

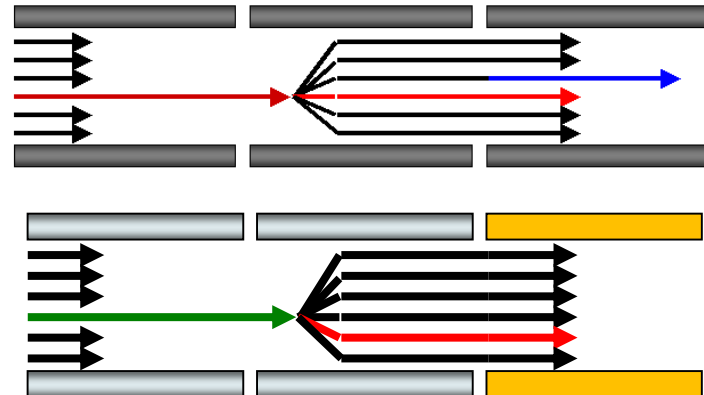


Skyline

Targeted Proteomics Environment

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

Brendan MacLean



Here Thanks to NIH Funding

- ▶ **NCI CPTAC** **2008 – 2011**
- ▶ **NIGMS** **2011 – 2016**
- ▶ **NCI LINCS** **2011 – 2019**



Instrument Vendor Partnerships

▶ 3 years



▶ 2 years



▶ 1 year



▶ Next year



User Community After 6 Years

- ▶ 3500+ registered users
- ▶ 2500+ support posts
- ▶ 270 registered for this meeting
- ▶ 53 abstracts at ASMS mention Skyline
- ▶ 350+ citations of original paper

- ▶ Chinese and Japanese translations
 - ▶ 43 volunteer reviewers



Teaching Targeted Proteomics

- ▶ *Weeklong course, Zurich (July 2013)*
- ▶ *Weeklong course, Zurich (February)*
- ▶ *ABRF workshop, Albuquerque (March)*
- ▶ *Weeklong course, Seattle (April)*
- ▶ *US HUPO workshop, Seattle (April)*
- ▶ **ASMS short course, Baltimore (June)**
- ▶ *EPS workshops, Brixen (August)*
- ▶ *Weeklong Course, Barcelona (October)*
- ▶ *Short course, Seoul (October)*
- ▶ *Short course, Mumbai (December)*



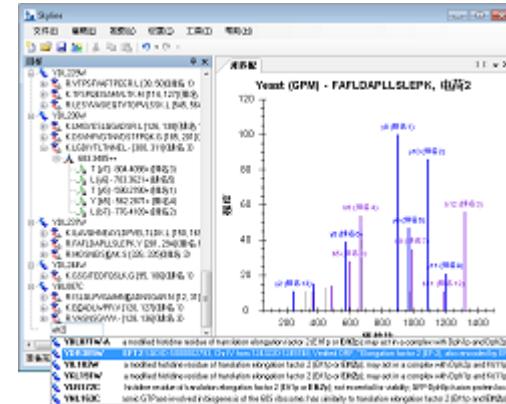
Chinese and Japanese Translations

- ▶ 2 years of preparation
- ▶ New testing tools
- ▶ Skyline 2.5 Chinese and Japanese
 - ▶ Released May 5th

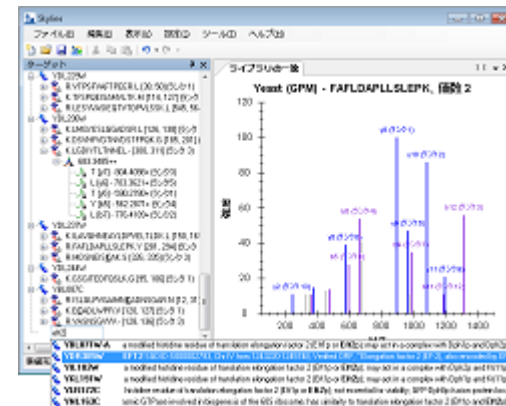
▶ Method Editing tutorial

- ▶ 5 more in review
- ▶ Other languages? (\$30,000)

靶向方法编辑 (25 页)



ターゲットメソッドの編集 (25ページ)



SkylineTester Critical Test Tool

The screenshot displays the Skyline Tester application window. The title bar reads "Skyline Tester". The menu bar includes "File", "Find", "Select build", "Options", and "Help". Below the menu bar is a tabbed interface with tabs for "Forms", "Tutorials", "Tests", "Build", "Quality", "Nightly", and "Output". The "Forms" tab is active, showing a "View Skyline forms" section. On the left, there is a "Language" dropdown menu set to "Chinese" and an "Options" section with a checked checkbox for "Show form names". The main area contains a table of forms with the following data:

