



Multi-Instrument, Skyline-based Comparison of DIA Peptide Detection and Statistical Confidence Tools

Dario Amodei

Postdoctoral Scholar, Skyline Developer

Mallick Lab

Stanford University

6/15/2014

Acquisition Methods

Targeted

Selected Reaction
Monitoring (SRM)

Few proteins
Good reproducibility
Good sensitivity

Discovery

Data Dependent
Acquisition (DDA)

Many proteins
Poor reproducibility
Poor sensitivity

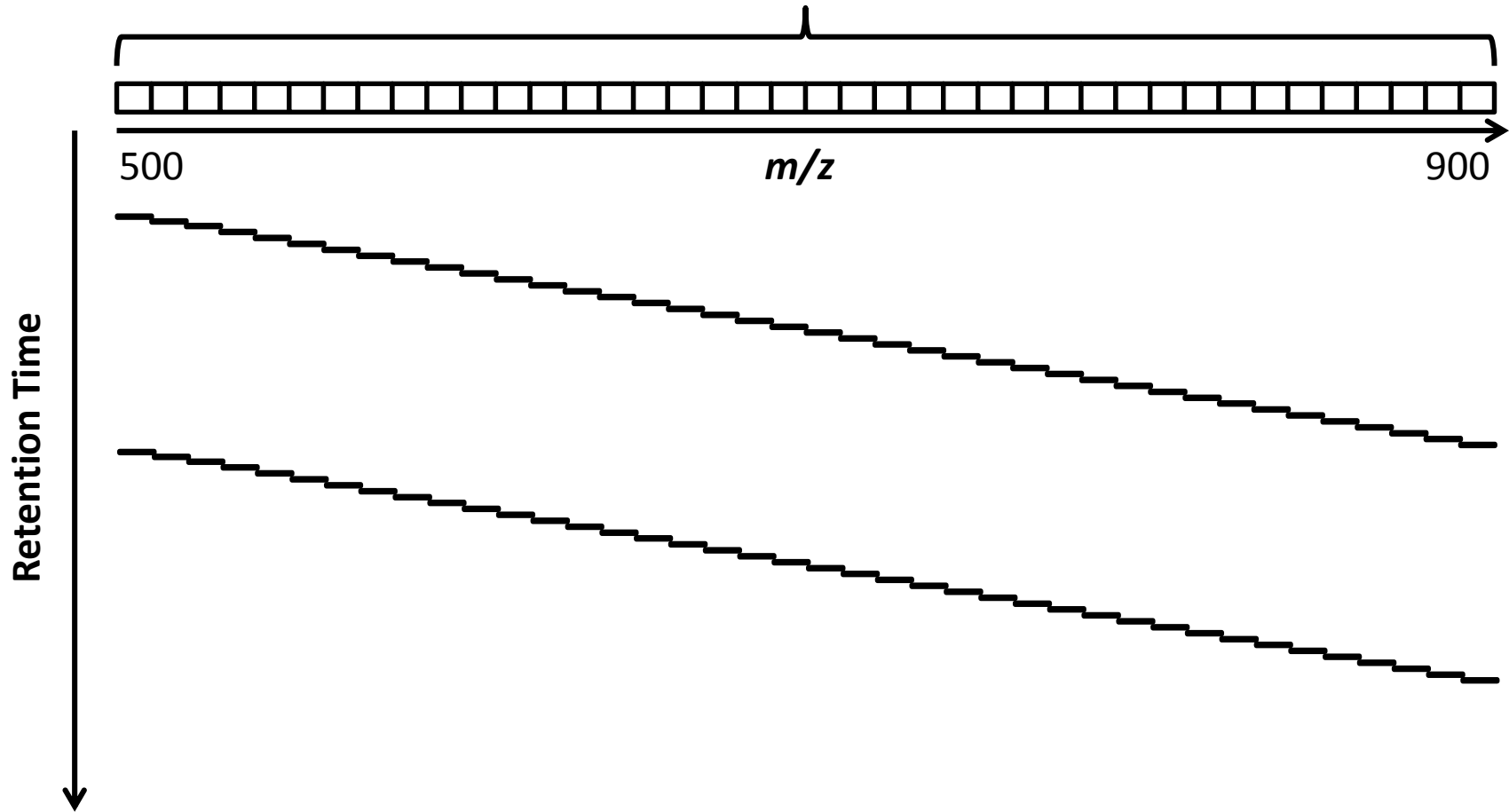
**Data Independent
Acquisition (DIA)**

e.g. SWATH-MS

“Best of both worlds?”

Data Independent Acquisition (DIA)

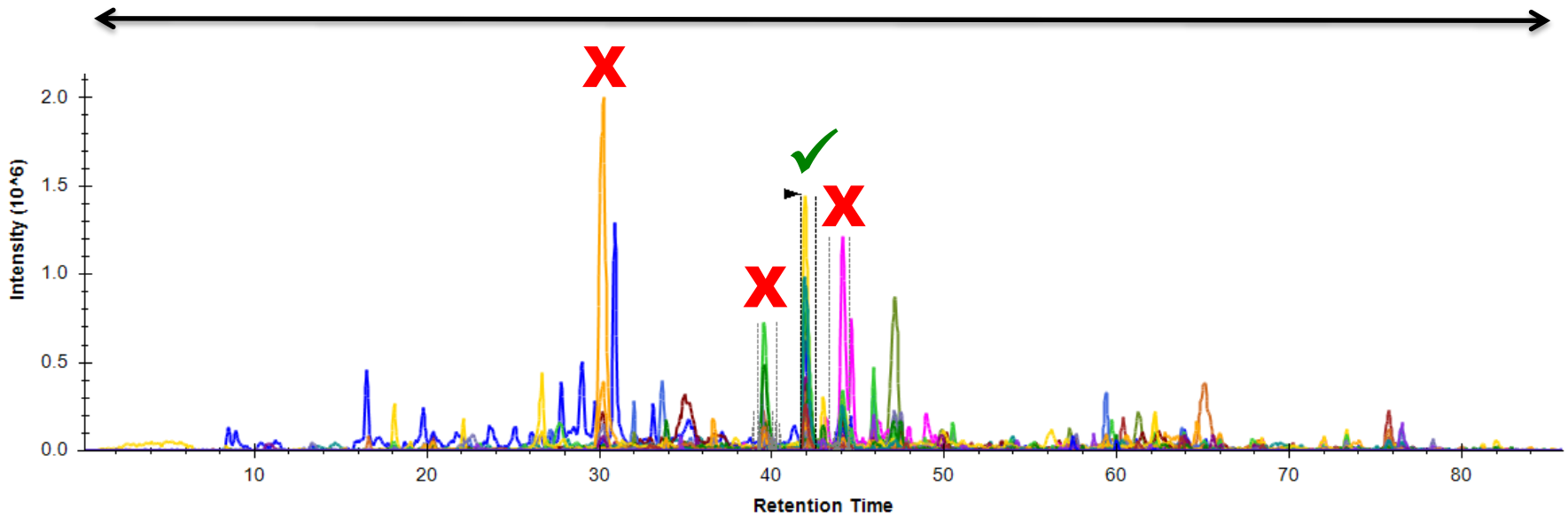
40 $10\ m/z$ -wide windows = 400 m/z



Peptide Detection in DIA Data

FEIELLSLDDDSIVNHEQDLPK *S. cerevisiae* lysate (soluble) 10 *m/z* wide window DIA (Q-Exactive)

Data is full chromatogram + can have lots of interferences



Which of these peaks is the actual peptide?

How sure are we?

1000's of peptides to look at, need to automate the process

How do I process my DIA data?

- **My experience as a user (circa 2013):**
 - Skyline could import DIA data but not good at peptide detection (peak picking), was still relatively SRM-gearred
 - Other tools (Open Swath, Spectronaut, Peak View) were stronger on peak picking, but I preferred Skyline's rich environment / features / usability
- **Task (Brendan, Dario, Don Marsh):**
 - Can we bring good peak picking to Skyline, using insights from these other tools as needed?

