



Skyline Tutorial Webinar #7

iRT Retention Time Prediction with Skyline

With
Brendan MacLean (Principal Developer)

Agenda

- ▶ Welcome from the Skyline team!
- ▶ **iRT Retention Time Prediction**
- ▶ Introduction with Brendan MacLean
 - ▶ Overview of iRT key concepts
- ▶ Tutorial with Brendan MacLean
 - ▶ Calibrating and building an iRT library
 - ▶ Using the iRT library for retention time prediction
- ▶ Audience Q&A – submit questions to Google Form:
<https://skyline.gs.washington.edu/labkey/qa4skyline.url>



Prior Knowledge and Consistency

- ▶ Based on empirical measurement
- ▶ Powerful enough to be used cross-lab / cross experiment
- ▶ More powerful run-to-run

- ▶ Relative ion abundance
 - ▶ Library: Spectral and chromatogram
 - ▶ Prediction: Zhang, Anal. Chem., 2004
- ▶ Retention time
 - ▶ Library: iRT (and AMT)
 - ▶ Prediction: Krokhin, Anal. Chem., 2006 (SSRCalc)



Chromatography-based Quantification

- ▶ Hypothesis testing (Verification)
- ▶ SRM
- ▶ PRM
- ▶ MSI chromatogram extraction (DDA)
- ▶ Data independent acquisition (DIA)