Form	Test	Seen
GraphSpectrum	TestMs1Tutorial	1
ImmediateWindow	ImmediateWind...	0
ImportDocResultsDlg	TestImportDoc	1
ImportIonMobilityFromSpectralLibraryDlg	TestIonMobility	1
ImportPeptideSearchDlg.ChromatogramsPa...	TestMs1Tutorial	1
ImportPeptideSearchDlg.FastaPage	TestMs1Tutorial	1
ImportPeptideSearchDlg.MatchModsPage	TestMs1Tutorial	1
ImportPeptideSearchDlg.Ms1FullScanPage	TestMs1Tutorial	1
ImportPeptideSearchDlg.SpectraPage	TestMs1Tutorial	1

At the bottom right of the window is a "Run" button. The status bar at the bottom indicates "bin (64 bit)" and a timer showing "0:00:00".

SkylineTester

- ▶ Automated access to any form in any language
- ▶ Automated access to tutorial screenshots
- ▶ Overnight runs of 340 tests in all languages
- ▶ Detects memory leaks

- ▶ Running in hospitality suites
 - ▶ Agilent
 - ▶ Thermo
 - ▶ Waters



Storing and Processing

- ▶ Cloud storage for raw data
- ▶ Integrated with Skyline
- ▶ Cloud chromatogram extraction
- ▶ Free introduction (<http://chorusproject.org>)
 - ▶ Amazon hosted and guaranteed
- ▶ Open access