DIA in Skyline (circa mid-2013)

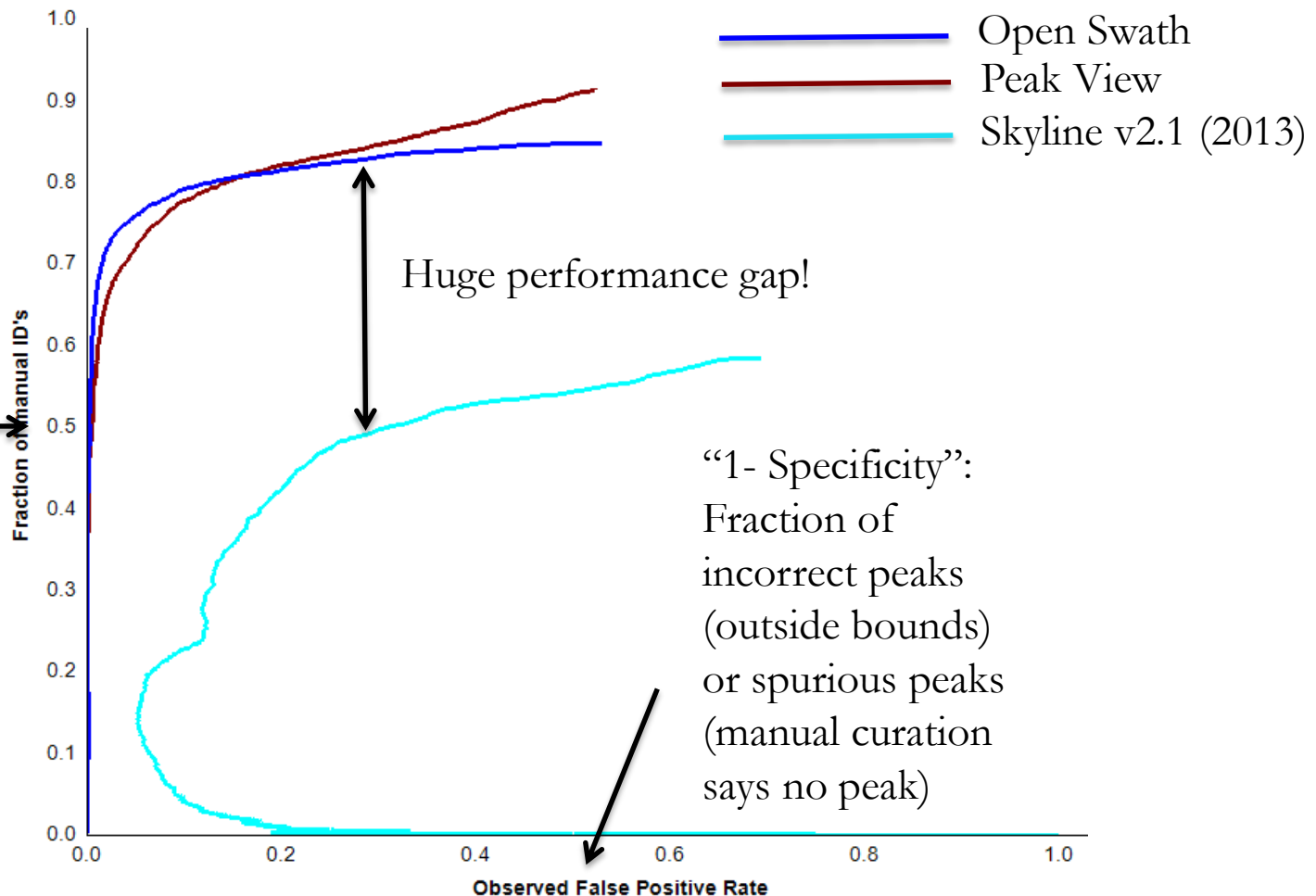
Dataset from Rost et al, *Nat. Biotech*, 2014 -- 344 heavy peptides spiked-in human background
10 dilution points x 3 replicates -- ABSCIEX 5600 SWATH 25 m/z windows

10350 total peptide-runs

80% of peptides had manually curated peak

ROC plot comparison

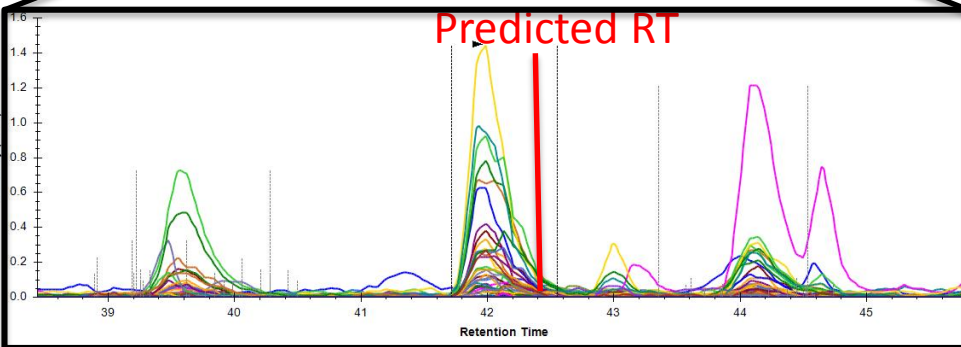
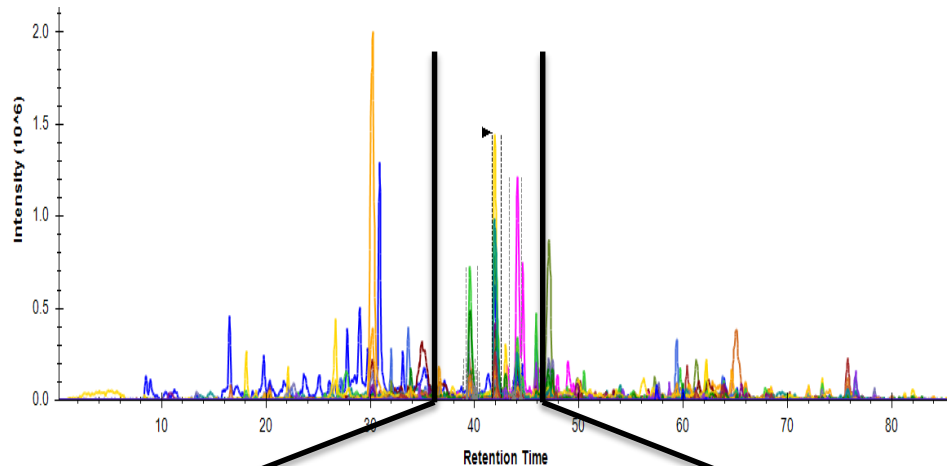
“Sensitivity”: What fraction of all curated peaks were correctly identified (automated pick within curated bounds)?



“1 - Specificity”: Fraction of incorrect peaks (outside bounds) or spurious peaks (manual curation says no peak)

Problem 1: Full RT Range

- Initially, Skyline did not restrict the range to the predicted retention window by default
- Most users ended up importing the whole chromatogram making peak picking difficult



Transition Settings

Prediction Filter Library Instrument Full-Scan

MS1 filtering

Isotope peaks included: None
Precursor mass analyzer:

Peaks: Resolution: m/z

Isotope labeling enrichment:

MS/MS filtering

Acquisition method: DIA
Product mass analyzer: TOF

Isolation scheme: Resolving power:

This is now the default!

Use only scans within 5 minutes of MS/MS IDs

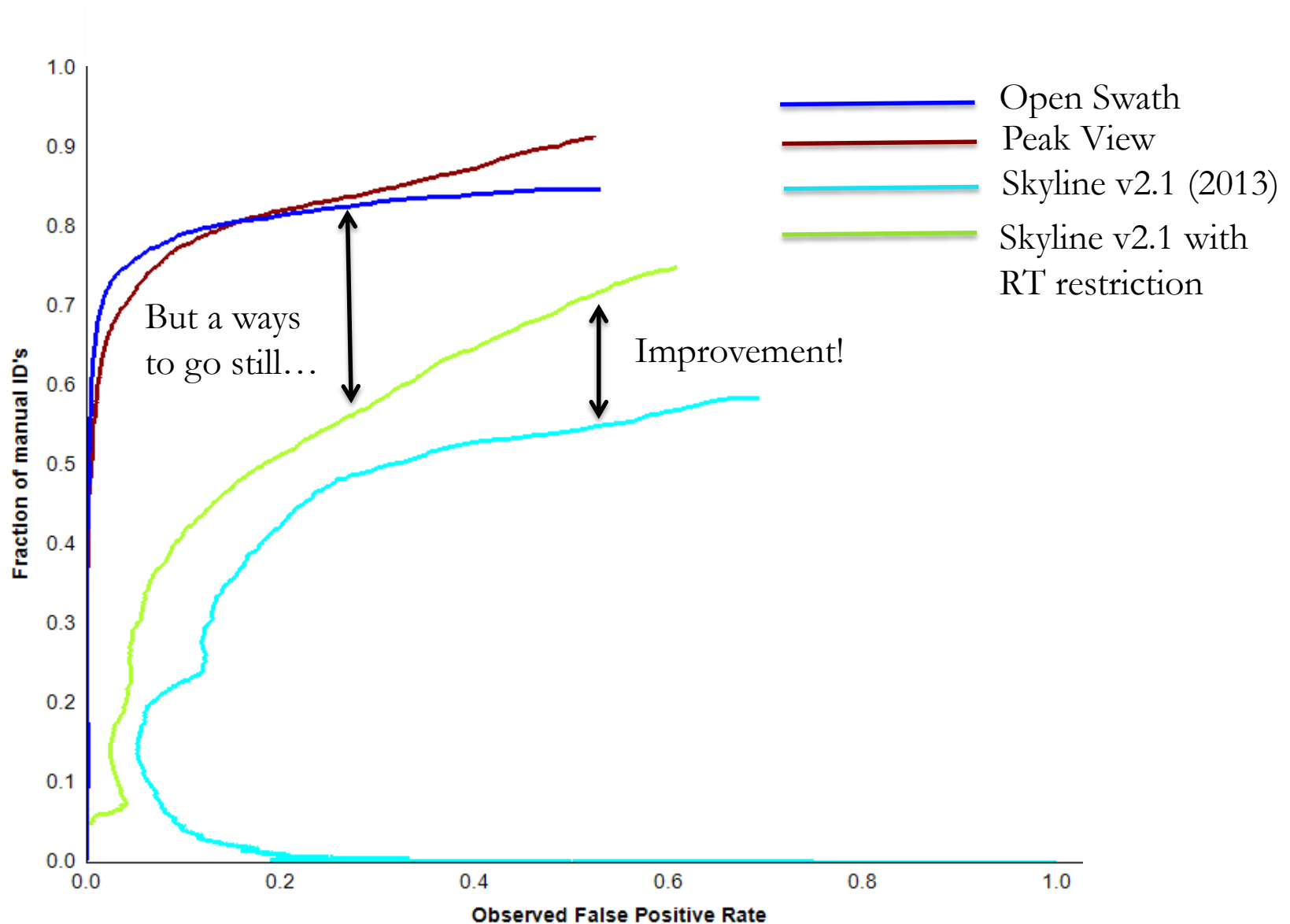
Use only scans within 4 minutes of predicted RT

