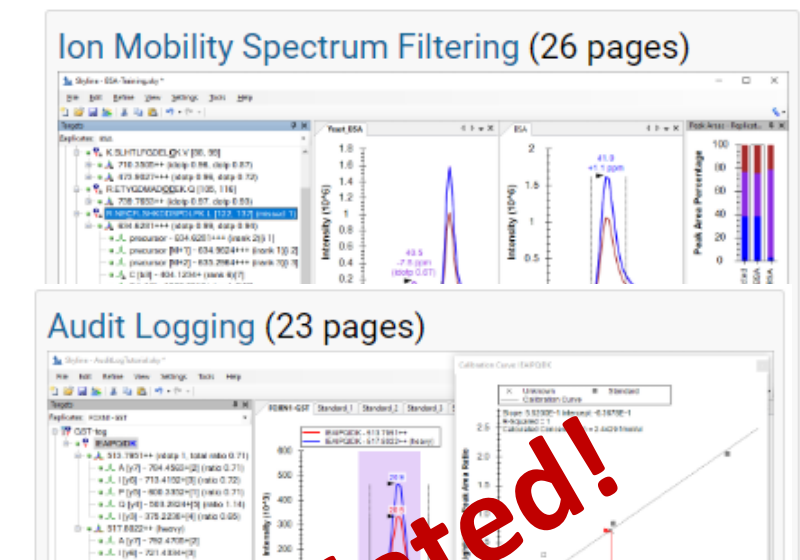
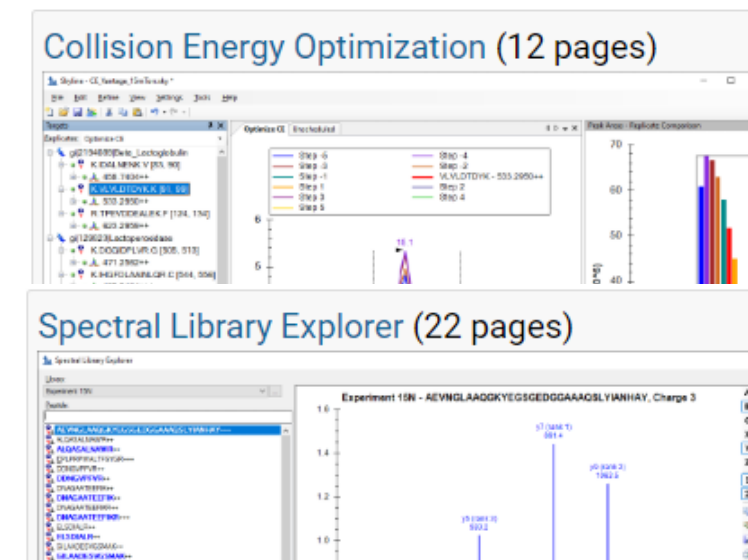
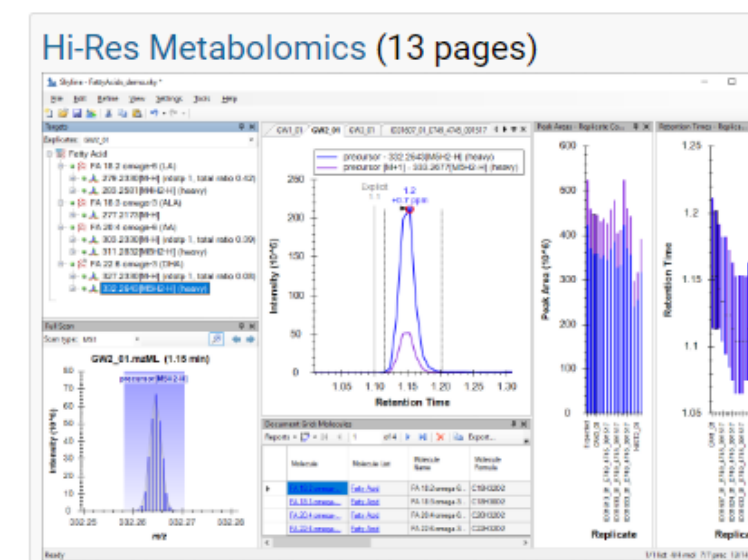
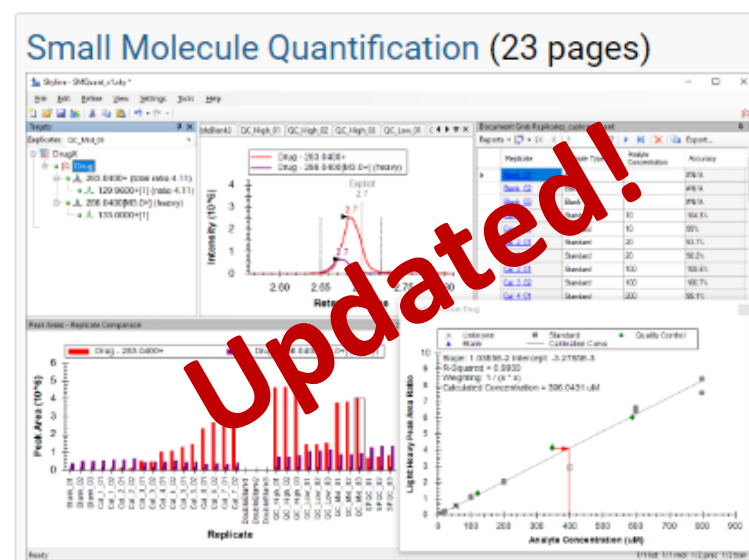
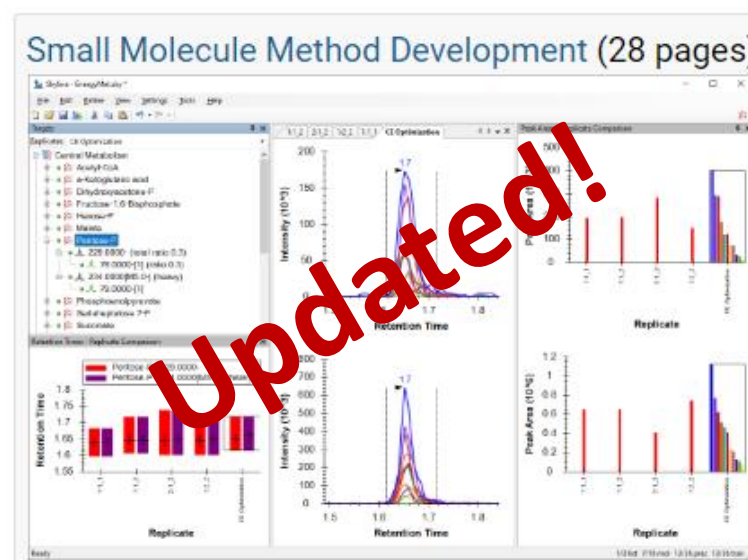
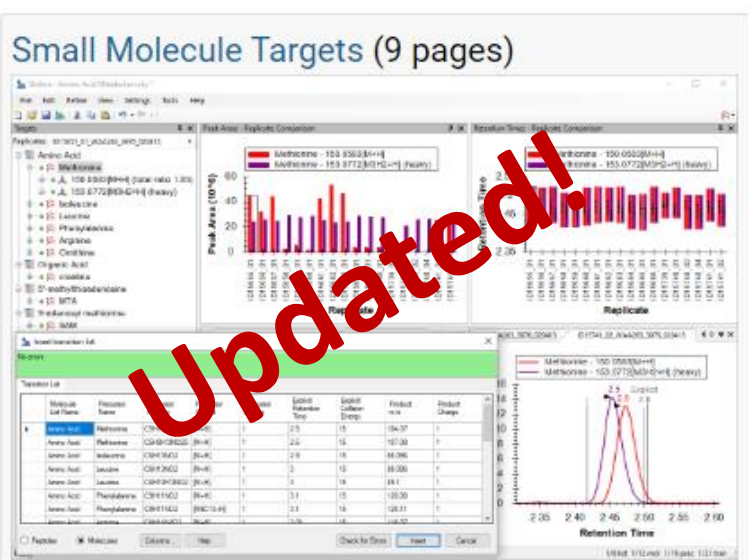
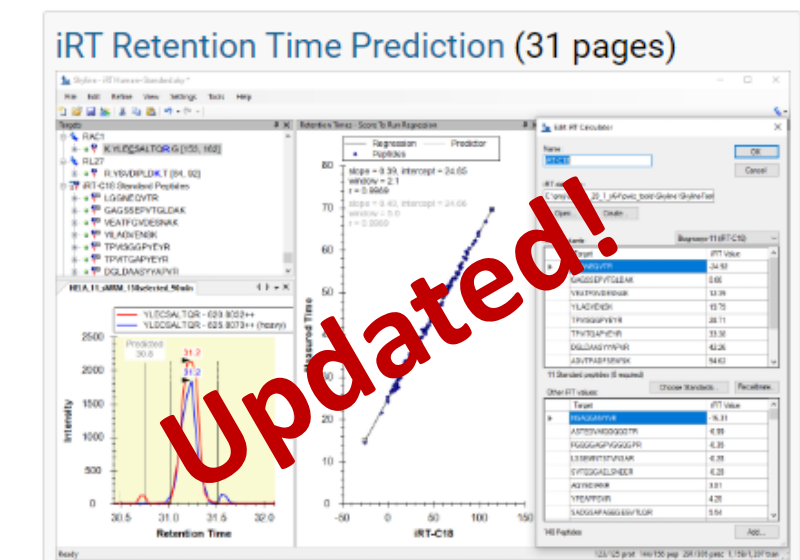
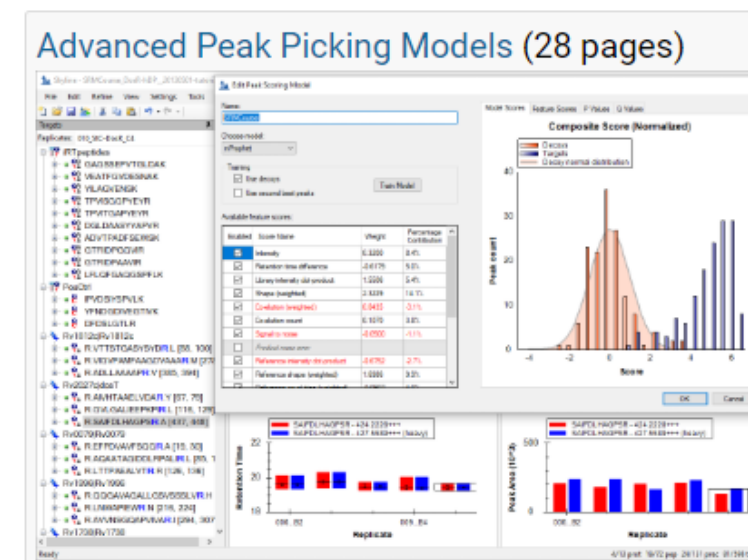
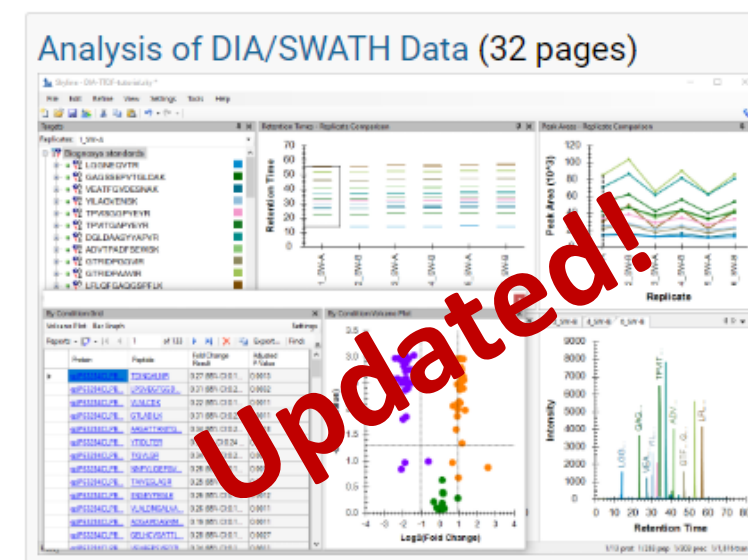
