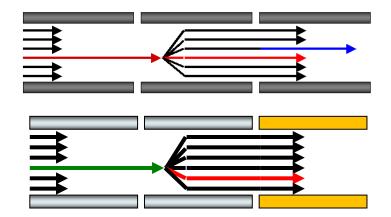


Targeted Proteomics Environment

Status of the Skyline open-source software project four years after its inception

Brendan MacLean





One Year

2011

- NCI funding ending in August
- Thursday poster at ASMS
- Broke ankle (twice)
- Hard drive died

2012

- Great new funding
- User meeting!
- New developers
- Progress on multiple fronts

This Meeting

Mike: 20-40 people, under \$1000

Sponsors















I 50+ people registered!

User Community After 4 Years

570 registered users

• 4 new papers in May

- Platform Independent and Label-free Quantitation of Proteomic Data using MSI Extracted Ion Chromatograms in Skyline – Mol. Cel. Prot.
- Label-Free Quantitation of Protein Modifications by Pseudo-Selected Reaction Monitoring with Internal Reference Peptides – J. Prot. Res.
- Using iRT, a Normalized Retention Time for More Targeted Measurement of Peptides - Proteomics
- The Development of Selected Reaction Monitoring Methods for Targeted Proteomics via Empirical Refinement - Proteomics
- > 25 abstracts at ASMS mention Skyline
- ▶ 75 citations of original paper (after 2 1/2 years)
 - ▶ 30 in 2012

Software Development After 4 Years

- Strong professional development team
- Grad student contributions
 - Jarrett Egertson
- Undergraduate internship program
 - Shannon Joyner Carnegie Melon University
 - Daniel Broudy Harvard University
- Growing outside contributions
 - Matthew Chambers Tabb Lab Vanderbilt
 - Lucia Espona Pernas Aebersold Lab ETH
 - David Cox AB SCIEX
 - Kevin Crowell PNNL

Brendan MacLean

- Lead developer and architect
- > 20+ years of professional software development
 - Big companies (Microsoft & BEA)
 - Small companies (Westside & LabKey)
 - Academia (Fred Hutchinson & U. of Washington)
- 9 years of proteomics

Focus

- Experimental structure
- All things Skyline...



Skyline File View



Nick Shulman

I7 years of professional software development

5 years at Microsoft & 12 years with Brendan

Creator of

- Custom reports & Results grid
- Custom annotations
- Background proteomes
- Topograph protein turnover

Focus – Peak Integration

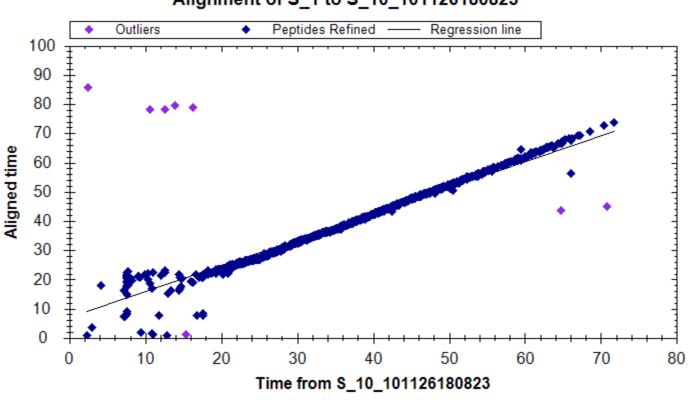
- Retention time alignment for MSI filtering
- mProphet algorithm