Include all matching scans

OK Cancel

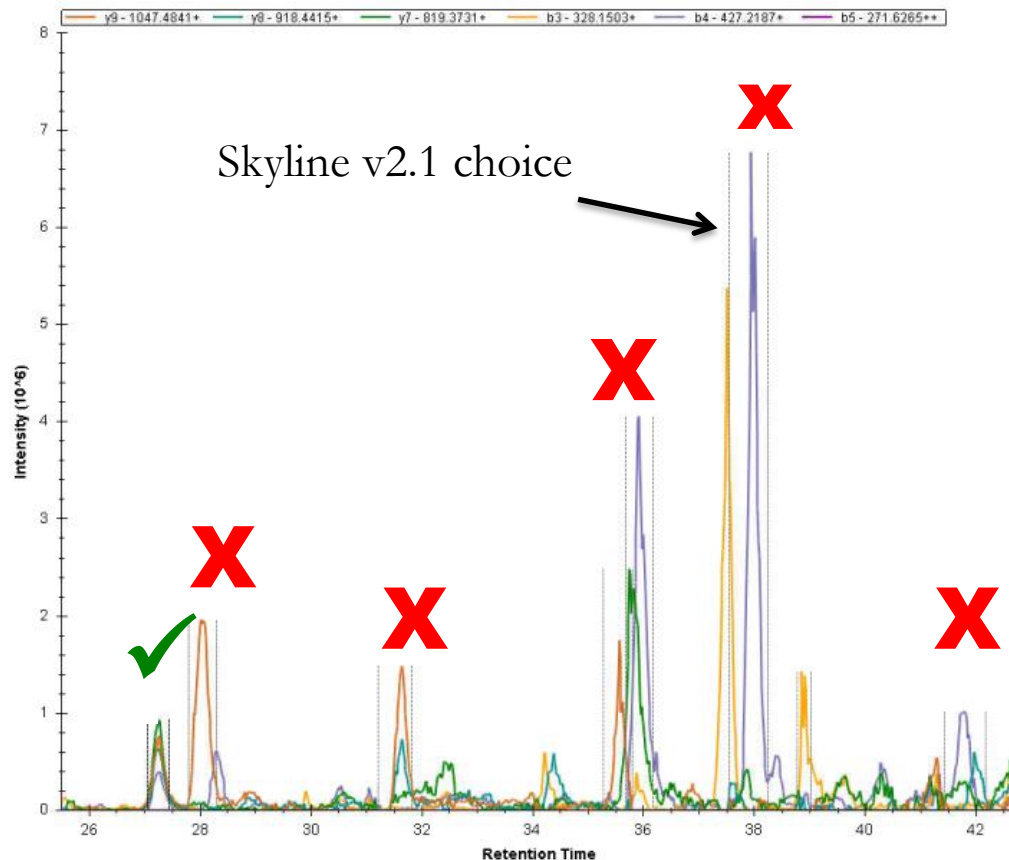
Impact of Chromatogram Restriction

ROC plot comparison



Problem 2: Peak Scoring

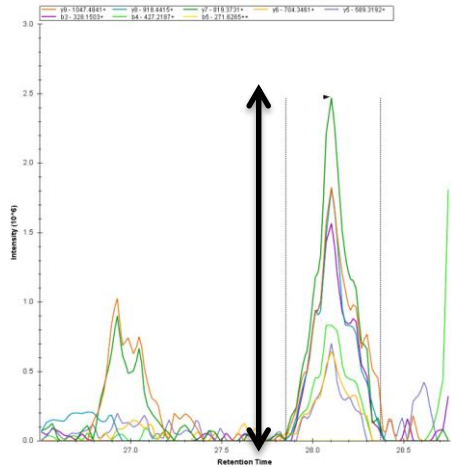
- In 2013, Skyline picked peaks based on intensity and crude co-elution measures
- Workflow geared towards SRM – Skyline suggests a peak, but you inspect manually, so OK if sometimes wrong



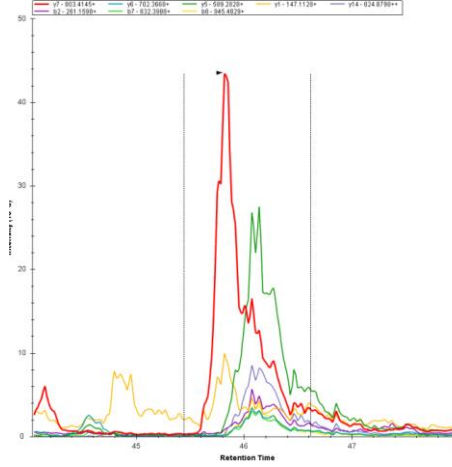
The mProphet Algorithm

Use machine learning (linear discriminant analysis) to form a composite score based on these (and other) indicators of peak quality, using decoy peptides to train (Reiter et al 2011)