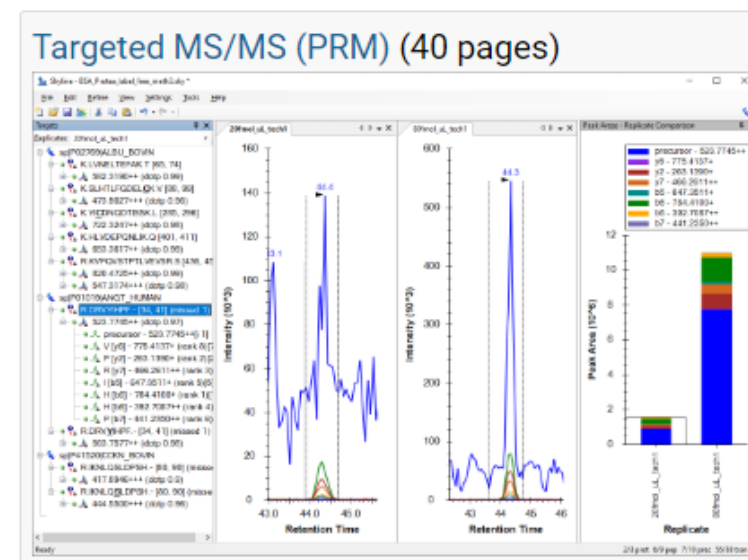
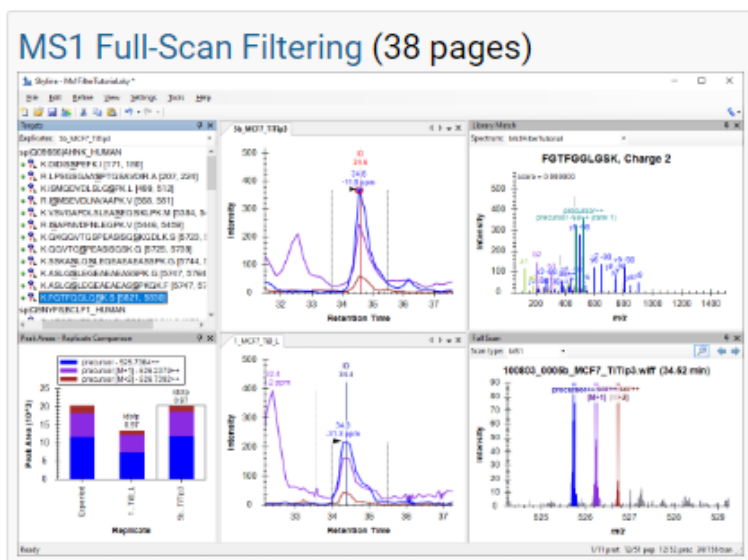
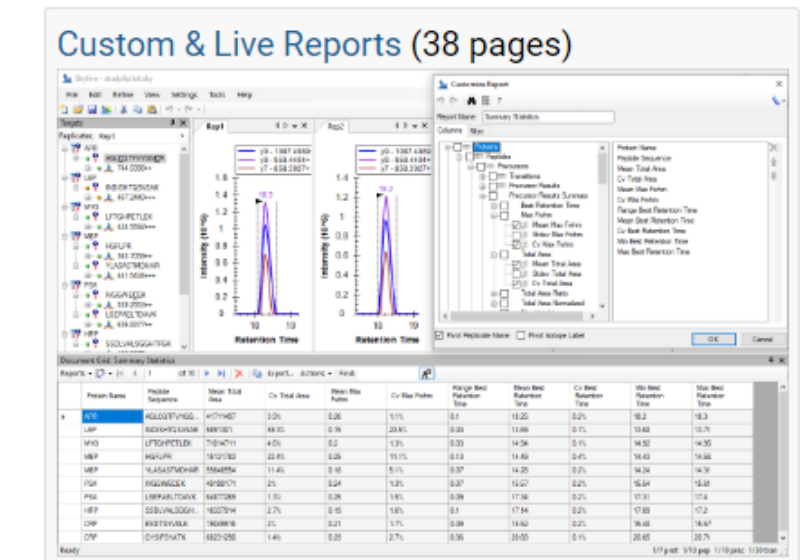
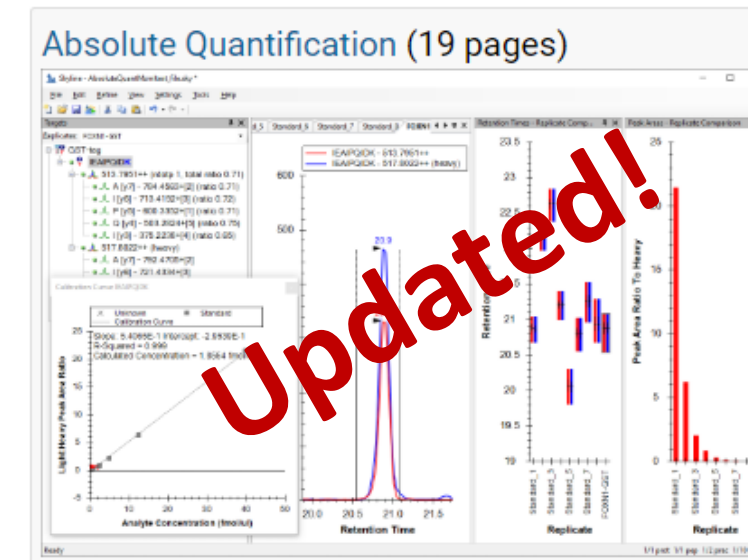
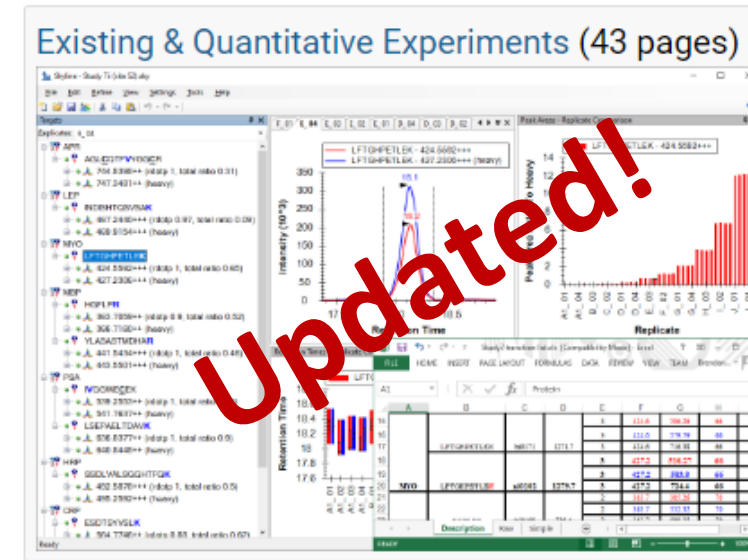
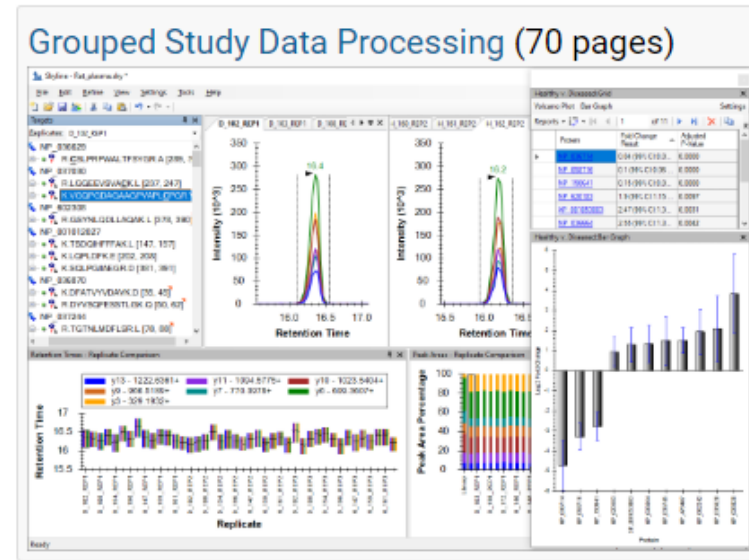
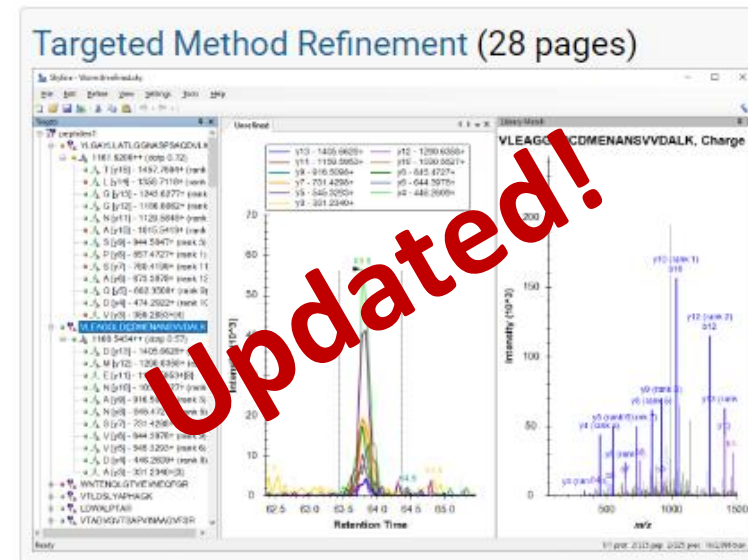
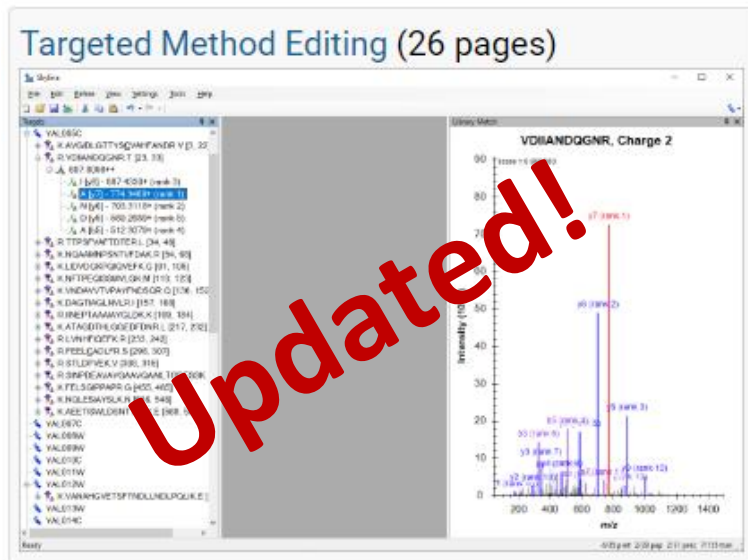