Acquisition	Targeted	Survey
More Selective	PRM	DIA
Less Selective	SRM	DDA

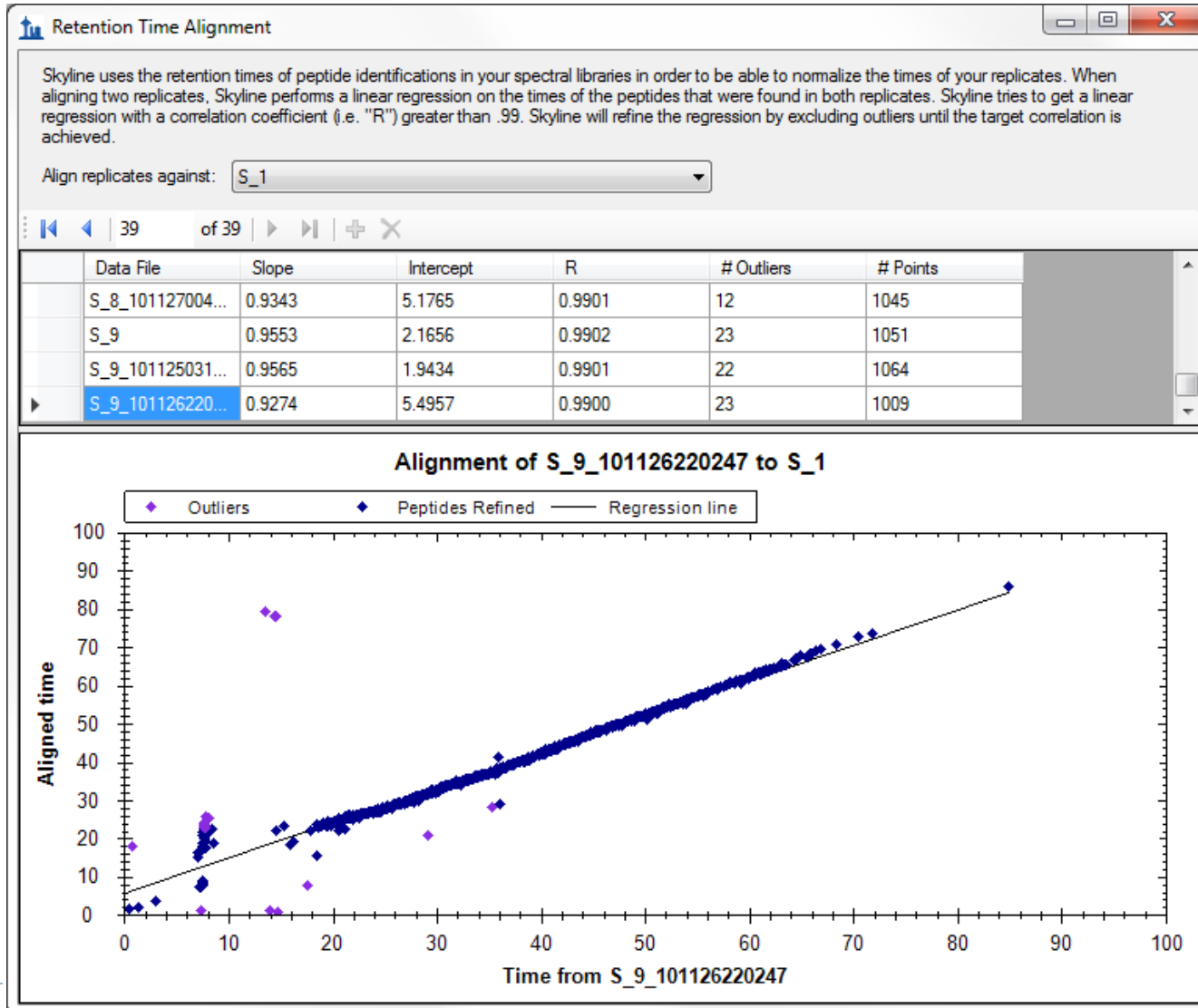
Scheduling & Detection

Source