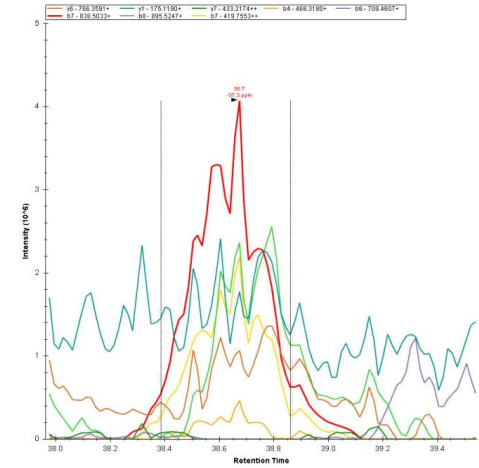
Intensity



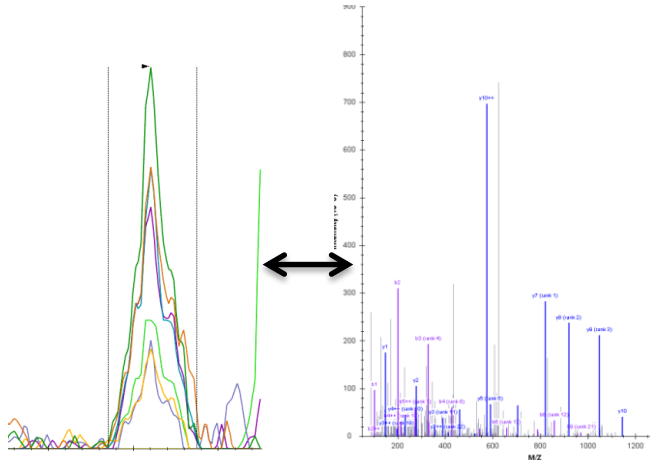
Co-elution



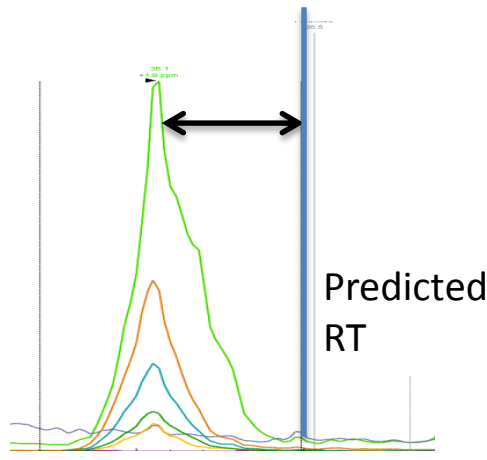
Shape



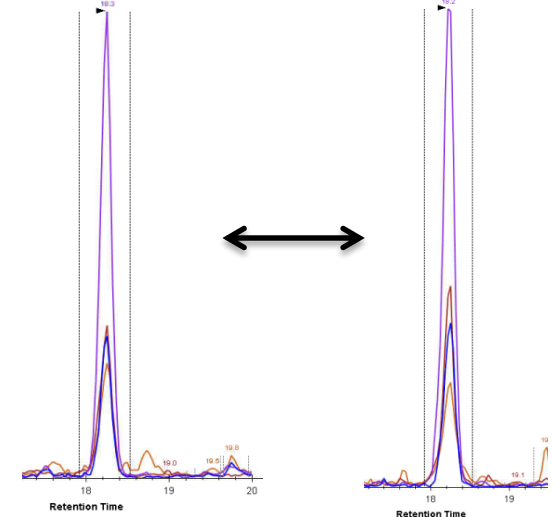
Spectral Library Correlation



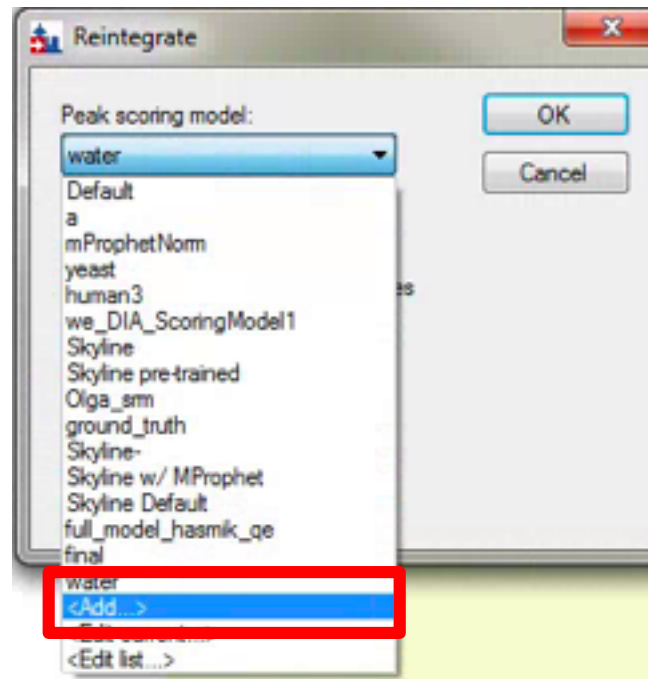
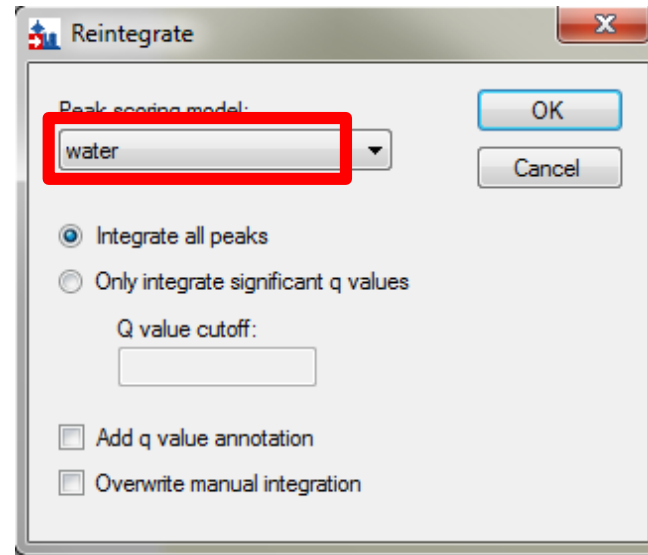
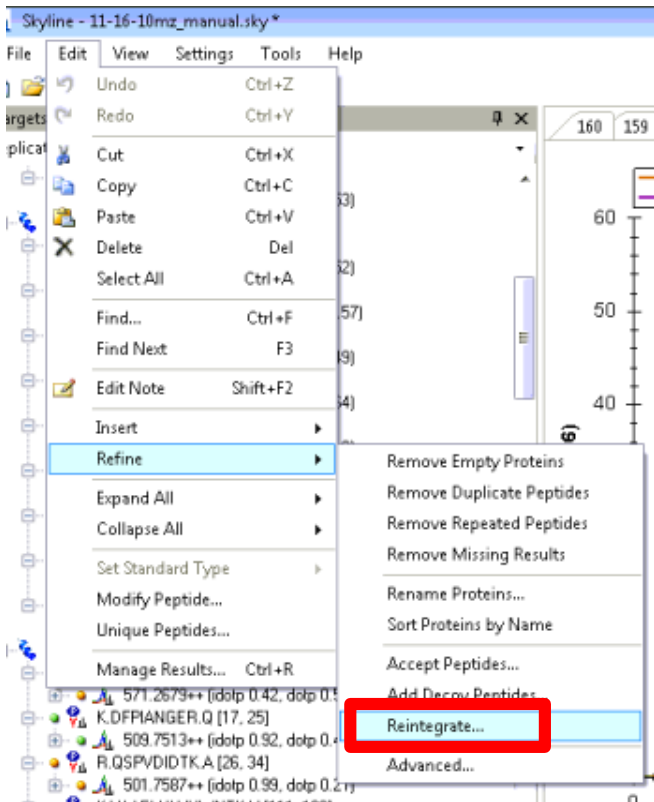
Retention Time



Comparisons w/ isotope standard



Using mProphet in Skyline



Using mProphet in Skyline

Edit Peak Scoring Model

Name:

Choose model:
mProphet

Training

Use decoys

Use second best peaks

Train Model

Available feature scores:

Enabled	Score Name	Weight	Percentage Contribution
<input checked="" type="checkbox"/>	Intensity		
<input checked="" type="checkbox"/>	Retention time difference		
<input checked="" type="checkbox"/>	Retention time difference ...		
<input checked="" type="checkbox"/>	Library intensity dot-product		
<input checked="" type="checkbox"/>	Shape (weighted)		
<input checked="" type="checkbox"/>	Co-elution (weighted)		
<input checked="" type="checkbox"/>	Co-elution count		
<input checked="" type="checkbox"/>	Signal to noise		
<input checked="" type="checkbox"/>	Product mass error		
<input type="checkbox"/>	Reference intensity dot-pr...		
<input type="checkbox"/>	Reference shape weight		

Model Scores

Feature Scores | P Values | Q Values

Train a model to see composite score

Peak count

Score

OK Cancel

Using mProphet in Skyline

Edit Peak Scoring Model

Name:

Choose model: mProphet

Training

Use decoys Use second best peaks Train Model

Available feature scores:

Enabled	Score Name	Weight	Percentage Contribution
<input checked="" type="checkbox"/>	Intensity	0.0664	1.6%
<input checked="" type="checkbox"/>	Retention time difference	-1.0154	25.1%
<input checked="" type="checkbox"/>	Retention time difference ...	0.1268	-11.4%
<input checked="" type="checkbox"/>	Library intensity dot-product	1.6845	9.0%
<input checked="" type="checkbox"/>	Shape (weighted)	4.2500	27.4%
<input checked="" type="checkbox"/>	Co-elution (weighted)	-0.1856	12.3%
<input checked="" type="checkbox"/>	Co-elution count	0.1801	3.4%
<input checked="" type="checkbox"/>	Signal to noise	0.6370	11.5%
<input checked="" type="checkbox"/>	Product mass error	-0.0449	5.3%
<input type="checkbox"/>	Reference intensity dot-pr...		
<input type="checkbox"/>	Reference shape (weight...		

Warning: calculators produced colinear scores

Model Scores Feature Scores P Values Q Values

Composite Score (Normalized)

Legend: Decoys Targets Decoy normal distribution

Peak count

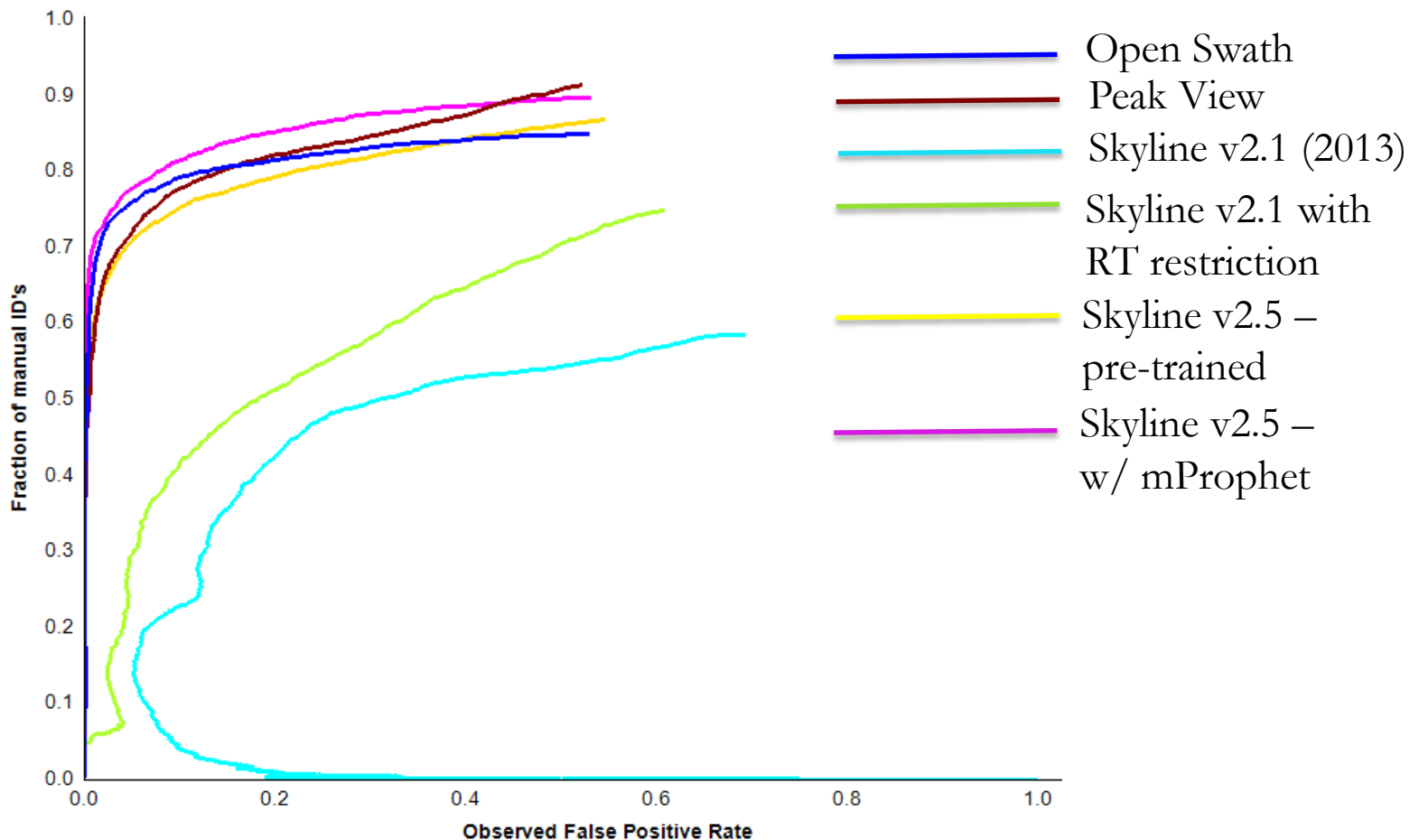
Score

OK Cancel

Swath Gold Standard Data (Human)