1.	Seattle
2.	Zhengzhou
3.	Chuo City
4.	New York
5.	Brisbane
6.	Cambridge
7.	Shanghai
8.	London
9.	Sydney
10.	San Diego

Viral Growth? Or Gartner Hype Cycle?



20 Tutorials – Working to Update All to 20.1



19 Tutorial Webinars – and Growing...

- Webinar #18:
DIA/SWATH Data Analysis



Brendan MacLean
(Principal Developer)



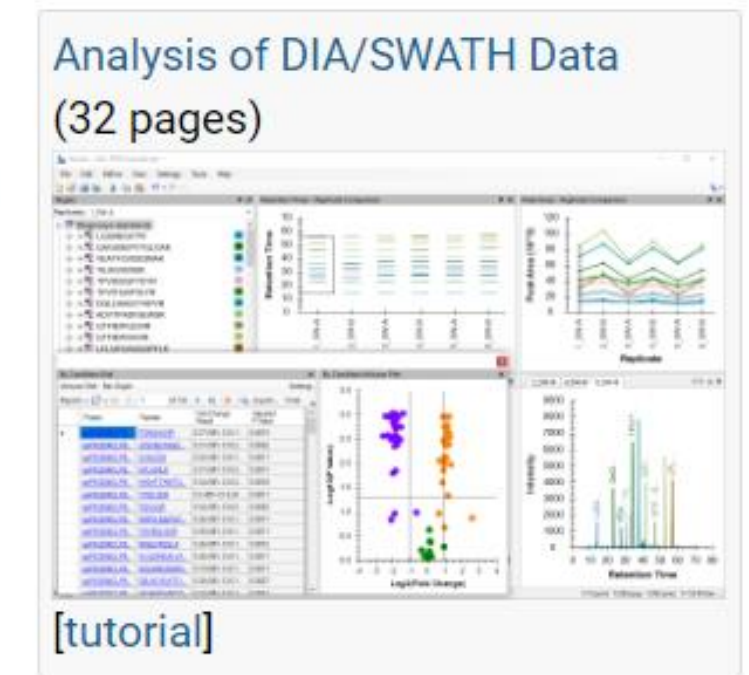
Presentation Slides



Ben Collins
(DIA/SWATH researcher)