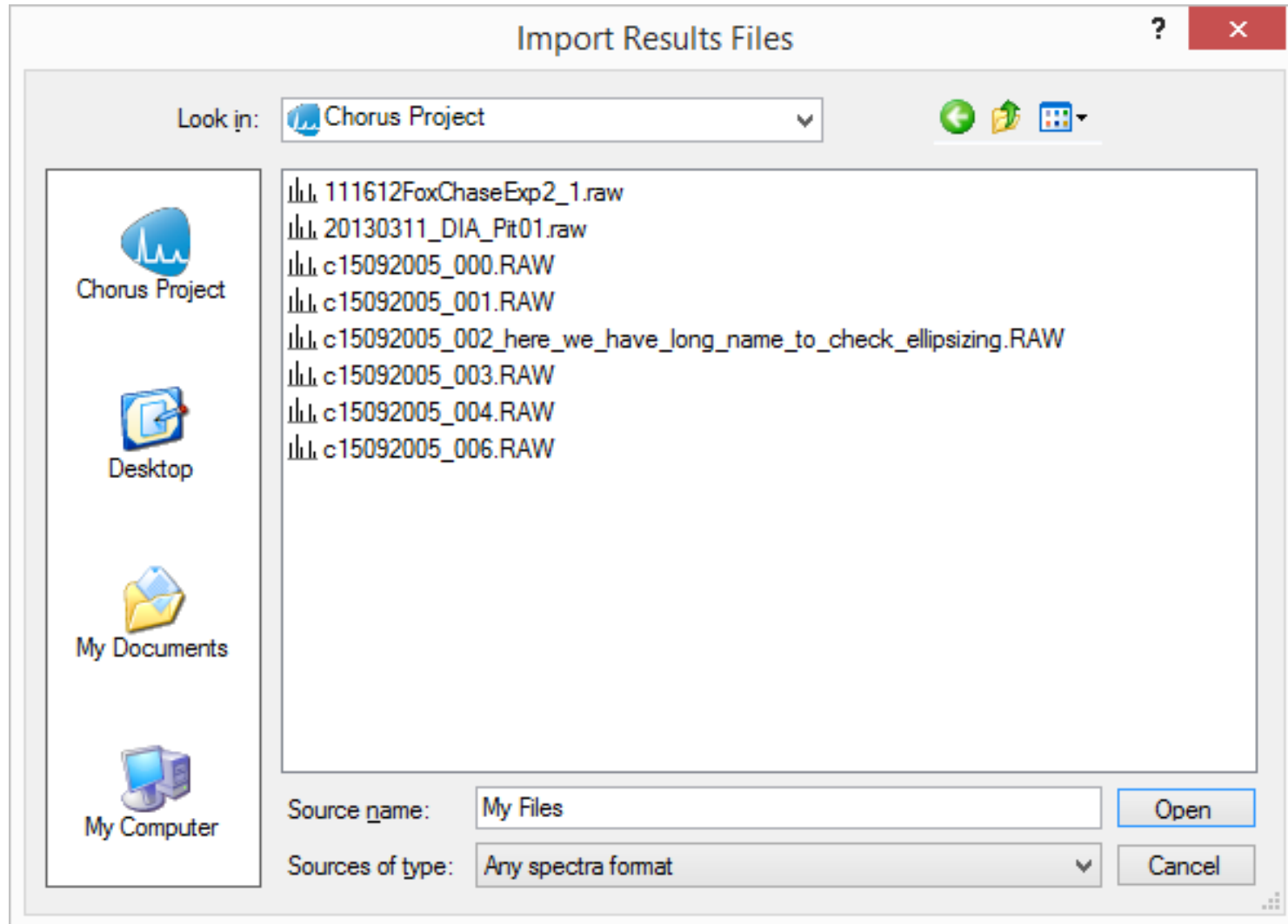


WOG 4:10pm - Brendan MacLean

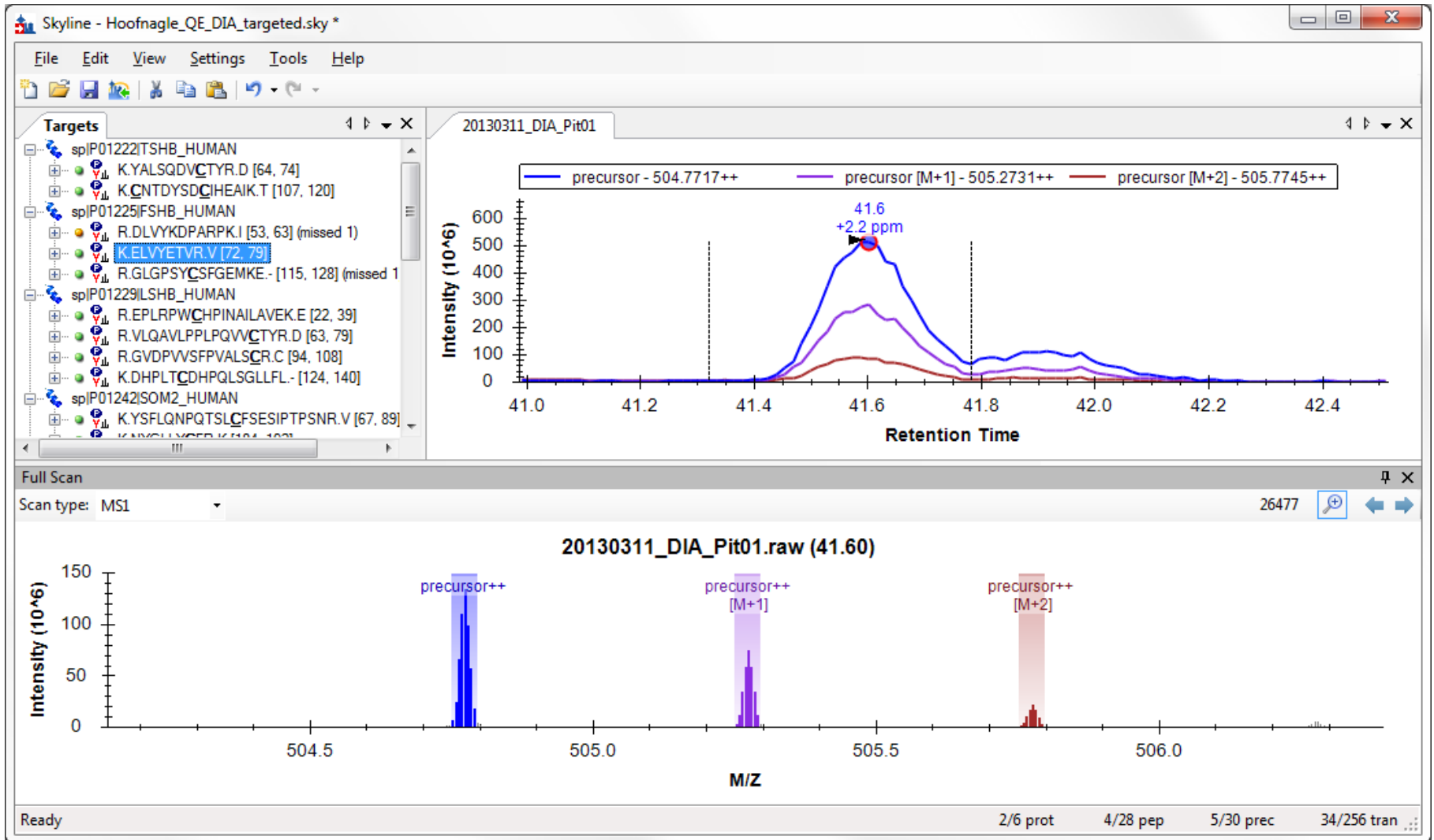
Rapid Processing of Large Scale Quantitative Proteomics Projects:
Integration of Skyline with the CHORUS Cloud