Skyline now matches or exceeds other tools.

ROC plot comparison



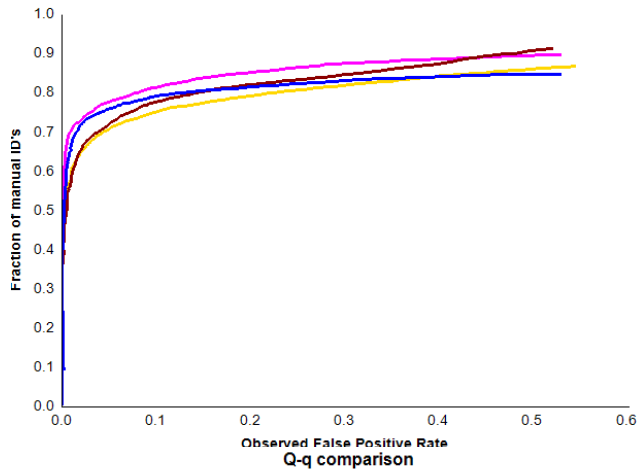
Results on Other Backgrounds

Open Swath
Peak View

Skyline v2.5 – pre-trained
Skyline v2.5 – w/ mProphet

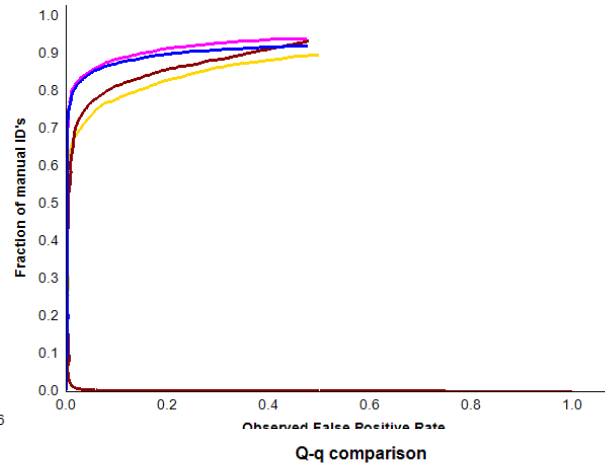
Human Background

ROC plot comparison



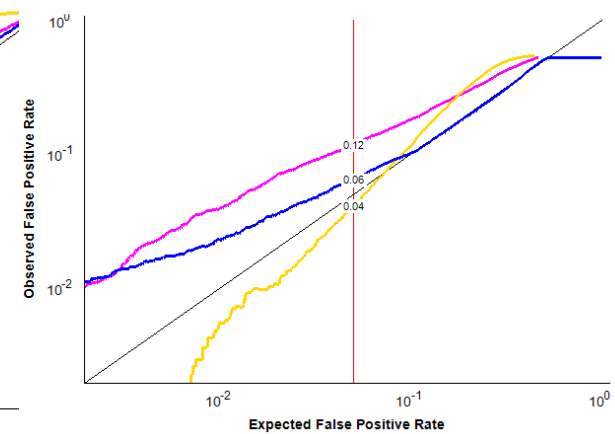
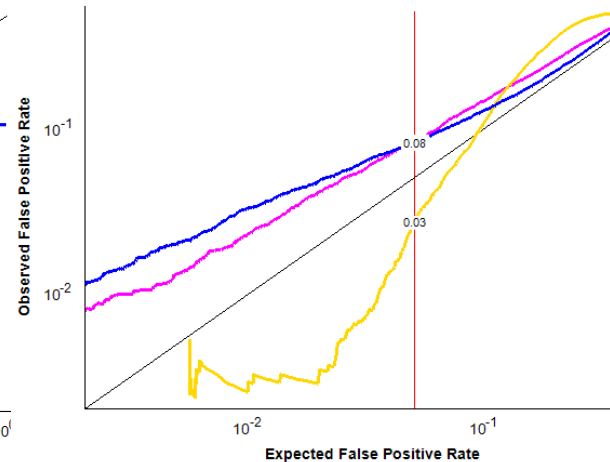
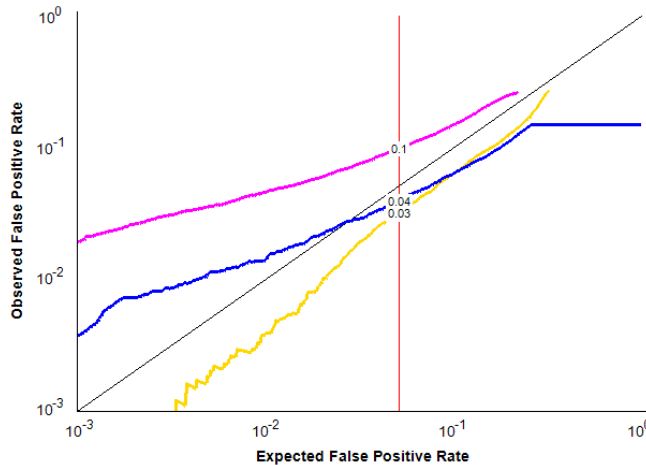
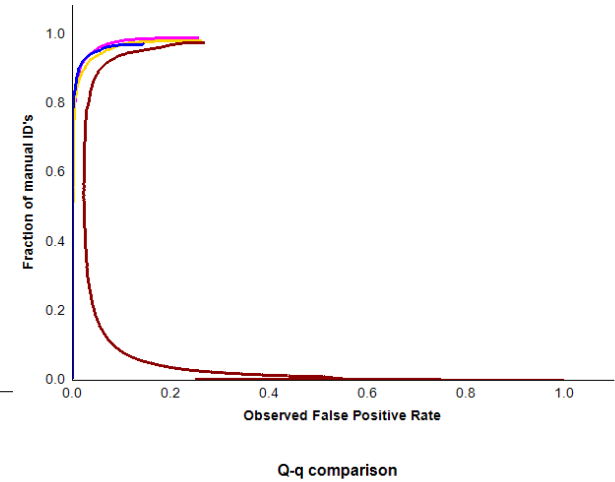
Yeast Background