Presentation Slides



- Webinar #19:
Ion Mobility Spectrum Filtering



Brendan MacLean
(Principal Developer)



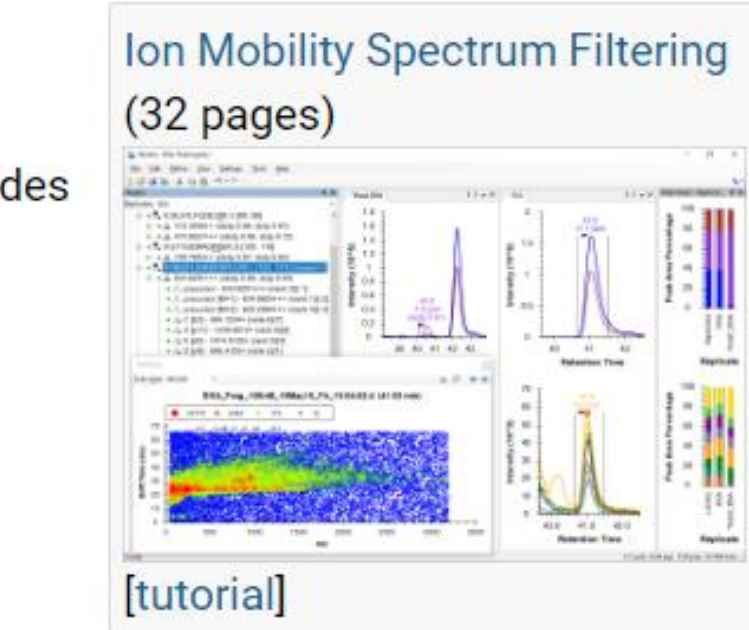
Presentation Slides



Erin Baker
(ion mobility researcher)



Presentation Slides



- Webinar #20:
dia-PASEF Data Analysis



Brendan MacLean
(Principal Developer)



Ben Collins
(DIA/SWATH researcher)

See also – 2020 May Institute at Northeastern -
<https://computationalproteomics.khoury.northeastern.edu/>

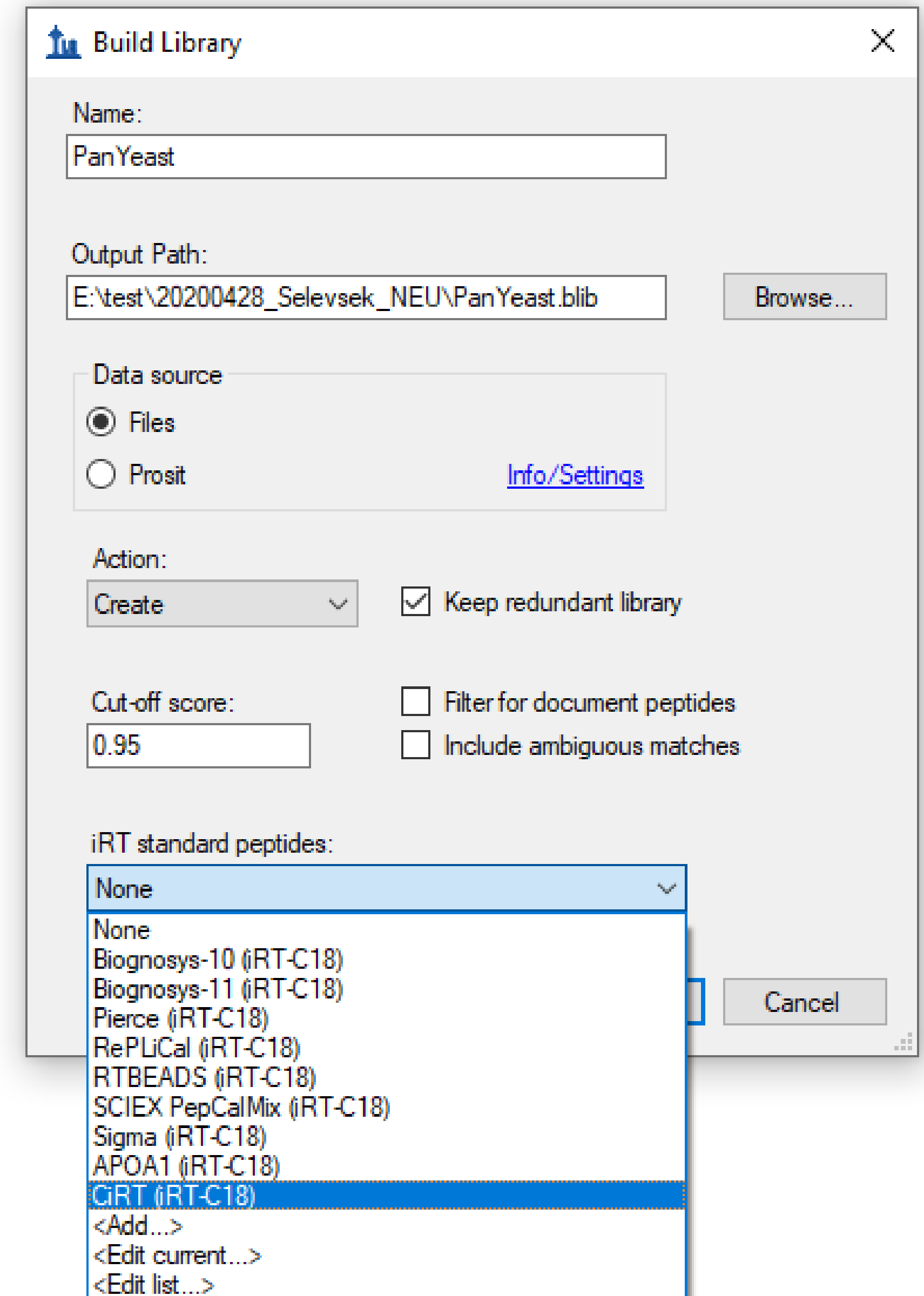
Improved iRT Support

- More understandable
- More automatic
- More use of CiRT and endogenous peptides
- Lowess and logarithmic regressions



Kaipo Tamura – NP 000

Improving iRT Integration in Skyline



dia-PASEF Data Processing

- Initial targeted integration took 5 hours per file proteomewide
- Switched to 3-array spectra (thanks to Hannes Röst)

header		2D spectrum				
precursor m/z	IMS	m/z	m/z	m/z	...	m/z
		intensity	intensity	intensity	...	intensity

63,386,406 per dia-PASEF file

header		3D spectrum				
precursor m/z	m/z	m/z	m/z	...	m/z	
	IMS	IMS	IMS	...	IMS	
	intensity	intensity	intensity	...	intensity	