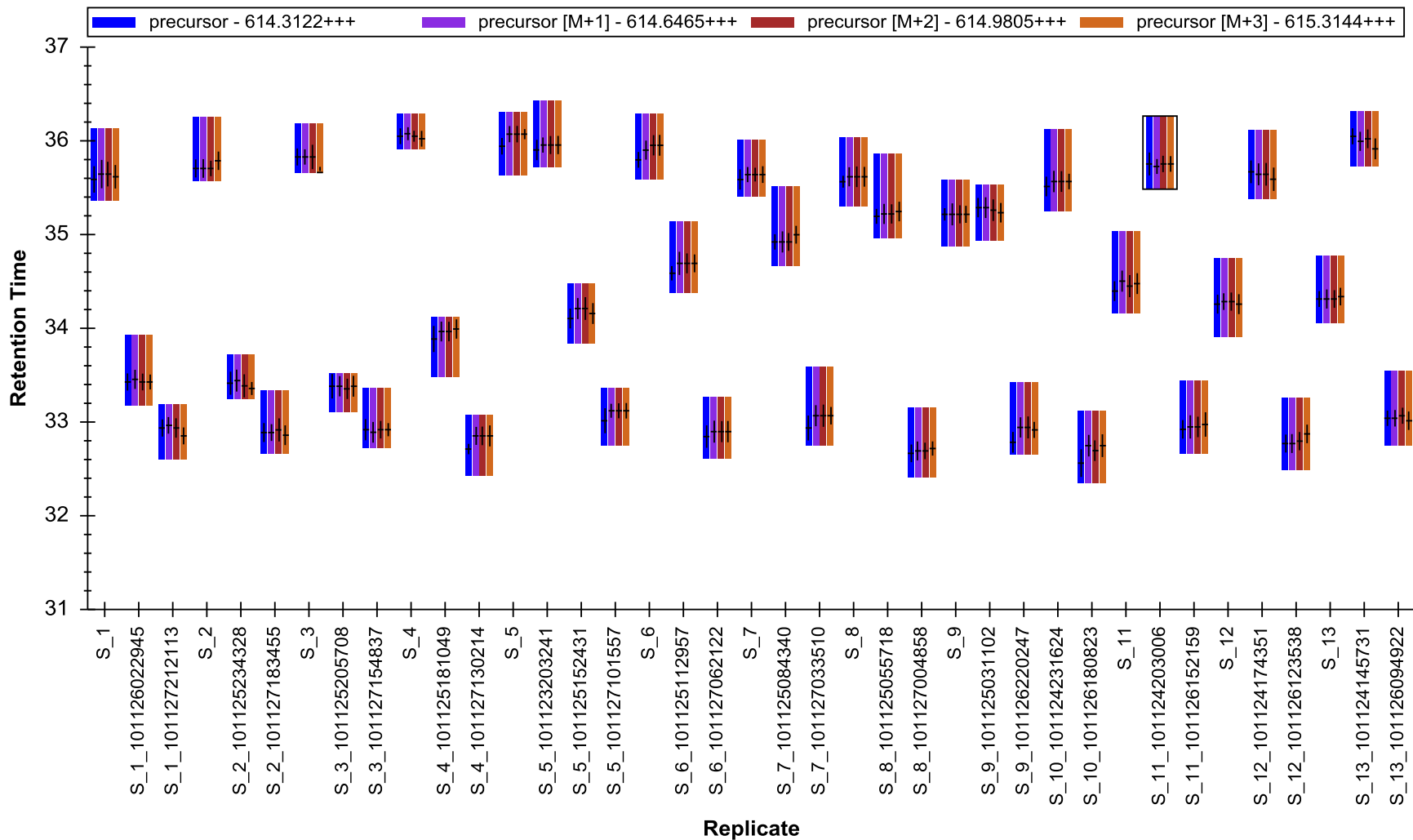
Extraction & Detection



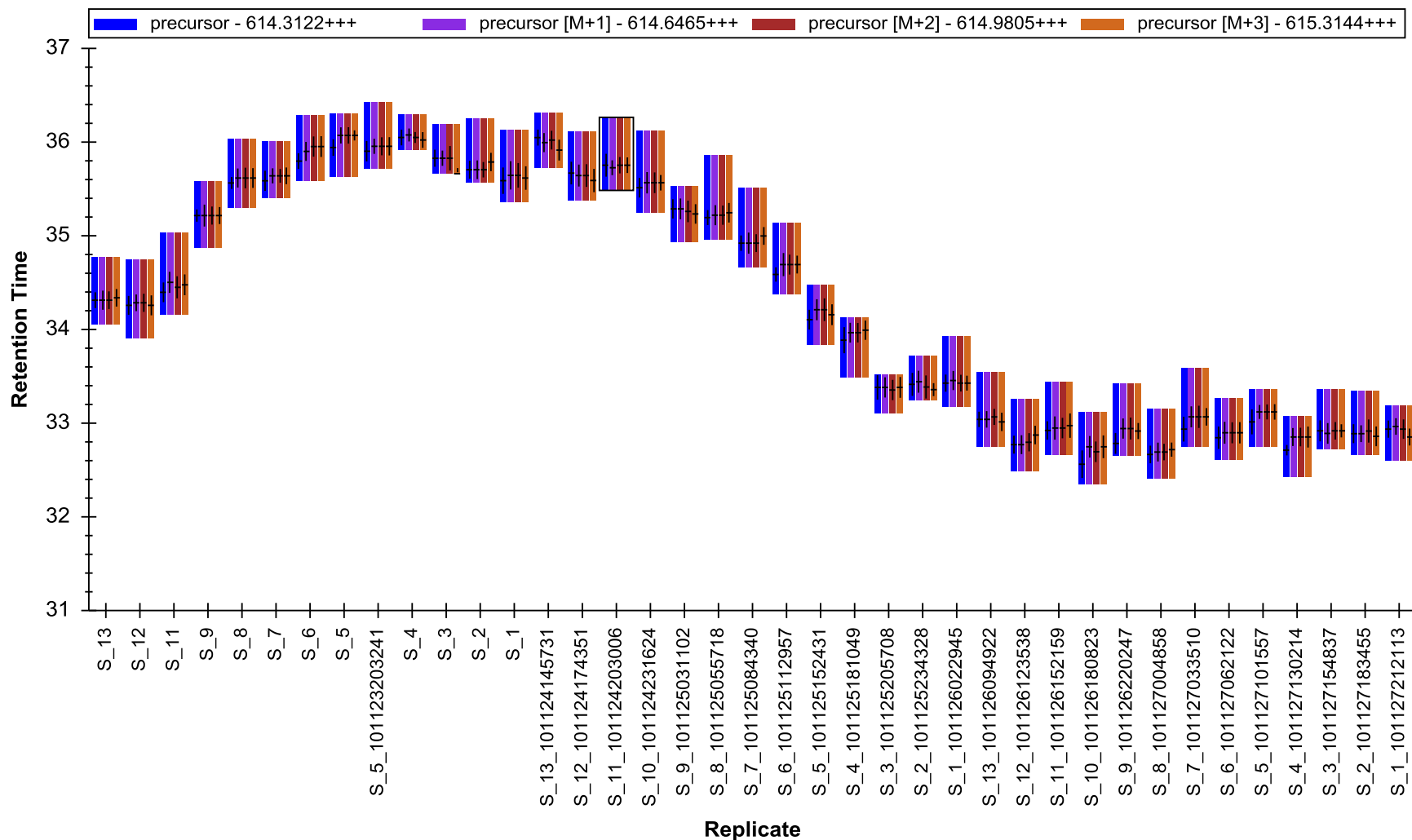
Retention Time Alignment by ID in DDA



Retention Times for SIVPSGASTGVHEALEMR