ROC plot comparison



No Background

ROC plot comparison



Problem 3: Isotopic Pairs

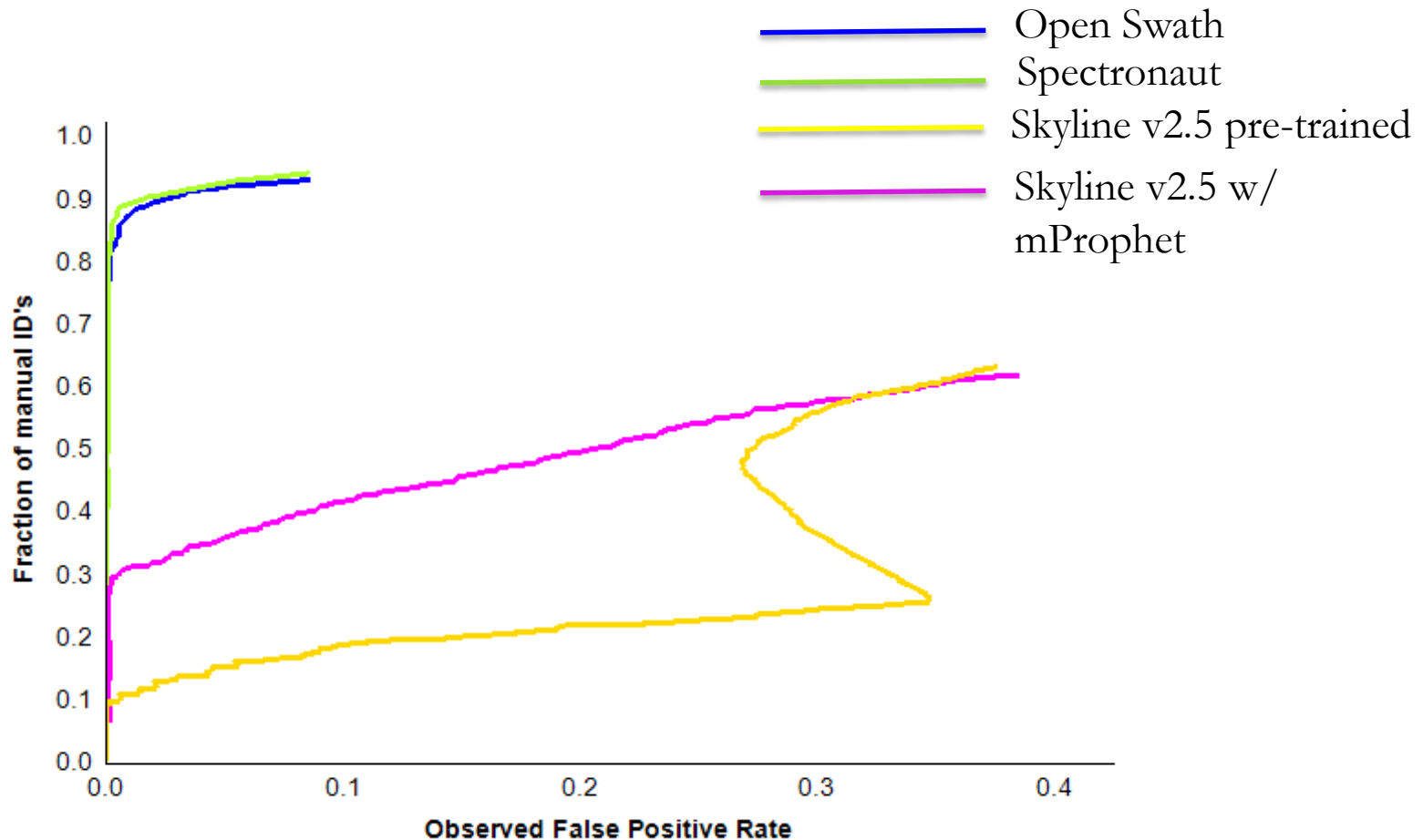
116 light/heavy synthetic peptide pairs spiked into depleted plasma background, 30 runs

Constant abundance heavy standards, light peptides in dilution series from 100 fm/ul to 0.01 fm/ul

4380 total peptide-file pairs

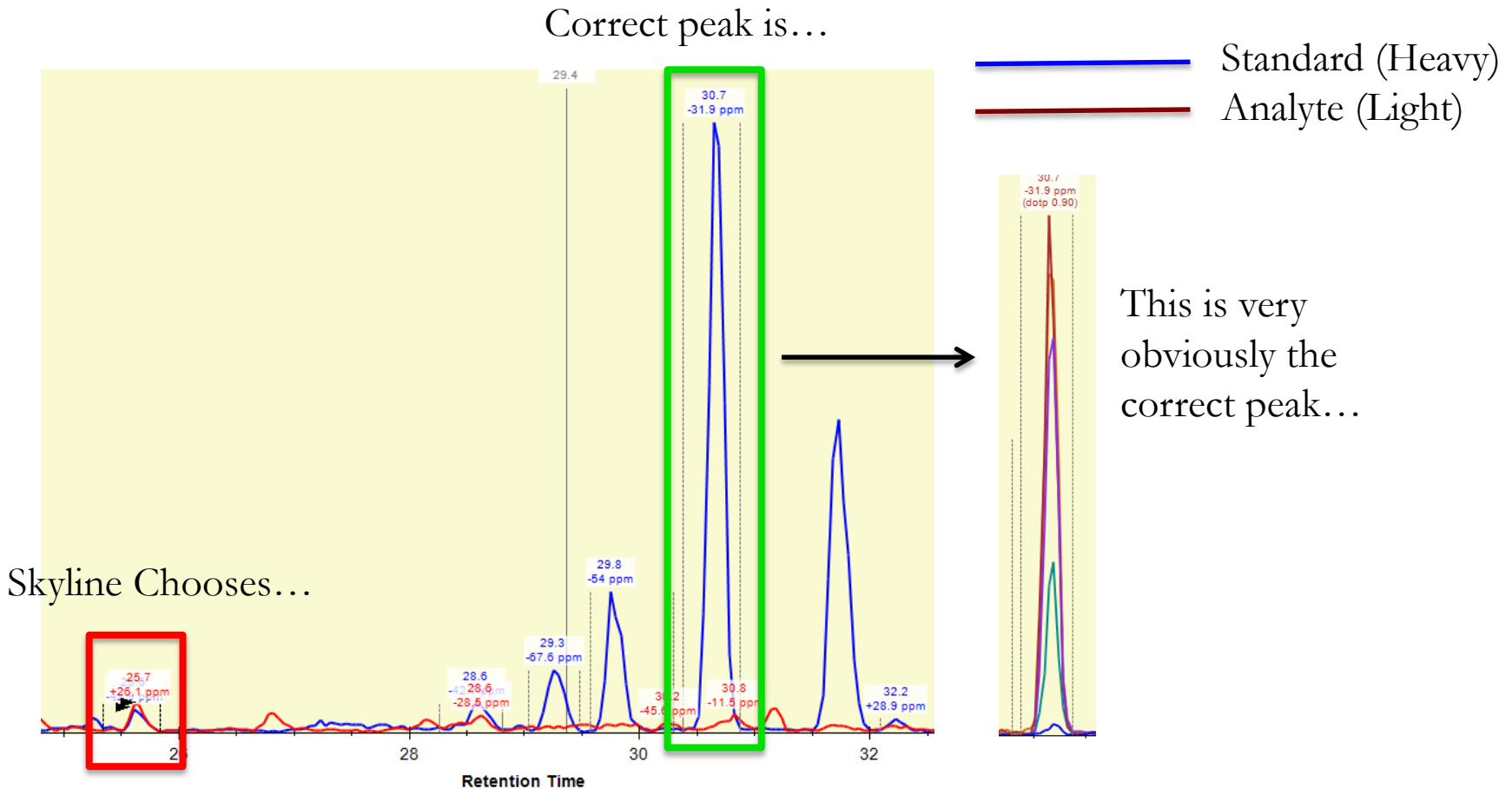
90% of peptide-runs had manually curated peak

ROC plot comparison



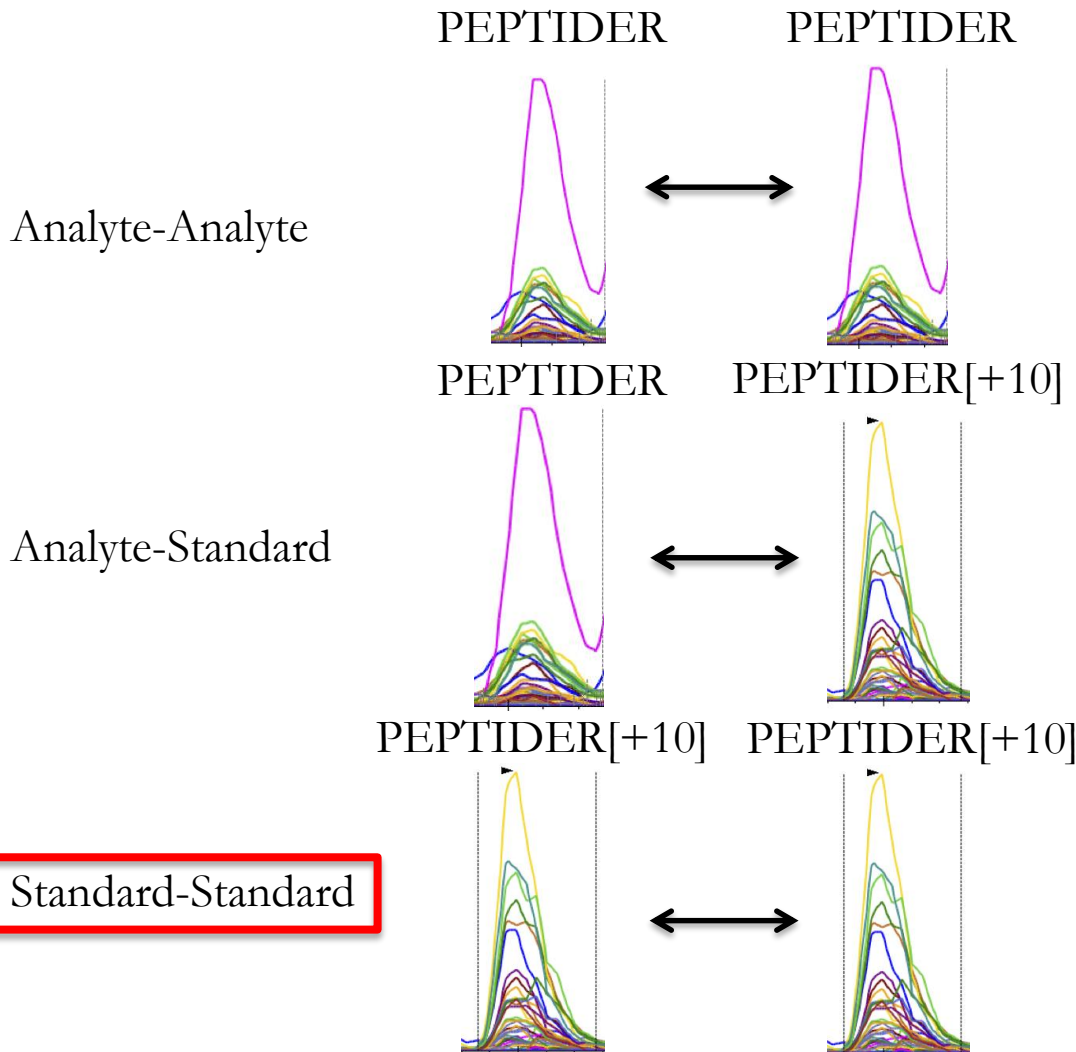
The cause of the problem

- mProphet only considers analyte-analyte and analyte-standard co-elution/shape/dotp
- If can't see analyte, peak will get a bad score even if standard is very easy to see