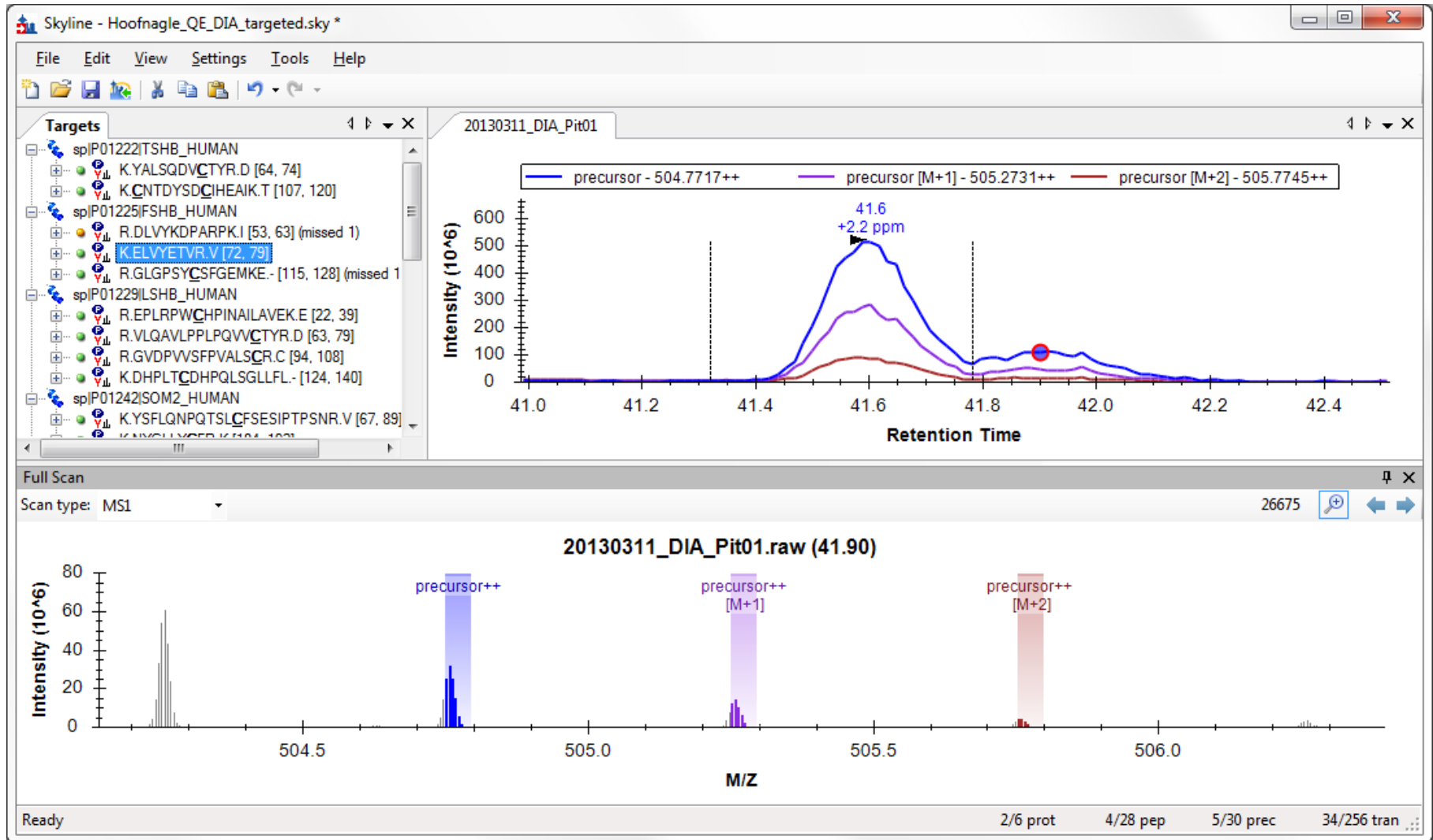
Skyline Direct Access to Chorus



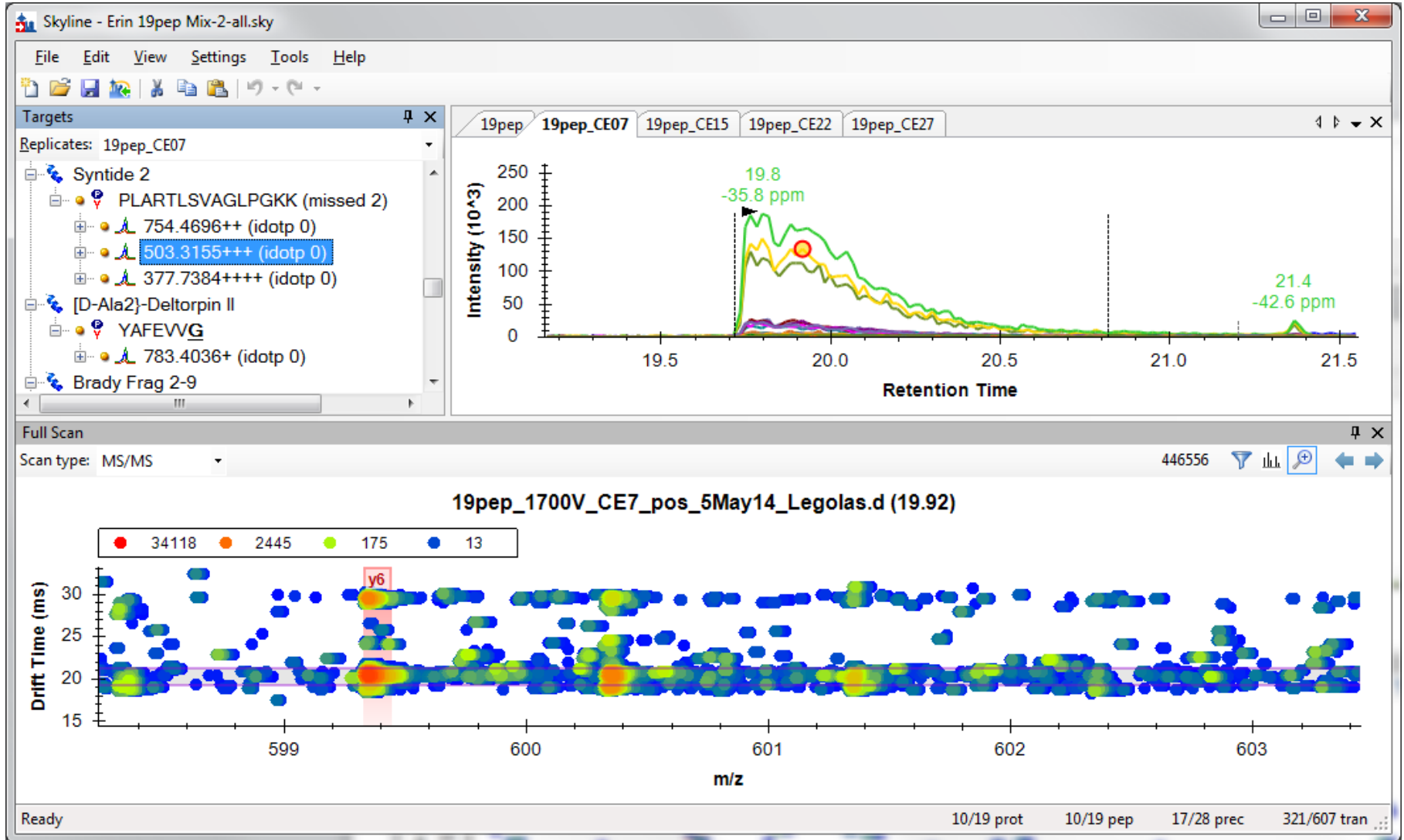