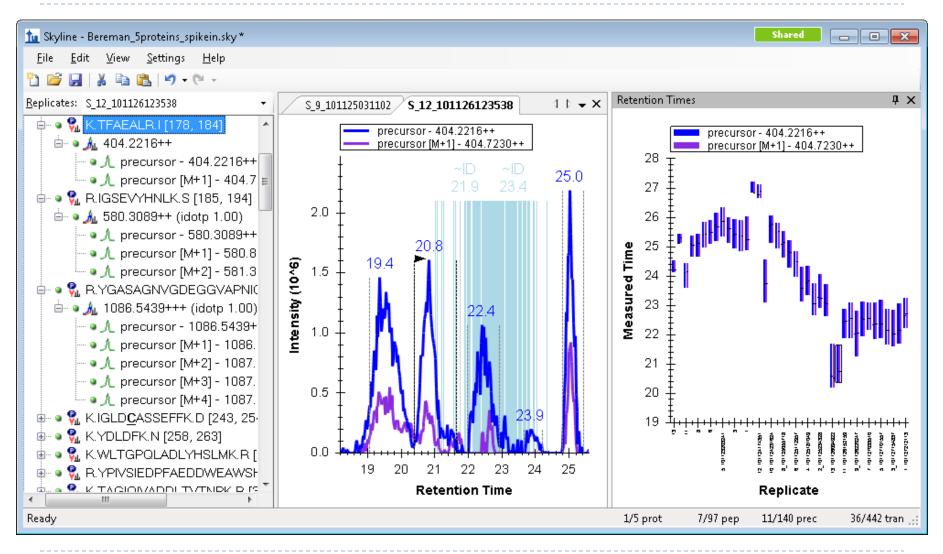
MS1 Filtering Retention Time Alignment

Aligning by linear regression of MS/MS peptide IDs



Alignment of S_1 to S_10_101126180823

MS1 Filtering Retention Time Alignment



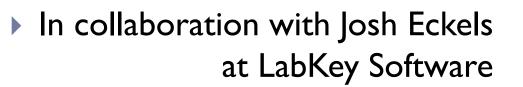
Vagisha Sharma

6 years of professional software development

- All in proteomics
- 4 years on proteomics repositories

Focus

Panorama WP 407 - Vagisha Sharma A private repository of targeted proteomics assays for Skyline







Panorama Peptide Details View

argeted MS	Folder		Targeted MS D	ashboard
	s > MRMer.zip >			
NNPETL	VALR			
eptide Sumr	nary			
File M	IRMer.zip			
Protein Y	(AL038W			
Sequence T	INNPETLVALR			
NeutralMass 1				
-	26.898569107055664			
	NNPETLVALR - 614.3382 NNPETLVAL <mark>R</mark> - 619.3423			
,	- 019.542	Strt (neavy) 2		
hart Width	400	Chart Height	400	
ynchronize Y-a	xis 🗹	Synchronize X-axis	s 🗹	
PDATE				
Chromatogr	rams			
Chromatog	rams			
Chromatogr	r ams silac_1_to_4 TNNPETLVALR		silac_1_to_4 TNNPETLVALR - 614.3382++ TNNPETLVALR - 619.3423++ (heavy)	
			silac_1_to_4 TNNPETLVALR - 614.3382++ 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,250 2,	
4,000	silac_1_to_4 TNNPETLVALR		TNNPETLVALR - 614.3382++ TNNPETLVALR - 619.3423++ (heavy) 2,250 2,250 2,250 - 26.9	
	silac_1_to_4 TNNPETLVALR		TNNPETLVALR - 614.3382++ TNNPETLVALR - 619.3423++ (heavy) 2,250 2,250 2,000 2,250	
4,000 - 3,500 -	silac_1_to_4 TNNPETLVALR		TNNPETLVALR - 614.3382++ TNNPETLVALR - 619.3423++ (heavy) 2,250 2,250 2,250 2,200 2,200 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750	
4,000 - 3,500 -	silac_1_to_4 TNNPETLVALR		TNNPETLVALR - 614.3382++ TNNPETLVALR - 619.3423++ (heavy) 2,250 2,250 2,250 2,200 2,200 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750	
4,000 - 3,500 -	silac_1_to_4 TNNPETLVALR		TNNPETLVALR - 614.3382++ TNNPETLVALR - 619.3423++ (heavy) 2,250 2,250 2,250 2,200 2,200 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750	
4,000 - 3,500 -	silac_1_to_4 TNNPETLVALR		TNNPETLVALR - 614.3382++ TNNPETLVALR - 619.3423++ (heavy) 2,250 2,250 2,250 2,200 2,200 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750	
4,000 - 3,500 -	silac_1_to_4 TNNPETLVALR		TNNPETLVALR - 614.3382++ TNNPETLVALR - 619.3423++ (heavy) 2,250 2,250 2,250 2,200 2,200 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750	
4,000 - 3,500 -	silac_1_to_4 TNNPETLVALR		TNNPETLVALR - 614.3382++ 2,250 2,250 2,000 2,250 1,750 2,250 1,500 1,500 1,250 1,250	
4,000	silac_1_to_4 TNNPETLVALR		TNNPETLVALR - 614.3382++ TNNPETLVALR - 619.3423++ (heavy) 2,250 2,250 2,250 2,200 2,200 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750 1,750	
4,000 - 3,500 - 3,500 - 2,500 - 1,500 - 1,000 -	silac_1_to_4 TNNPETLVALR		TNNPETLVALR - 619.3423++ (heavy) 2,250 2,000 1,750 1,500 1,250 500	
4,000 · 3,500 · 2,500 · 2,500 · 1,500 ·	silac_1_to_4 TNNPETLVALR		TNNPETLVALR - 619.3423++ (heavy) 2,250 2,000 1,750 1,500 1,250 1,250 500 250	
4,000 - 3,500 - 3,500 - 2,500 - 1,500 - 1,000 - 500 - 0 -	silac_1_to_4 TNNPETLVALR	27.5 29.0	TNNPETLVALR - 619.3423++ (heavy)	20.0
4,000 · 3,500 · 3,3000 · 2,500 · 1,500 · 1,000 · 500 ·	silac_1_to_4 TNNPETLVALR	27.5 28.0	TNNPETLVALR - 619.3423++ (heavy) 2,250 2,000 1,750 1,500 1,250 1,250 500 250	28.0
4,000 - 3,500 - 3,500 - 2,500 - 1,500 - 1,000 - 500 - 0 -	silac_1_to_4 TNNPETLVALR		TNNPETLVALR - 619.3423++ (heavy) 2,250 2,000 1,750 1,500 1,250 500 250 0 26.0 26.5 27.0 27.5 28.0 TNNPETLVALR - 619.3423++ (heavy) 2,250 2,000 1,250 1,250 2,000 1,250 2,000 2,250 2,000 1,250 2,000 2,250 2,000 2,250 2,000 2,000 1,250 2,000 2,000 1,250 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,000 2,	