Adding Scores for Synthetic Peptides

Solution: add standard-standard co-elution/shape/dotp scores



Choose model:
mProphet

Training

Use decoys

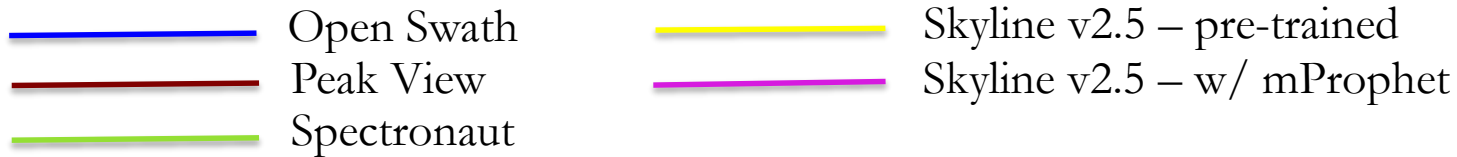
Use second best peaks

Train Model

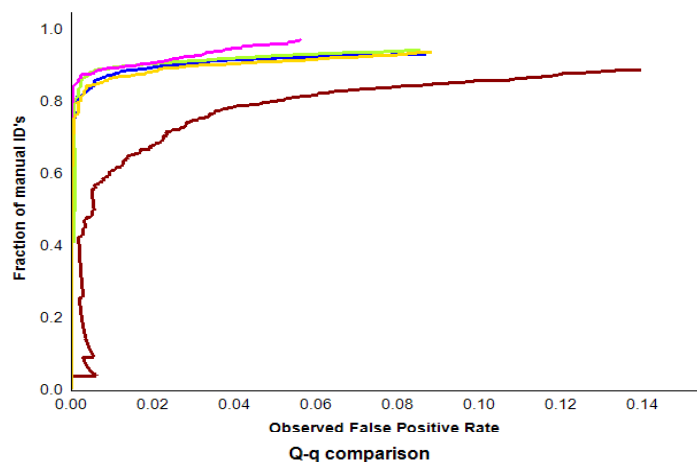
Available feature scores:

Enabled	Score Name	Weight	Percentage Contribution
<input checked="" type="checkbox"/>	Reference intensity dot-pr...	4.7239	8.6%
<input checked="" type="checkbox"/>	Reference shape (weight...	-4.1214	-9.0%
<input checked="" type="checkbox"/>	Reference co-elution (wei...	-0.0522	2.2%
<input checked="" type="checkbox"/>	Reference co-elution count	0.4179	7.7%
<input checked="" type="checkbox"/>	Standard Intensity	1.9076	31.8%
<input checked="" type="checkbox"/>	Standard library dot-product	3.3553	12.1%
<input checked="" type="checkbox"/>	Standard signal to noise	0.5878	9.5%
<input checked="" type="checkbox"/>	Standard product mass er...	-0.0194	6.9%
<input checked="" type="checkbox"/>	Standard shape (weighted)	3.8236	17.8%
<input checked="" type="checkbox"/>	Standard co-elution (weig...	-0.0423	3.2%
<input type="checkbox"/>	Standard product shape		

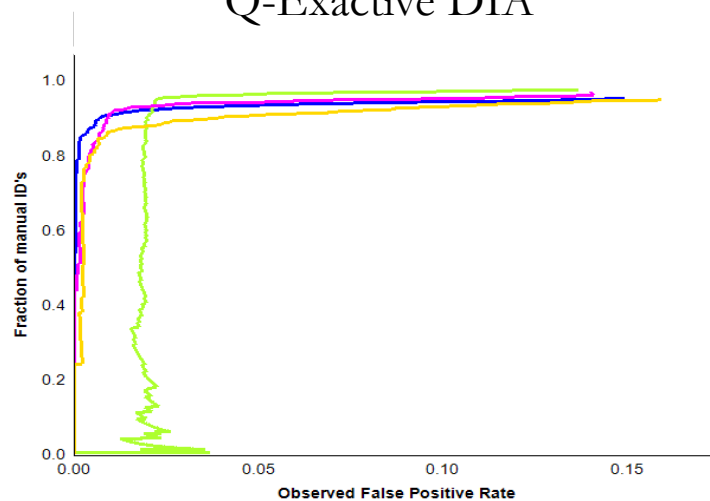
Pairs/Heavy Peptides



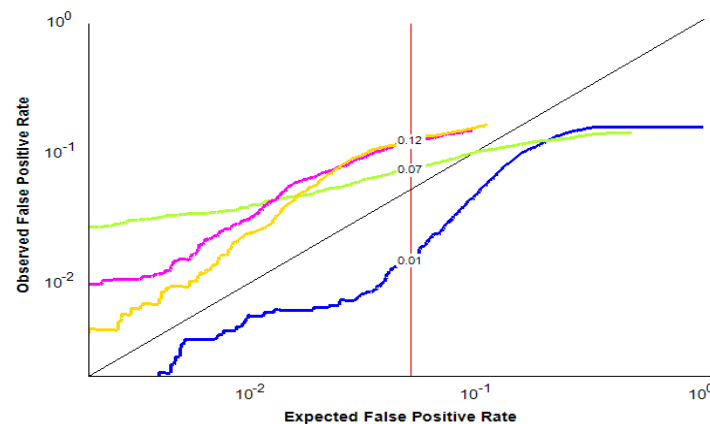
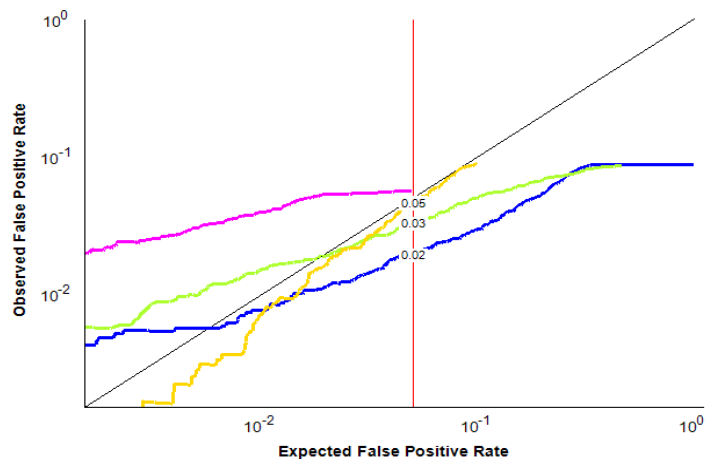
SWATH DIA