Full Scan Spectrum View



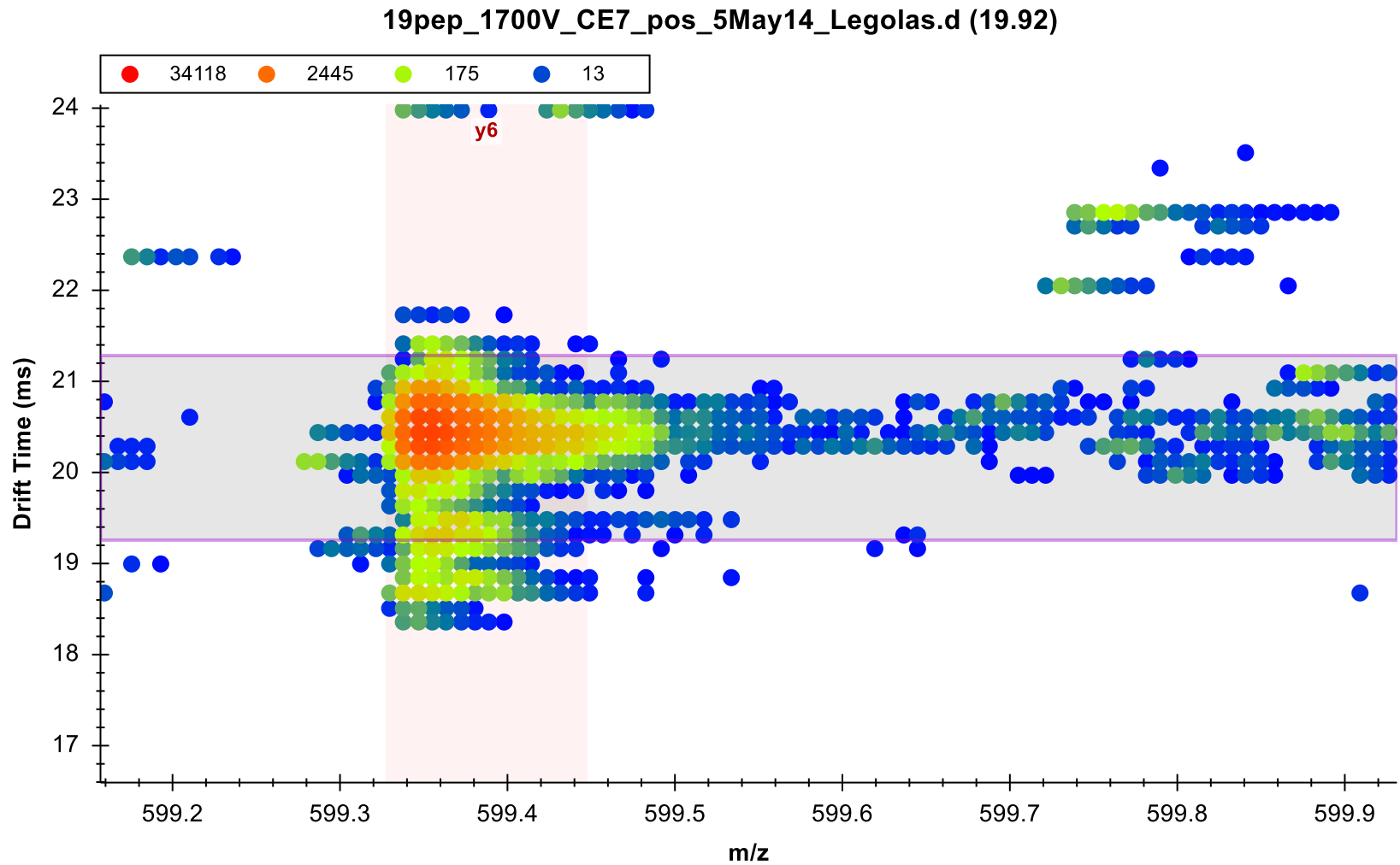
“Like taking the blinders off”