Don Marsh

35 years of professional software development

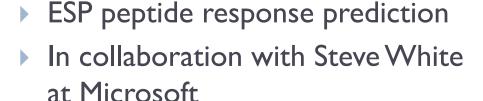
- Big companies Apple and Microsoft
- Medium Tagilent and Stride Micro
- Co-founder of two start-up companies, one acquired by Microsoft

Contributed: 64-bit Skyline and DIA Isolation Schemes

Lots of stress testing

Focus

- Full-scan filtering
- Performance



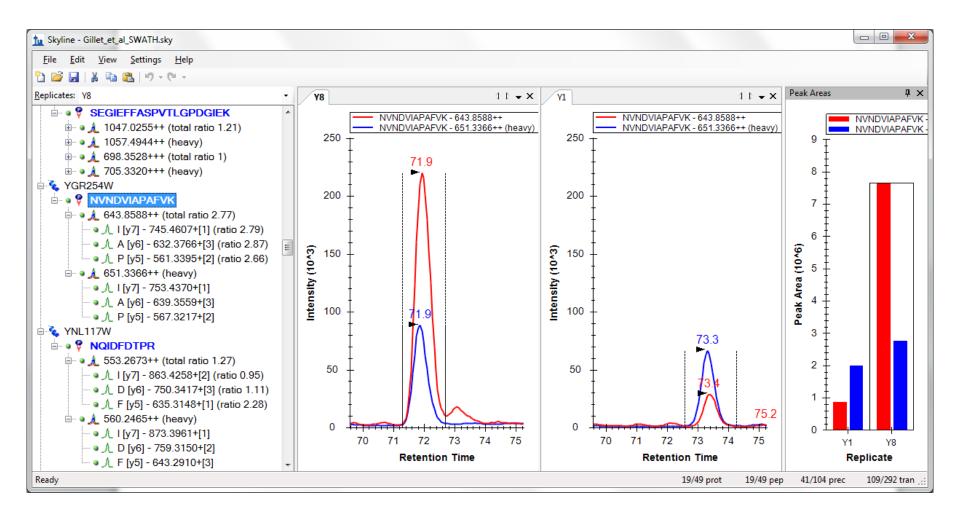


Skyline AB SCIEX SWATHTM Settings (32 x 25 m/z Extraction Windows)

Transition Settings	tu Ed	it Isolation Scheme		×	Π
Prediction Filter Library Instrument Full-Scan	Name:				
MS1 filtering		1	-1-	Cancel	
MS/MS filtering		Use results data isolation targ Isolation <u>w</u> idth: 2 Th	ets		
Acquisition method: Product mass analyzer:	<u>P</u> respect	ified isolation windows			11
DIA 🔻 TOF 👻		Start	End	Margin	4
	•	400.5	425.5	0.5	Ξ
		425.5	450.5	0.5	۲
Isolation scheme: Resolving power:		450.5	475.5	0.5	
SWATH - 10,000		475.5	500.5	0.5	
		500.5	525.5	0.5	
		525.5	550.5	0.5	
		550.5	575.5	0.5	
Filter only retention time scheduling windows	Margi <u>n</u> s:				
		Symmetric			•
OK Cancel				.4	

Gillet, L.C. et al. Mol. Cell. Prot. 2012.