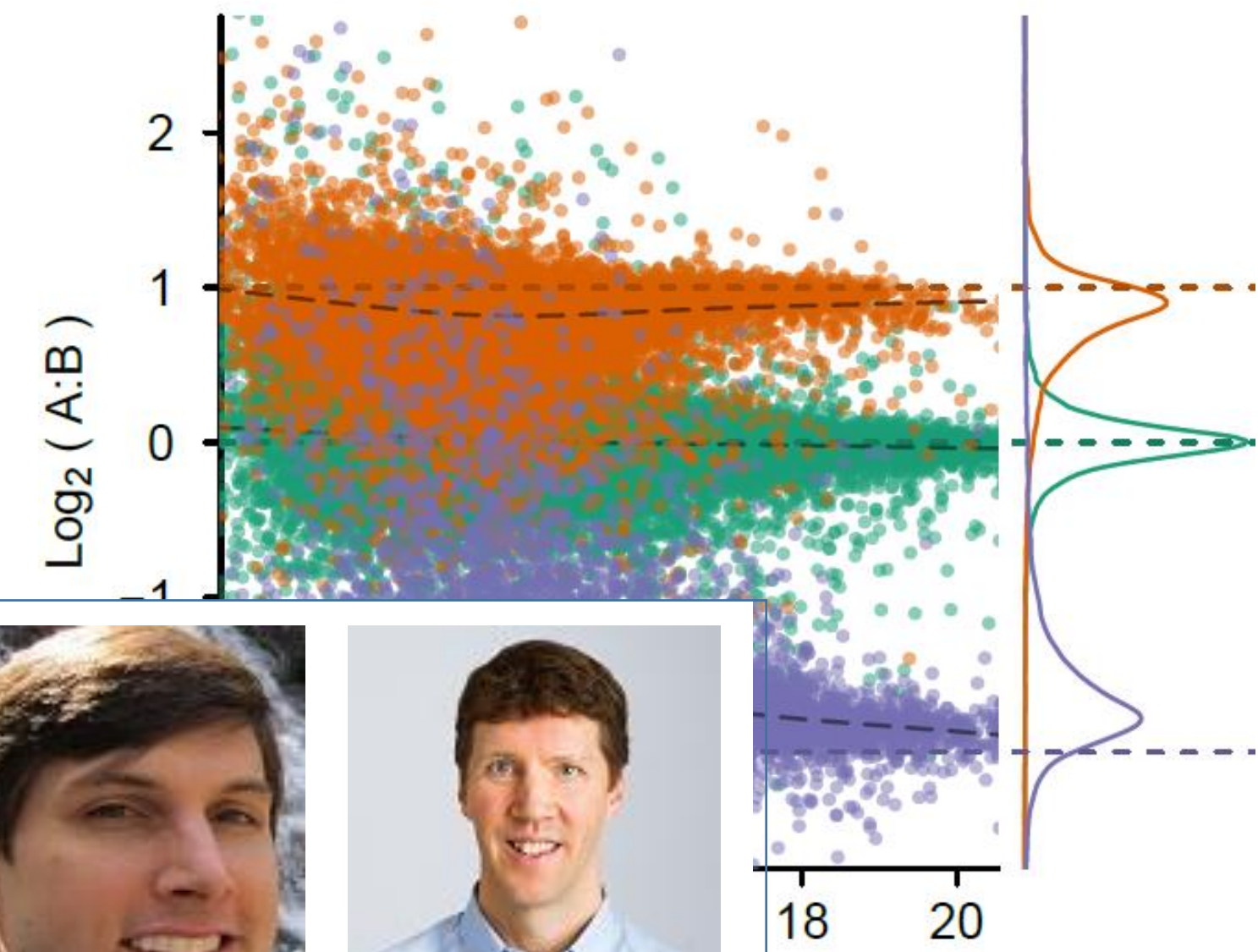
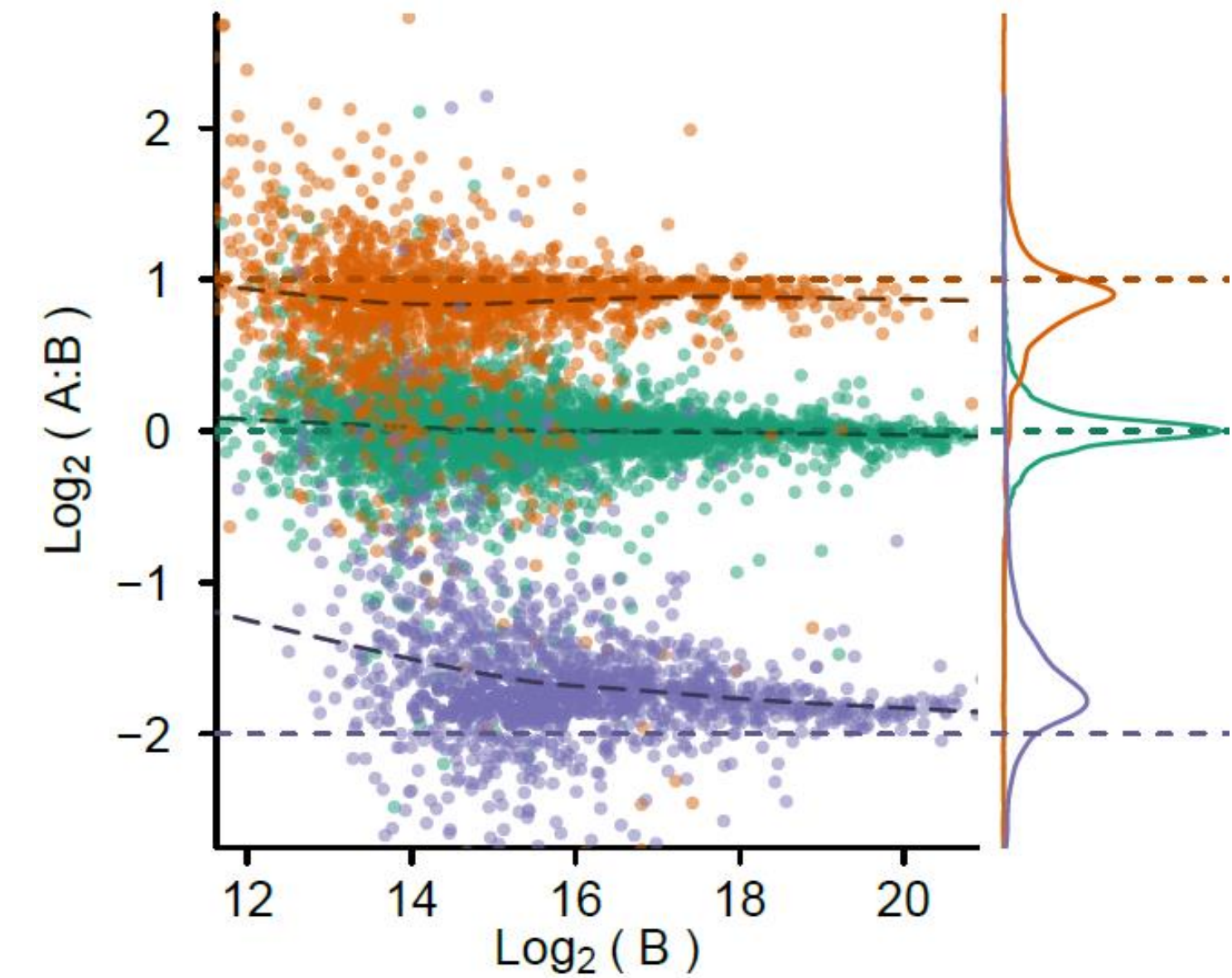
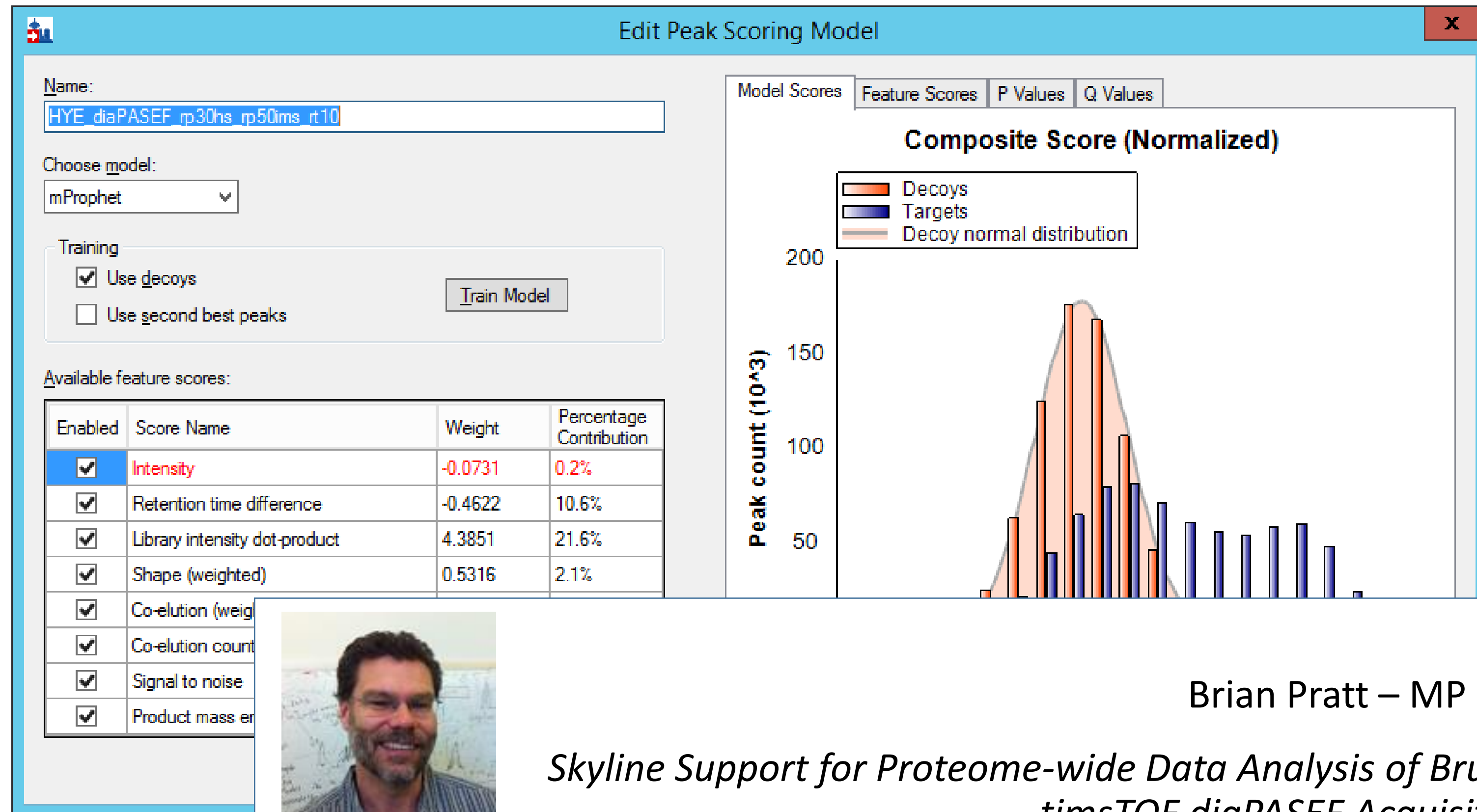
68,378 per dia-PASEF file