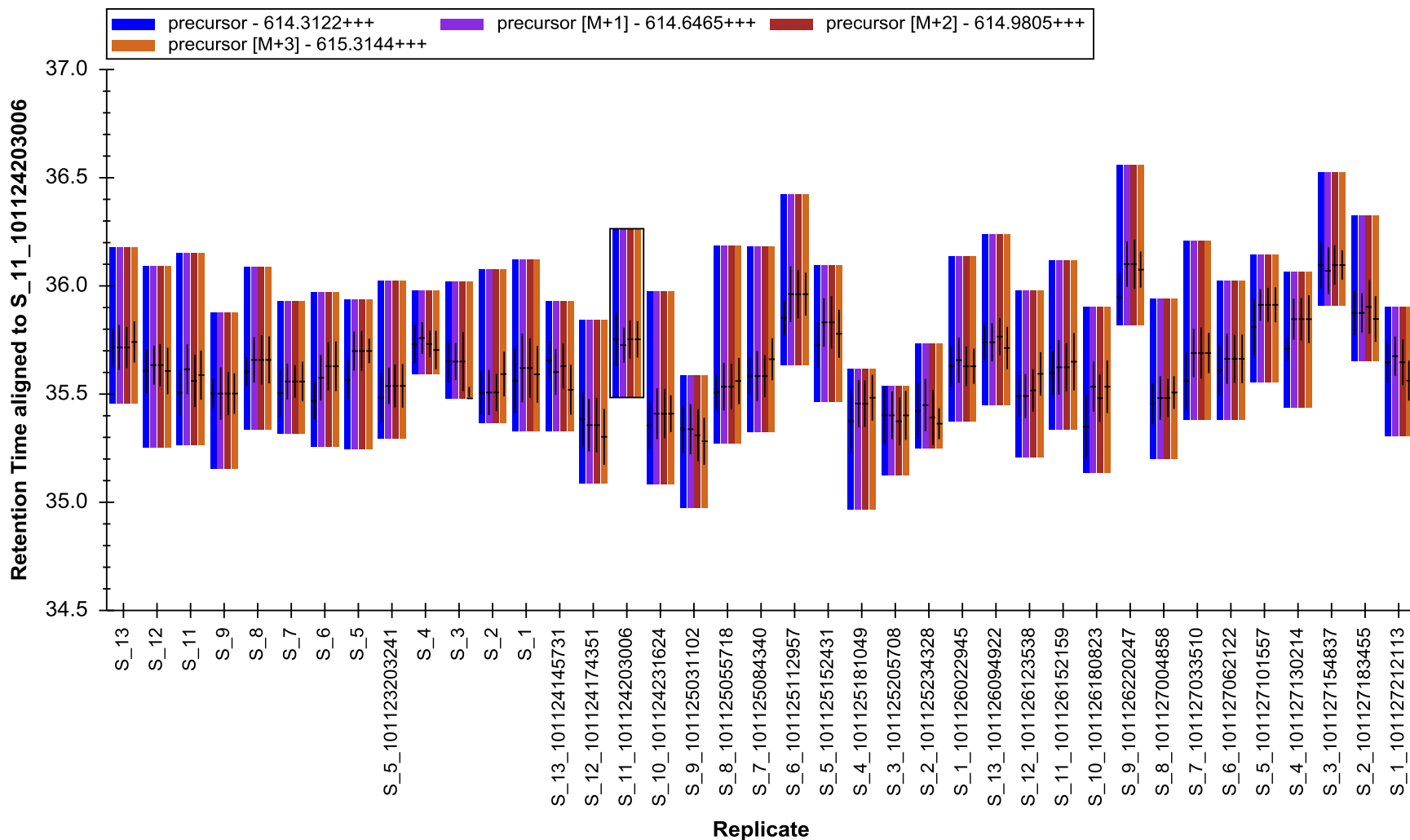


Retention Times for SIVPSGASTGVHEALEMR



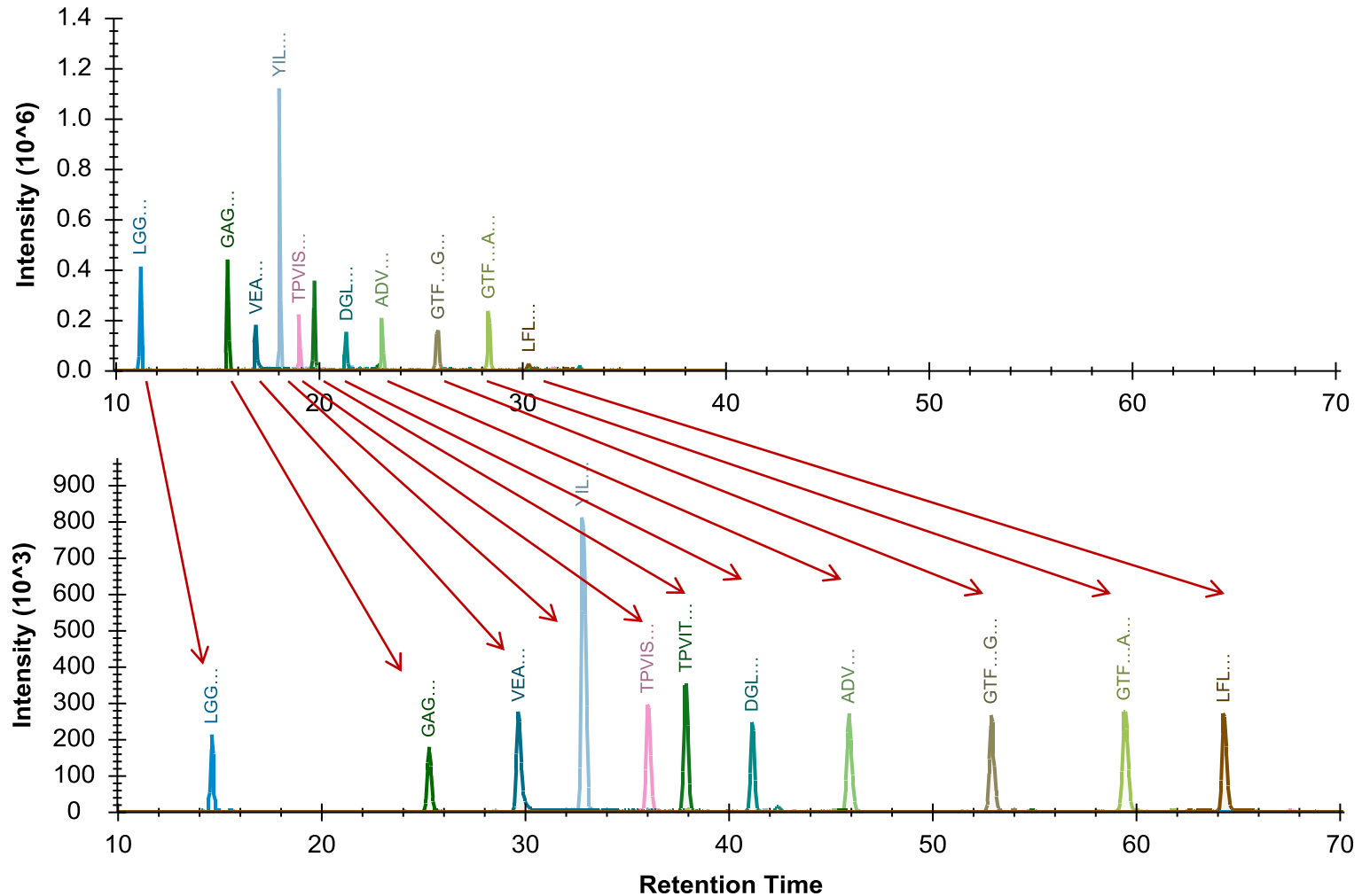
Acquired Time Order

