Skyline
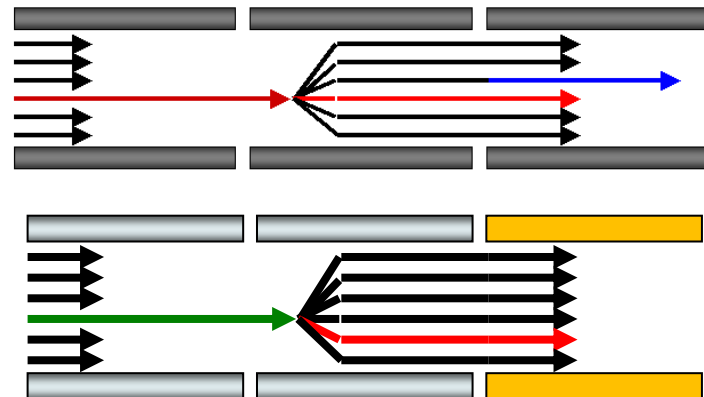
Targeted Proteomics Environment

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

Brendan MacLean

W

UNIVERSITY *of*
WASHINGTON

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

- 150+ 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 MS1 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 ½ 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
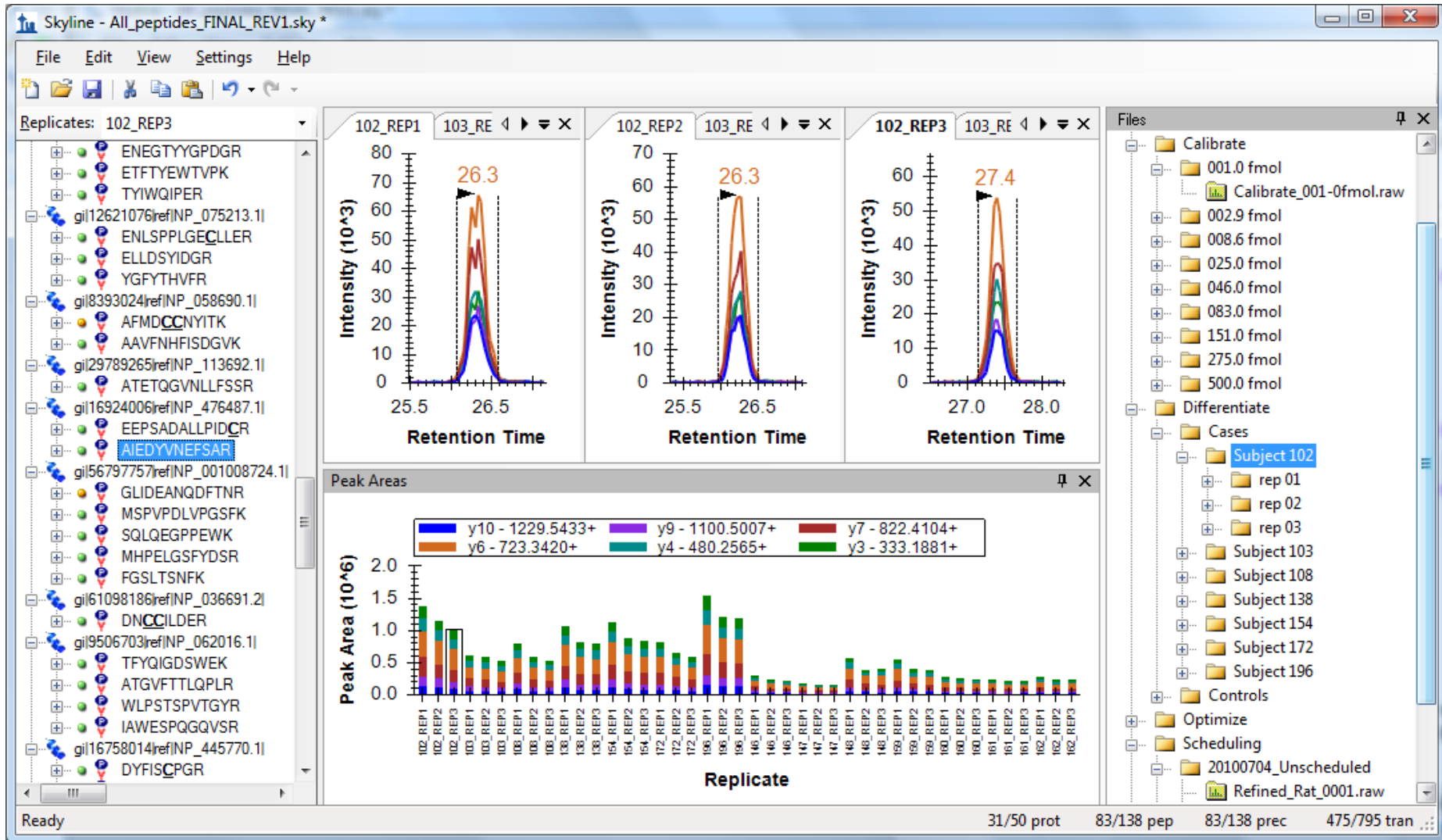 - David Cox – AB SCIEX
  - Kevin Crowell – PNNL