- Result: 60x improved perf + 2-3x for other IMS data

system	parallel files	import time (min)
Dell i7 4-core 16 GB of RAM and HDD	3	82
Dell i7 6-core 64 GB of RAM and SSD	6	42
Dell Xeon 24-core 192 GB of RAM and SSD	6	32

dia-PASEF Data Processing

- Proteomewide workflow with IMS filtering

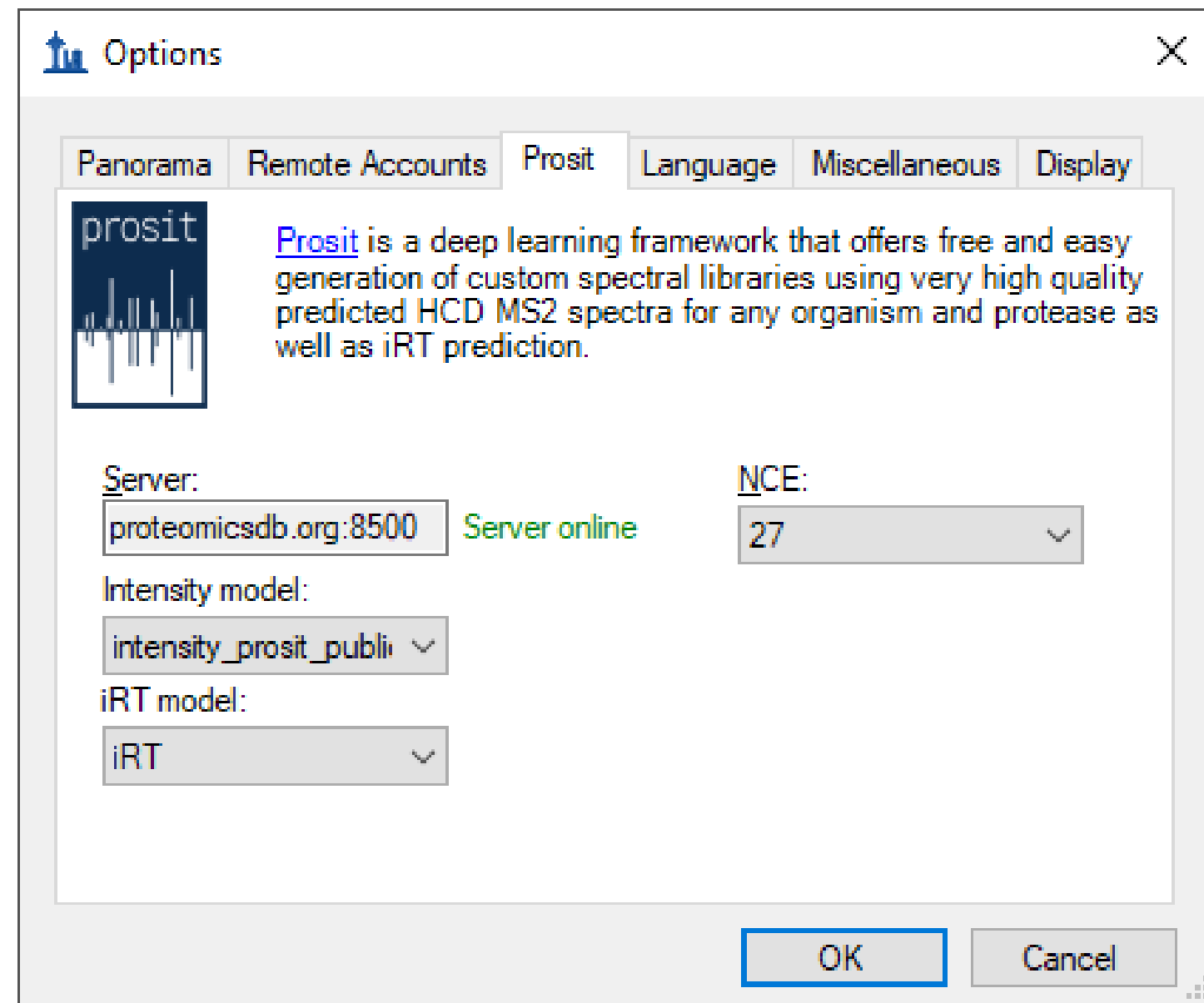


Brian Pratt – MP 253

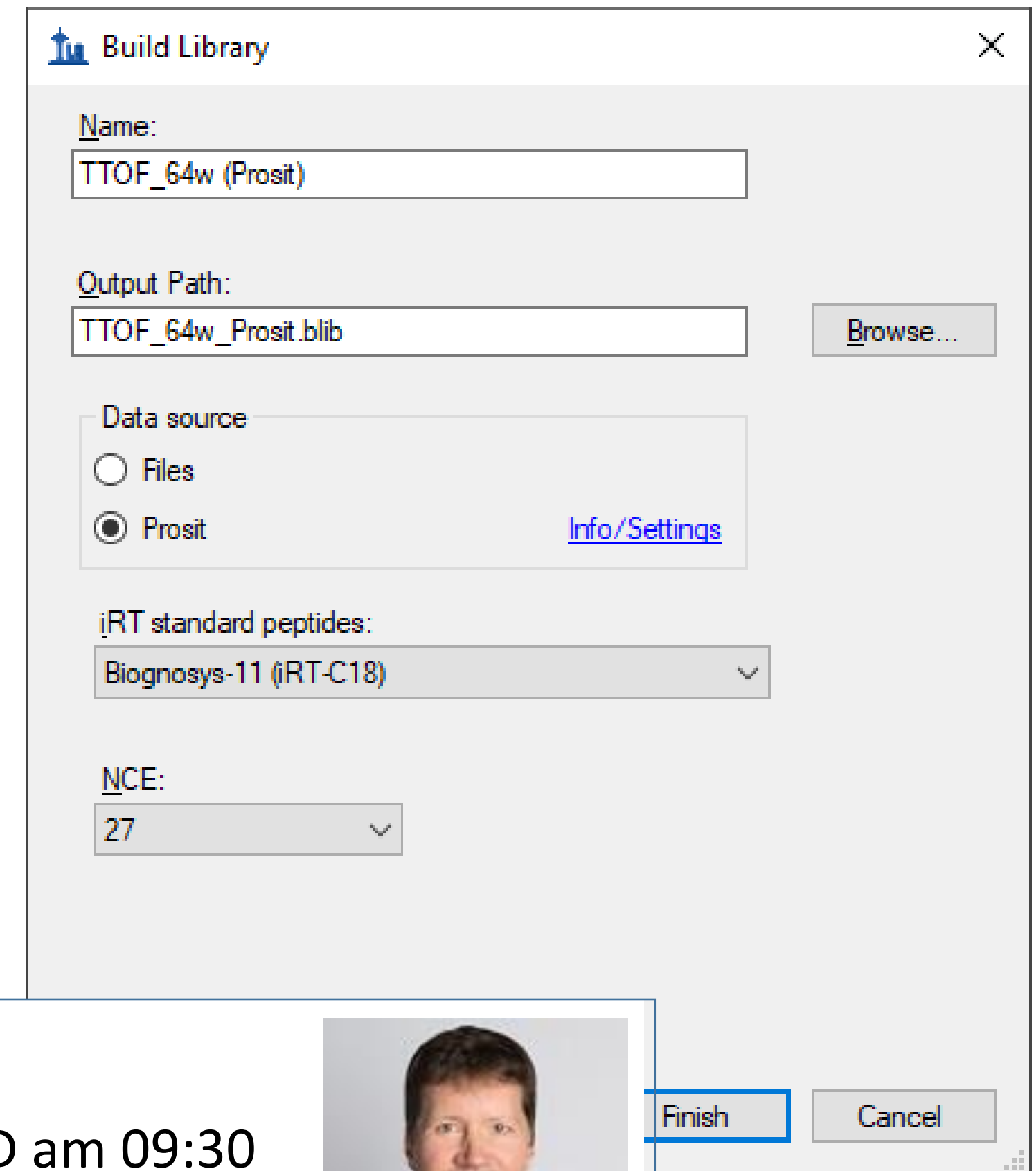
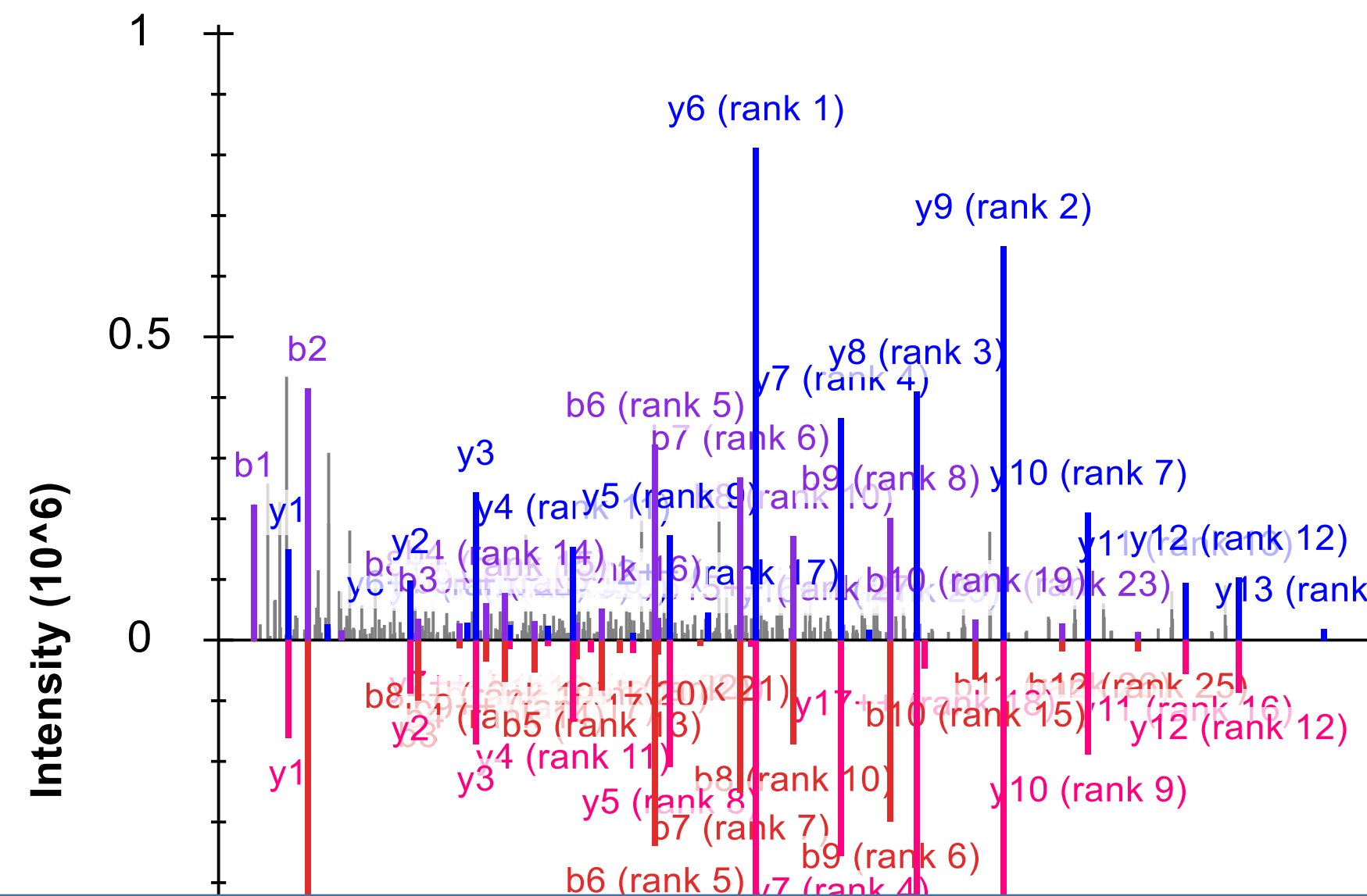