Retention Times for SIVPSGASTGVHEALEMR

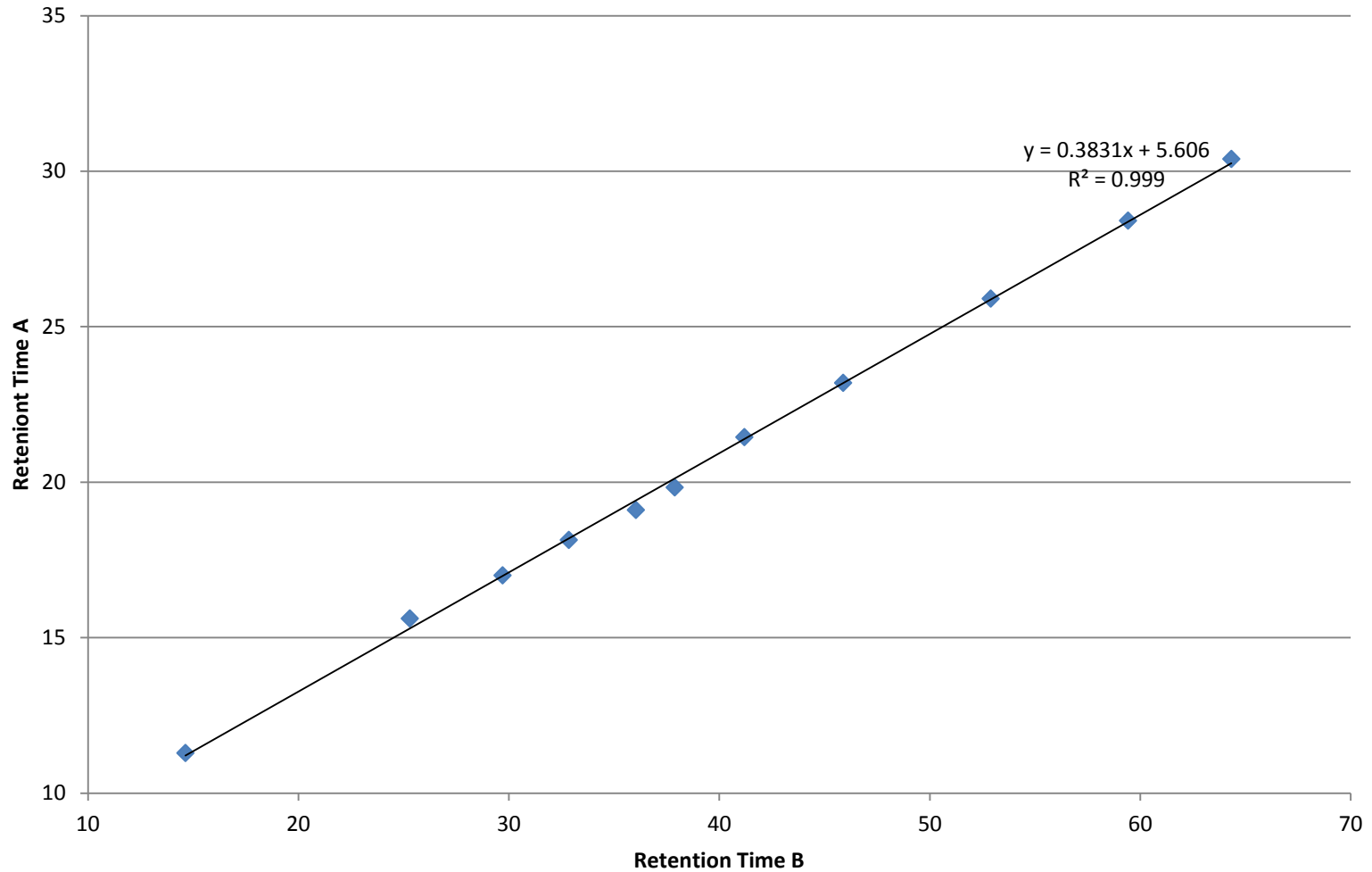


Aligned Times

iRT Standard Peptides



Retention Time Alignment by Standards