Exploring Ion Mobility MS/MS Scans

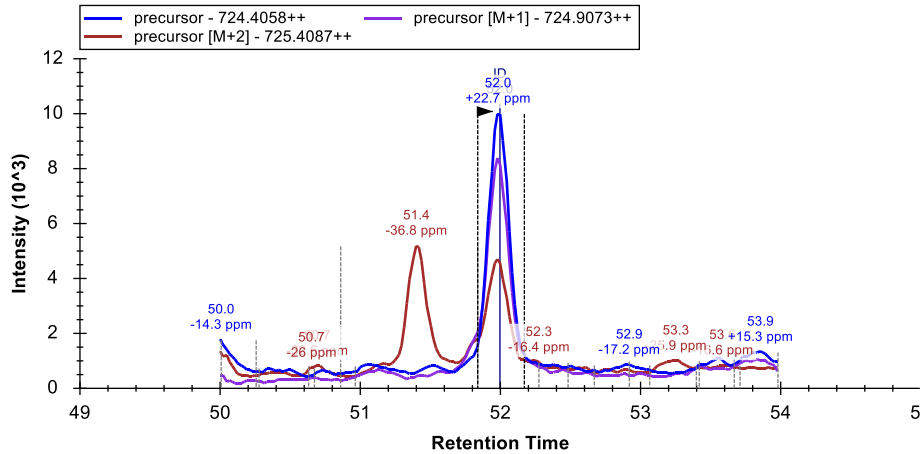


Exploring Ion Mobility MS/MS Scans

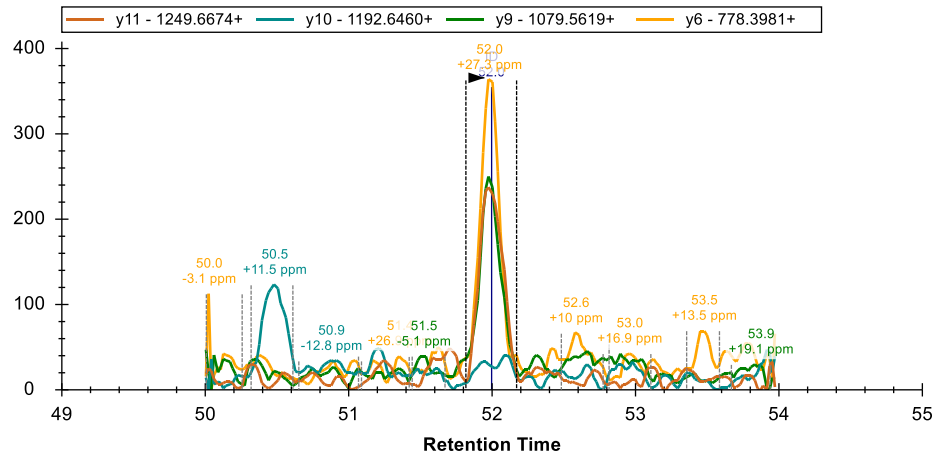
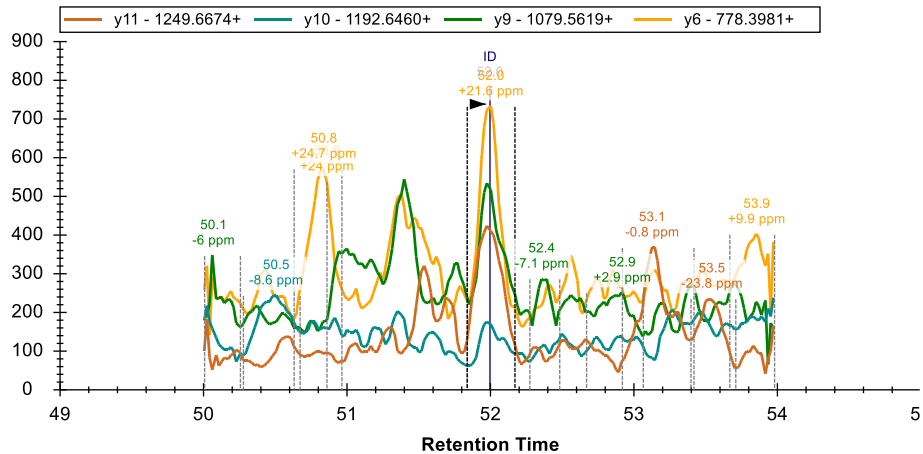
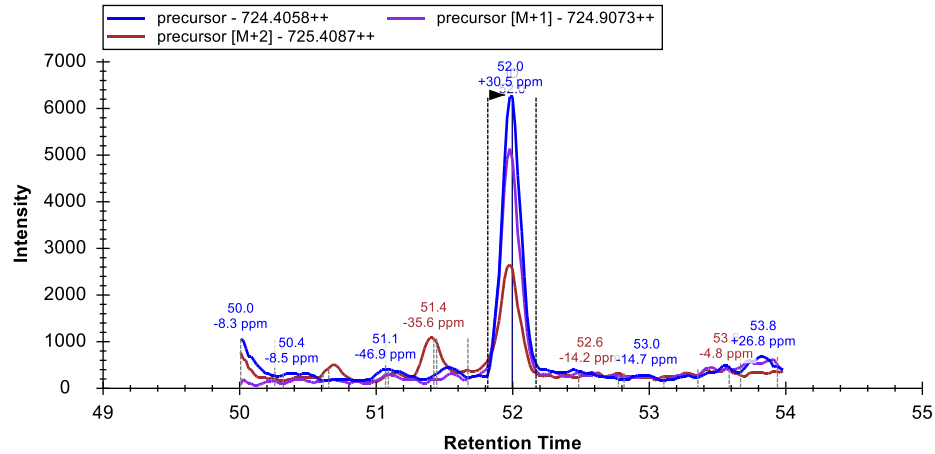


Yeast Peptides in Mouse Brain

Without IMS Drift Time Filtering



With IMS Drift Time Filtering, Rs=40



Aggregating and Publishing

- ▶ Publish fully annotated Skyline documents
 - ▶ Publication protocol in 2014
- ▶ Build chromatogram libraries
- ▶ Aggregate lab QC data (future)

- ▶ Free hosted version (<http://panoramaweb.org>)
 - ▶ 66 separate projects so far (LINCS, CPTAC and ABRF sPRG)
 - ▶ >780 data sets uploaded
 - ▶ User controlled security
- ▶ Locally installable server application
- ▶ Free and open source (Apache 2.0)