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
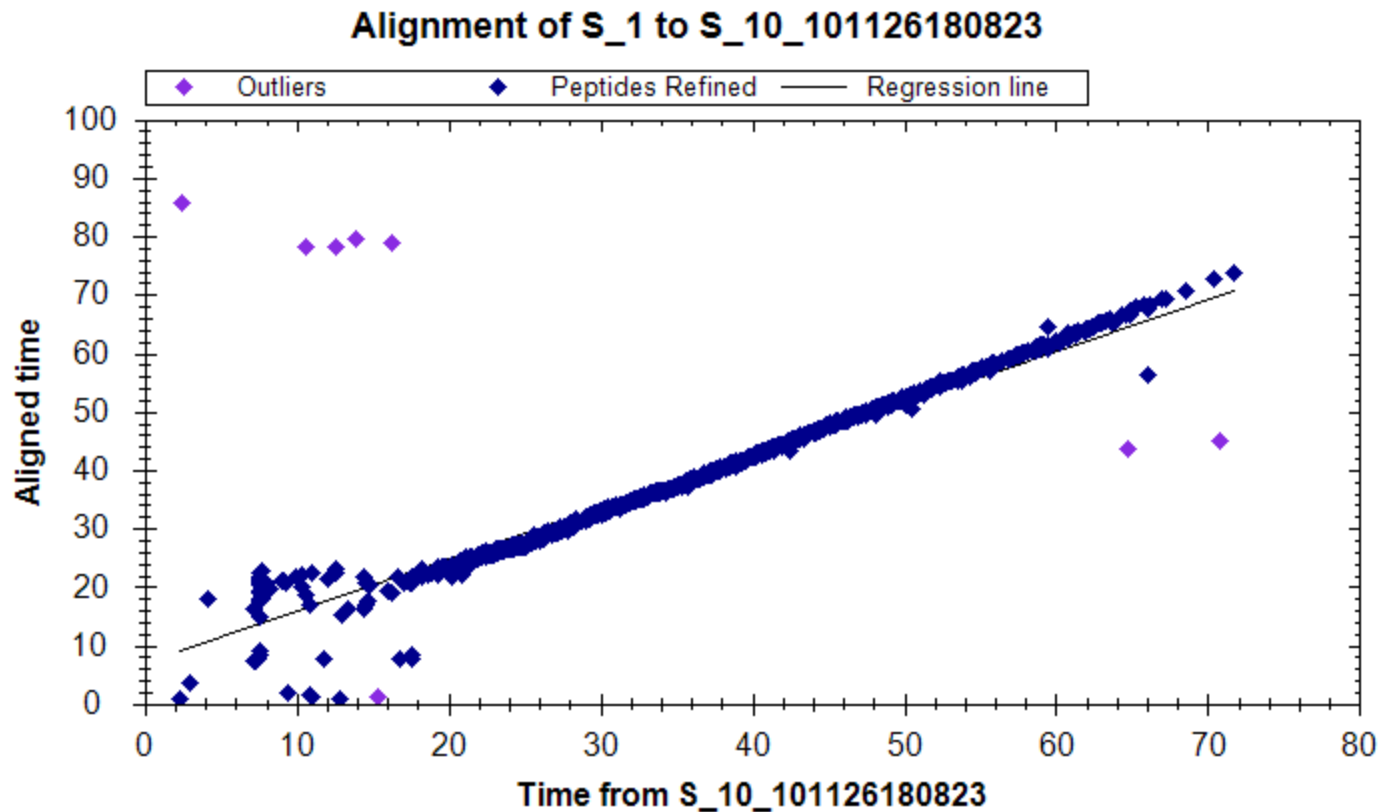
- ▶ 17 years of professional software development
  - ▶ 5 years at Microsoft & 12 years with Brendan
- ▶ Creator of
  - ▶ Custom reports & Results grid
  - ▶ Custom annotations
  - ▶ Background proteomes
  - ▶ Topograph – protein turnover