iRT Standard Attributes

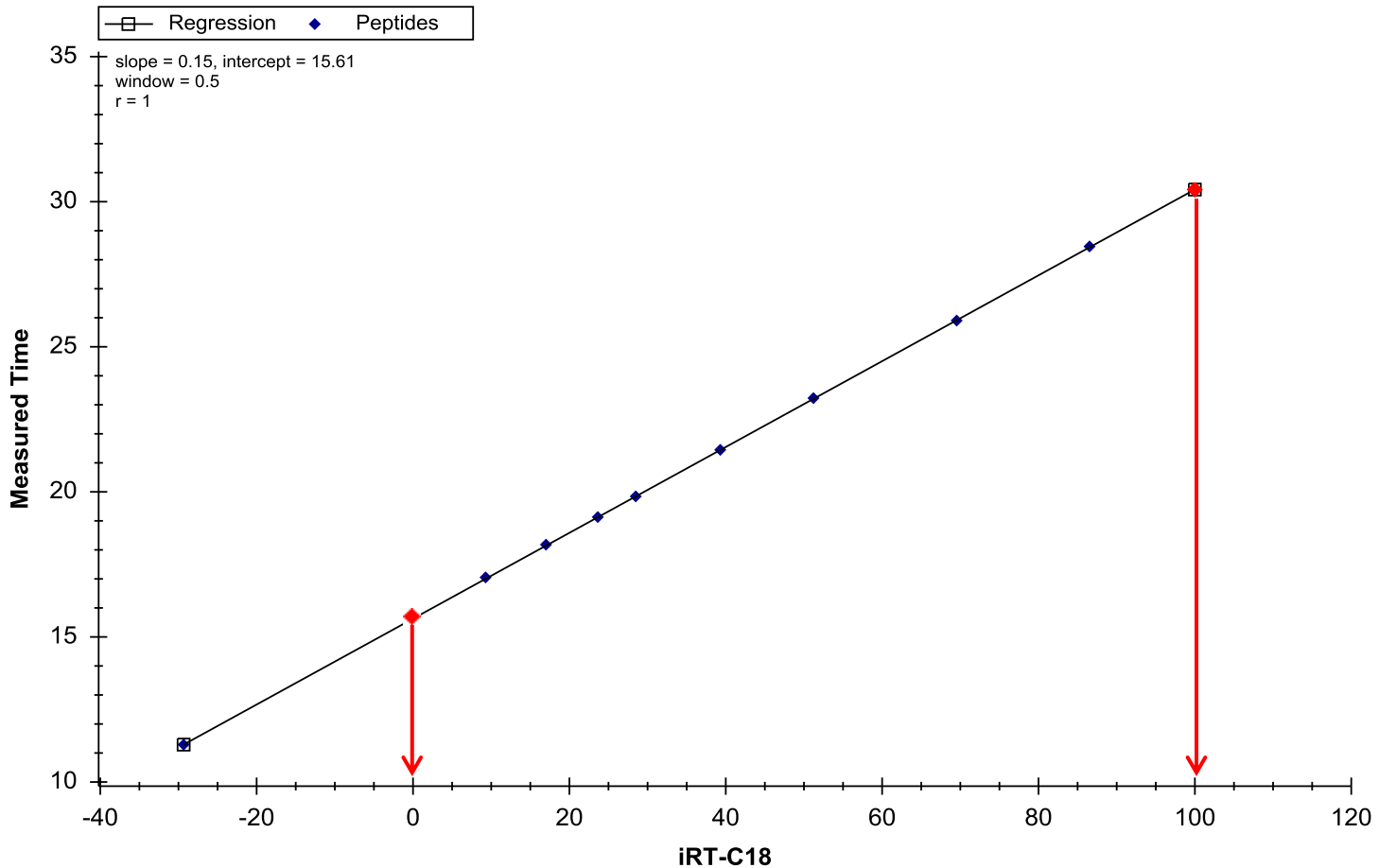
- ▶ 10-20 peptides
- ▶ Consistently measurable in sample
- ▶ Spanning gradient range of interest