Q-Exactive DIA



Q-q comparison

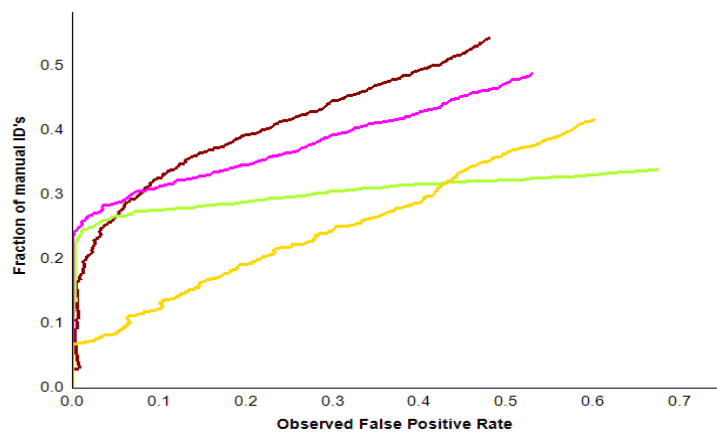


Light Peptides Only

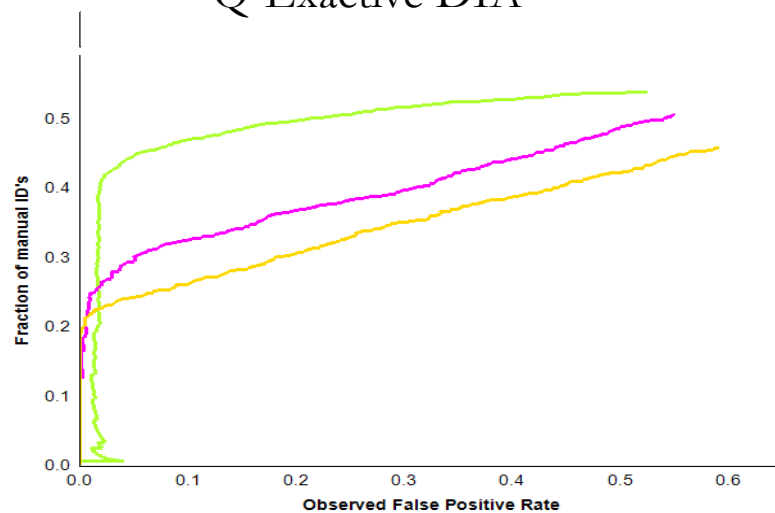
Open Swath
Peak View
Spectronaut

Skyline v2.5 – pre-trained
Skyline v2.5 – w/ mProphet

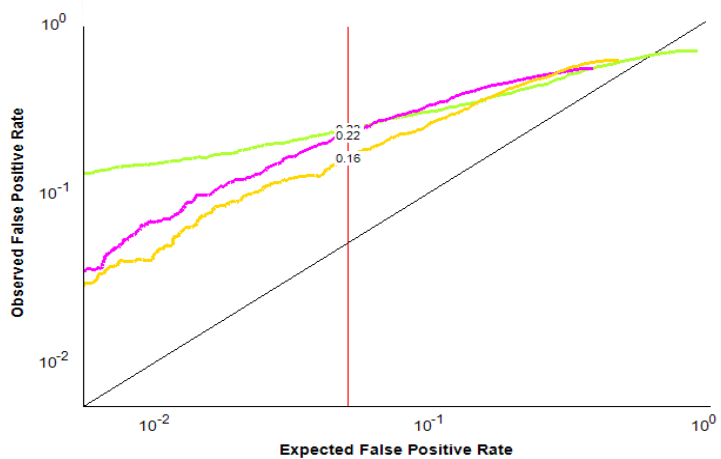
SWATH DIA



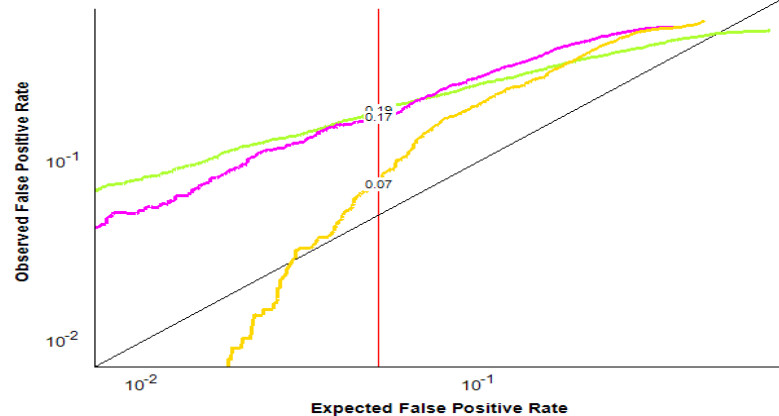
Q-Exactive DIA



Q-q comparison



Q-q comparison



Preliminary Conclusions

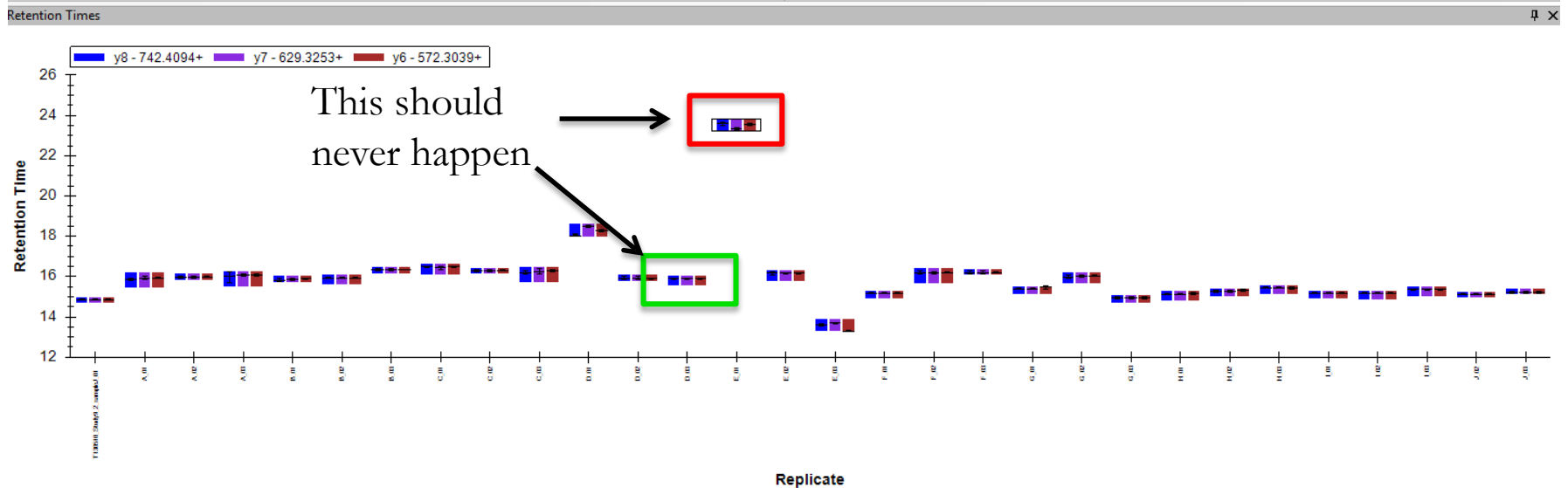
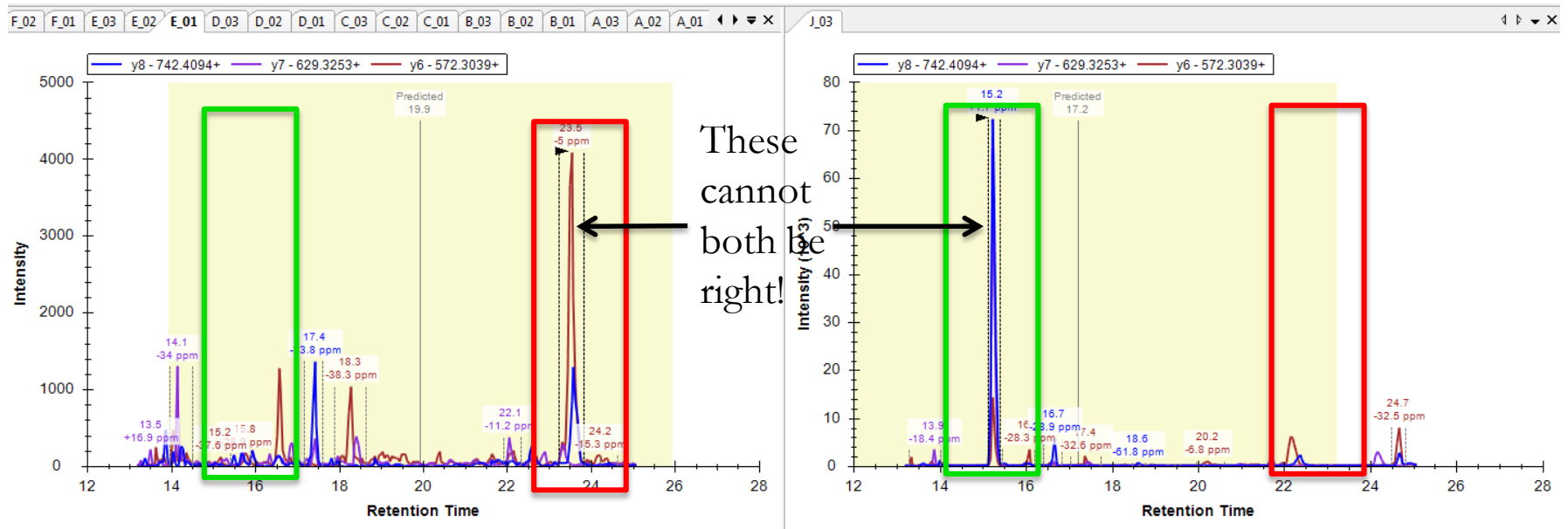
- Skyline's peak picking is now roughly equivalent to other tools for processing DIA data
- Proper treatment of isotope standards requires modification of published algorithms
- Most tools overstate statistical confidence

Help Us Make Skyline Better!

Send us your DIA data sets for testing Skyline against other tools and improving it.

- **We are interested in:**
 - Spiked-in synthetic peptides, especially dilution series
 - Biological data, especially if it has some ground truth (manually picked peaks, SILAC, isotope standards)
- **We will share anything we find with you**
- **We will keep your data confidential, aside from very high level peak picking statistics**

Future Plans – Cross Run Peak Picking



Future Peak Picking Plans (Winter Release)

- Multi-replicate peak picking
- Comparison of Skyline with other tools on larger number of DIA data sets
- Getting the statistics right

Acknowledgements

Stanford University

Parag Mallick
Seema Sharma

ETH Zurich

Hannes Rost
Reudi Aebersold
Lucia Espona Pernas

University of Washington

Brendan MacLean
Don Marsh

Purdue University

Olga Vitek

Dario's Poster: Thursday June 19th
(#035) 10:30 AM – 2:30 PM

Steve Tate

Michael MacCoss
David Johnson
Ying Sonia Ting

Broad Institute

Hasmik Keshishian

Special thanks to **Brendan MacLean** for creating Skyline and reviewing all my code!