Skyline Support for Proteome-wide Data Analysis of Bruker timsTOF diaPASEF Acquisition



Prosit Integration



Tricolor_SuperLibrary (rebuilt) vs. Prosit
TAFDEAIAELDTLNEESYK, Charge 3
dotp: 0.8014



Brendan MacLean – WOD am 09:30

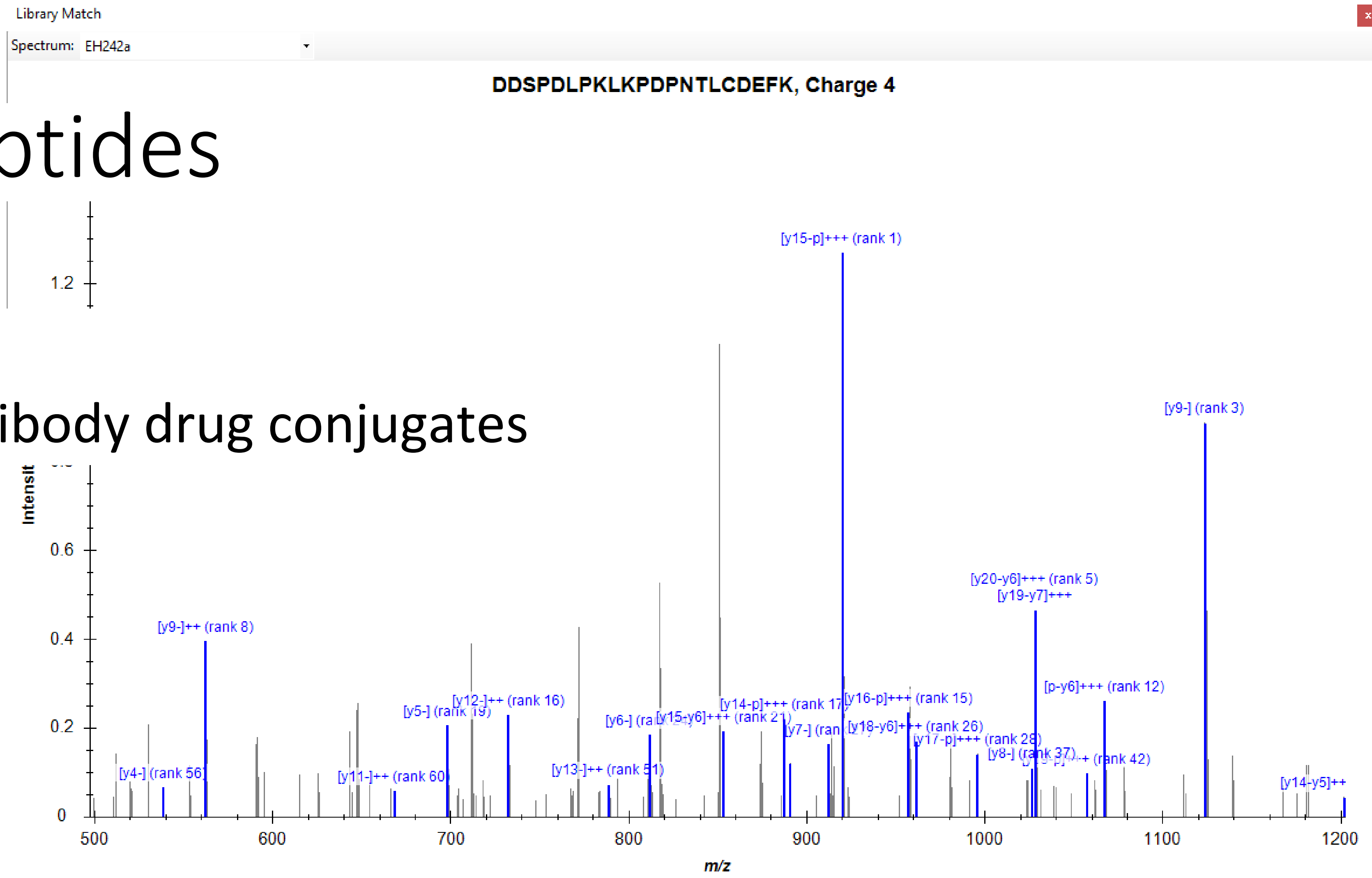
Skyline integrates the Prosit prediction server for proteome-wide DIA data analysis using on-demand fragment intensity and iRT prediction