AB SCIEX SWATHTM Data



Gillet, L.C. et al. Mol. Cell. Prot. 2012.

Quantitative Proteomics

Spectrum-based

- Spectral counting
- Isobaric tags
- Chromatography-based
 - SRM SRM: 220 abstracts, MRM: 390 abstracts
 - MSI chromatogram extraction
 - Targeted MS/MS
 - Data independent acquisition (DIA)
 DIA: 9 abstracts, SWATH: 18 abstracts



2010 Support Multiple Instrument Vendors

- SRM
- Exporting transition lists & native methods
- Importing native instrument output files
- AB SCIEX
- Agilent Technologies
- Thermo-Scientific
- Waters

2012 Support Multiple Instrument Vendors

- Full-Scan
- Exporting isolation lists & native methods
- Importing native instrument output files
- AB SCIEX
- Agilent Technologies
- Thermo-Scientific
- Waters

2012 Support Multiple Instrument Vendors

- Full-Scan
- Exporting isolation lists & native methods
- Importing native instrument output files
- ► AB SCIEX SWATH[™]
- Agilent Technologies
- Thermo-Scientific

Waters

DIA DIA & Multiplexed DIA MSe™



WOA 10am - Brendan MacLean

Targeted Proteomics Quantitative Analysis of Data Independent Acquisition MS/MS in Skyline

New Full-Scan Features for v1.2 (February)

- Integrated display of MS/MS peptide ID spectra in MSI chromatograms
- Peak picking in MS1 chromatograms based on MS/MS peptide ID
- Improved memory performance for full-scan chromatogram extraction
- New isotope dot-product score on MS1 full-scan filtered peaks, and expected relative isotope abundance in peak area plot and reports
- Faster MS/MS library loading
- Method export for Thermo and AB SCIEX
- Thermo Q Exactive data support

New Features for v1.2 (February)

- Command-line interface
- More accurate retention time prediction with integrated iRT support
- New enhanced Find with Find All
- Unexpected error form

New Features for v1.3 (June)

Advanced support for data independent acquisition (DIA)

- ► AB SCIEX SWATH[™]
- Agilent DIA
- Thermo Multiplexed DIA
- ► Waters MSeTM
- 64-bit version with higher memory limits
- Retention time alignment for MS1 filtering
- Auto-detect modifications in Spectral Library Explorer
- Decoy peptide and transition generation for FDR based peak picking

New Features for v2.1 (Fall)

- Panorama support
- Full-scan mass accuracy
- Data import performance
- Customizable Tools menu
- New algorithms
 - mProphet probability based peak picking
 - ESP peptide response prediction
- Experiment structure with File View
 - Quantitative statistics
 - Experiment statistics
- Agilent tMRM and Thermo iSRM support

Acknowledgments:

Skyline Team (emeriti)

- Eva Baker
- John Chilton
- Gregory Finney
- Barbara Frewen
- Mimi Fung
- Randall Kern
- Alana Killeen
- Daniela Tomazela

Broad Institute

- Sue Abbatiello
- Steve Carr
- Jake Jaffe

Duke

- Will Thompson
- Arthur Moseley

Buck Institute

- Birgit Schilling
- Matthew Rardin
- Brad Gibson

IMSB

- Rudolph Aebersold
- Ludovic Gillet
- Christina Ludwig
- Vanderbilt
 - Matthew Chambers
 - Amy Ham
 - Daniel Liebler

AB Sciex

- Fadi Abdi
- David Cox
- Christie Hunter
- Brent Lefebvre

Agilent Technologies

- Christine Miller
- Joe Roark
- Pat Perkins

Thermo-Scientific

- Markus Kellmann
- Andreas Kuehn
- Vlad Zabrouskov

Waters

- Laurence Firth
- James Langridge
- Roy Martin
- Kieran Neeson
- Keith Richards

This work is funded by grants from NIH/NIGMS, NIH/NHGRI, Agilent Technologies and Thermo-Fisher Scientific.