- ▶ Biognosys
- ▶ Pierce
- ▶ Sigma Aldrich
- ▶ Heavy reference peptides
- ▶ Analyte peptides – ApoA I



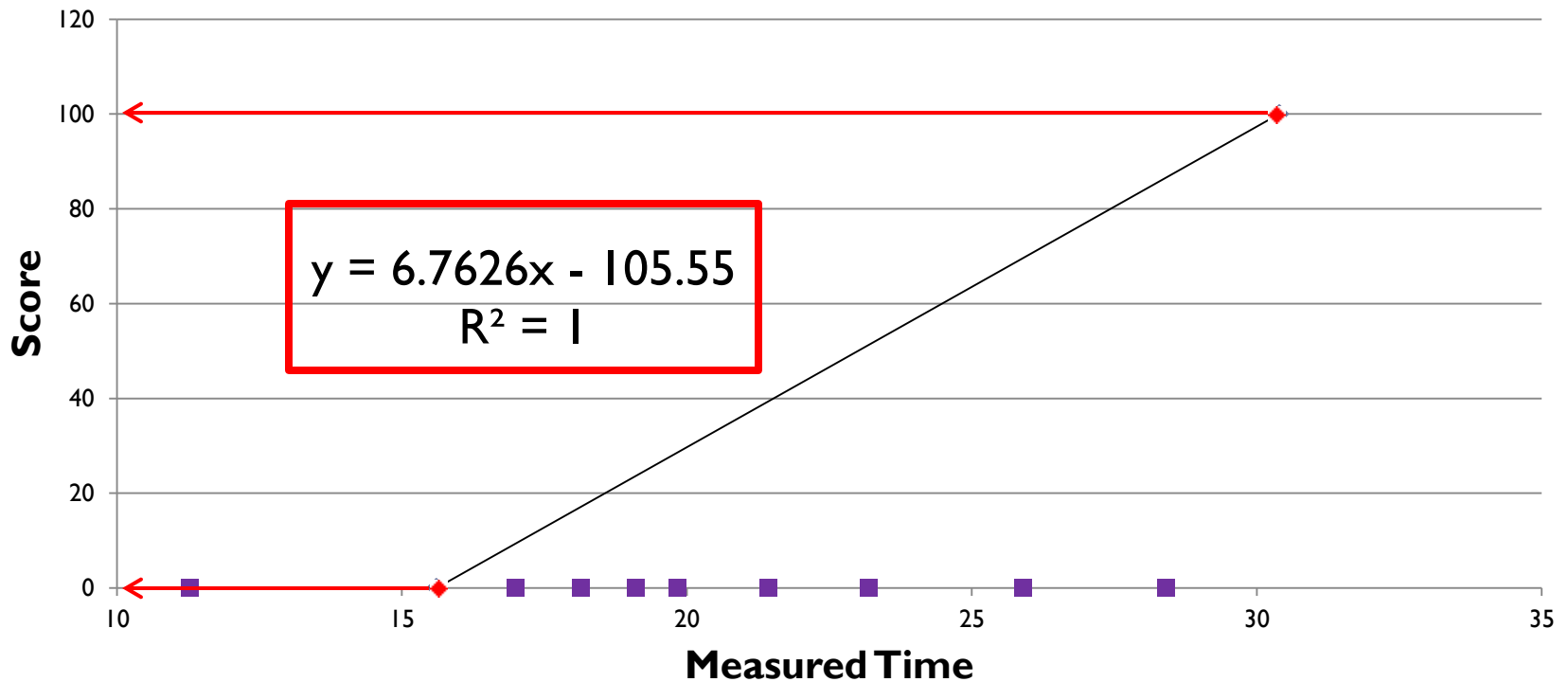
Defining an iRT Scale

▶ Retention time “independent”