m/z

See also – 2020 May Institute at Northeastern - <https://computationalproteomics.khoury.northeastern.edu/>

Crosslinked Peptides



- Chemical crosslinkers
- Disulfide bonds in antibody drug conjugates

Targets

Pe

DDSPDLPKLKPDNTLCDEFK-SLGKVGTR

850.6860++++ (dotp 0.99, dotp 0.93)

- precursor - 850.4342++++ (irank 4)[i 3]
- precursor [M+1] - 850.6849++++ (irank 1)[i 1]
- precursor [M+2] - 850.9355++++ (irank 2)[i 2]
- S [b3-] - 318.0932+ (rank 8)[7]
- P [y11-] - 1335.5885+ (rank 3)[3]
- P [y9-] - 1123.5088+ (rank 2)[2]
- F [y2-] - 294.1812+ (rank 10)[8]
- P [y9-] - 562.2581++ (rank 7)[6]
- P [y18-p] - 1027.8812+++ (rank 5)[9]
- P [y15-p] - 919.4932+++ (rank 1)[1]
- [y4] V - 432.2565+ (irank 4)[4]
- [y3] G - 333.1881+ (i
- [p-y6] G - 1066.8711-

680.7502, +5 (dotp 0.99,

DDSPDLPKLKPDNTLCDE

GACLLPKIETMR-EKVLTS

FKDLGEEHFK-DTHKSEIA



Nick Shulman – TP 082

Crosslinked peptide support in Skyline

Biopharmaceuticals MAM-QC

- Skyline Document Grid support
 - Modified area proportion
 - Attribute area proportion

Document Grid: WithGrouping			
Reports ▾ ↻ ⏪ ⏩ 32 of 50 ✕ 📄 Export... Actions ▾ Find:			
	Protein	Peptide Modified Sequence	Attribute Group ID
	NISTmAb_HC	QVTLR	
	NISTmAb_HC	Q[-17]VTLR	
	NISTmAb_HC	ESGPALVKPTQTLTLTC[+57]TFSGFSLSTAGMSVG...	
	NISTmAb_HC	QPPGK	

Configure Panorama Folder: /Panorama MAM

Create Project

Users / Permissions

Project Settings

Change File Root

Configure Panorama Folder

- Experimental data - A collection of published Skyline documents for various experimental designs
- Multi-attribute method (MAM)** - An experimental data folder variant with additional reporting for multi-attribute method analyses
- Chromatogram library - Curated precursor and product ion expression data for use in designing and validating future experiments
 - Rank peptides within proteins by peak area
- Quality control - System suitability monitoring of reagents and instruments

Finish

Document Summary

NIST PTM_2020-05-06_11-38-02.sky.zip 📄 37 MB ▾ 1 version

2 proteins, 50 peptides, 137 precursors, 411 transitions - 3 replicates, 2 lists - Skyline-daily (64-bit) 20.1.1.83 (d7f345585) - PTM Report, Peptide Map



Panorama

Biopharmaceuticals MAM-QC



Panorama

- Reports and tracking in Panorama

PTM Report

1 - 11 of 11

Modification	Sequence	Site Location	Chain	Sample Name		
				Sc_WCL-250ng-5ngNIST_rKCTi_3hRP-20cm_DE30_QE_3	Sc_WCL-250ng-5ngNIST_rKCTi_3hRP-20cm_DE30_QE_2	Sc_WCL-250ng-5ngNIST_rKCTi_3hRP-20cm_DE30_QE_1
Oxidation (M)	R.VGYMHWYQQKPGK.A	M32	NISTmAb_LC	13.59%	14.62%	17.89%
Oxidation (M)	K.VTNMDPADTATYYCAR.D	M87	NISTmAb_HC	12.11%	11.15%	11.31%
Oxidation (M)	R.WQQGNVFSCSVMHEALHNHYTQK.S	M431	NISTmAb_HC	19.59%	24.14%	22.15%
Oxidation (M)	K.DTLMISR.T	M255	NISTmAb_HC	44.55%	38.44%	41.02%
Oxidation (M)	-.DIQMTQSPSTLSASVGDR.V	M4	NISTmAb_LC	9.94%	9.85%	9.41%
Gln->pyro-Glu (N-term Q)	-.QVTLR.E	Q1	NISTmAb_HC	99.58%	99.54%	99.33%
G2F						3.80%
G1F						43.41%
G0F						48.80%
Deamidated (NQ)						7.39%
Lys clipping						84.96%

Josh Eckels – MP 252

Multi-Attribute-Method reporting with Panorama and Skyline

Panorama - Aggregating and Publishing



- Upload Skyline documents to private project
- Publish to **Panorama Public** – part of ProteomeXchange
 - 184 public data sets (75 more in review)
- Longitudinal instrument QC data
- Biopharmaceuticals MAM-QC
- Quant support
- Build chromatogram libraries
- Free hosted version (<http://panoramaweb.org>)
 - >450 separate projects
 - >8000 data sets uploaded (>13,000 QC docs)
- Locally installable server application (Panorama Partners)
 - Roche, Genentech, Regeneron, Merck, and NCI

Instrument Vendor Collaborators

▶ Agilent Technologies

- ▶ John Fjeldsted
- ▶ Marilyn Marx
- ▶ Christine Miller



▶ Bruker

- ▶ Sven Brehmer
- ▶ Jens Decker
- ▶ Stephanie Kaspar
- ▶ Gary Kruppa
- ▶ Markus Lubeck
- ▶ Pierre-Olivier Schmit



▶ AB Sciex

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



▶ Shimadzu

- ▶ Junko Iida
- ▶ Norio Mukai
- ▶ Toshiya Matsubara
- ▶ Kiriko Matsuo
- ▶ Jun Watanabe



▶ Thermo-Scientific

- ▶ Mary Blackburn
- ▶ Aaron Gahadjar
- ▶ Sebastien Gallien
- ▶ Bahvin Patel
- ▶ Vlad Zabrouskov



▶ Waters

- ▶ Martin Lunt
- ▶ Ronan O'Malley
- ▶ Keith Richards
- ▶ Hans Vissers



Skyline Team



Brendan MacLean



Nick Shulman



Brian Pratt



Vagisha Sharma



Rita Chupalov



Kaipo Tamura



Matt Chambers



Nat Brace



Brian Connolly



Tobi Rohde