- ▶ Focus – Peak Integration
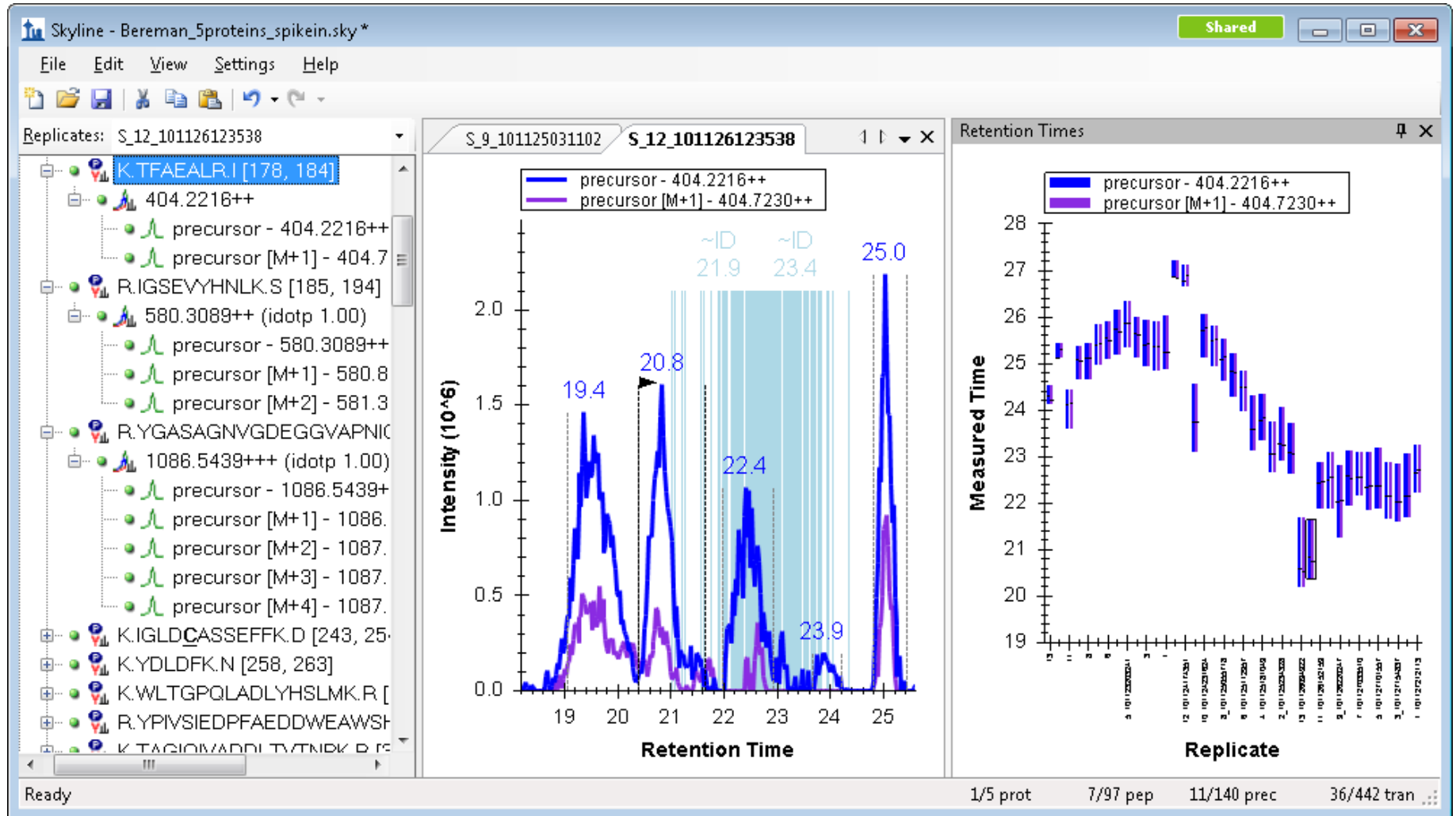 - ▶ Retention time alignment for MS1 filtering
  - ▶ mProphet algorithm

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
A private repository of targeted proteomics assays for Skyline

WP 407 - Vagisha Sharma

- In collaboration with Josh Eckels at LabKey Software

Panorama Peptide Details View

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

  ‣ ESP peptide response prediction

  ‣ In collaboration with Steve White at Microsoft

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

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

AB SCIEX SWATH™ 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

  ▸ MS1 chromatogram extraction

  ▸ Targeted MS/MS

  ▸ Data independent acquisition (DIA)
    DIA: 9 abstracts, SWATH: 18 abstracts

Skyline

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   DIA

▸ Thermo-Scientific   DIA & Multiplexed DIA

▸ Waters   MSe™

Skyline

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 MS1 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 MSe™
- 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

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