Panorama Partnership Program

- ▶ 2 additional labs
- ▶ Seeking leading edge targeted proteomics infrastructure
- ▶ Local Panorama server installation
- ▶ Direct collaboration with Skyline/Panorama team
- ▶ Full support for 1 year

- ▶ Josh Eckels (LabKey Software)



6 External Tools

Msstats (Vitek)



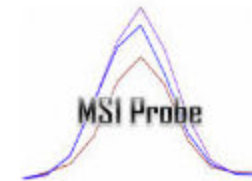
grouped study statistics

QuaSAR (Carr)



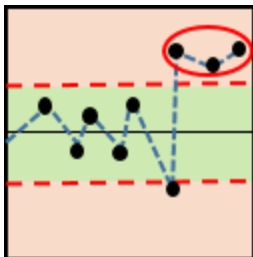
response curve statistics

MSI Probe (Gibson)



MSI filtering statistics

SProCoP (MacCoss)



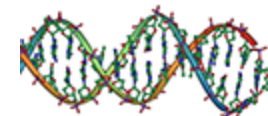
system suitability

Protter (Wollscheid)



transmembrane topology

Population Variation



mutation frequency

Replicate Annotations and Live Reports

Define Annotation

Name:
Condition

Type:
Value List

Values:
Disease
Healthy

Applies To:

- Proteins
- Peptides
- Precursors
- Transitions
- Replicates
- Precursor Results
- Transition Results

Results Grid

Replicate Name	SubjectId	BioReplicate	Run	Condition
D_172_REP1	D172	6	6	Disease
D_172_REP2	D172	6	20	Disease
D_172_REP3	D172	6	34	Disease
D_196_REP1	D196	7	7	Disease
D_196_REP2	D196	7	21	Disease
D_196_REP3	D196	7	35	Disease
H_146_REP1	H146	8	8	Healthy
H_146_REP2	H146	8	22	Healthy
H_146_REP3	H146	8	36	Healthy
		9	9	Healthy
		9	23	Healthy
		9	37	Healthy
		10	10	Healthy
		10	24	Healthy
		10	38	Healthy
		11	11	Healthy
		11	25	Healthy
		11	39	Healthy

Filter:

Annotation Settings

Annotations are extra pieces of data which you can attach to elements in a Skyline document. Use this dialog to control which annotations are available in this document, as well as to define new annotations.

- SubjectId
- BioReplicate
- Run
- Condition
- Concentration

Edit List...

OK Cancel

Live Reports

- ▶ 100x faster
- ▶ Responsive to document changes
- ▶ Direct editing and pasting
- ▶ Filtering
- ▶ Linked to other views



What's Next?

- ▶ **More of the same**
 - ▶ Peak picking investigation and improvements
 - ▶ Production release of Chorus integration
 - ▶ Complete translations to Chinese and Japanese
 - ▶ More great visualizations
 - ▶ More great support and instruction
 - ▶ Keep of with innovation from 6 instrument vendors
- ▶ **Integrated calibration**
- ▶ **Integrated differential statistics**
- ▶ **Integrate with search from Sangtae Kim**



Skyline Team

▶ Nick Shulman



▶ Don Marsh



▶ Brian Pratt



▶ Dario Amodei



▶ Vagisha Sharma



▶ Kaipo Tamura



▶ Yuval Boss

▶ Jarrett Egertson

▶ Max Horowitz-Gelb

▶ Danny Broudy

▶ Trevor Killeen



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▶ U. of Wa.

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- ▶ Jim Bolinger
- ▶ Jimmy Eng
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- ▶ D. R. Mani

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- ▶ Matthew Rardin
- ▶ Brad Gibson

▶ Duke

- ▶ Will Thompson
- ▶ Arthur Moseley

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- ▶ Christina Ludwig
- ▶ Olga Schubert
- ▶ Hannes Röst
- ▶ George Rosenburger
- ▶ Lucia Espona Pernas

▶ PNNL

- ▶ Sam Payne
- ▶ Sangtae Kim

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- ▶ Meena Choi
- ▶ Olga Vitek

▶ Stanford

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- ▶ Parag Mallick

▶ Vanderbilt

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- ▶ Daniel Liebler
- ▶ David Tabb



Instrument Vendor Collaborators

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- ▶ Shripad Torvi



▶ Bruker

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- ▶ Kiriko Matsuo



▶ Thermo-Scientific

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- ▶ Markus Kellmann
- ▶ Andreas Kuehn
- ▶ Vlad Zabrouskov



▶ Waters

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- ▶ Keith Richards