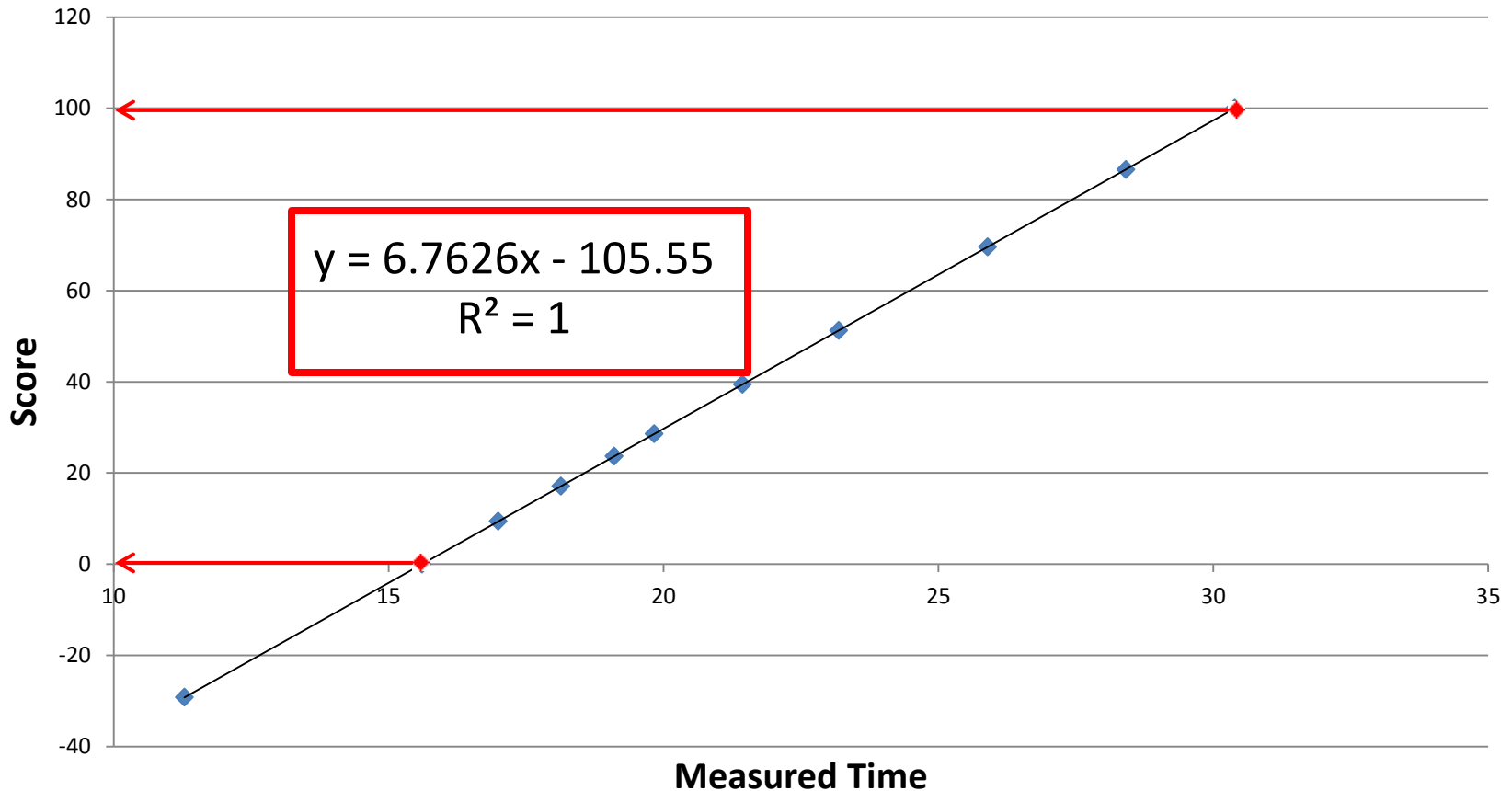
Defining an iRT Scale

- ▶ Points on a line (score = time * slope + intercept)

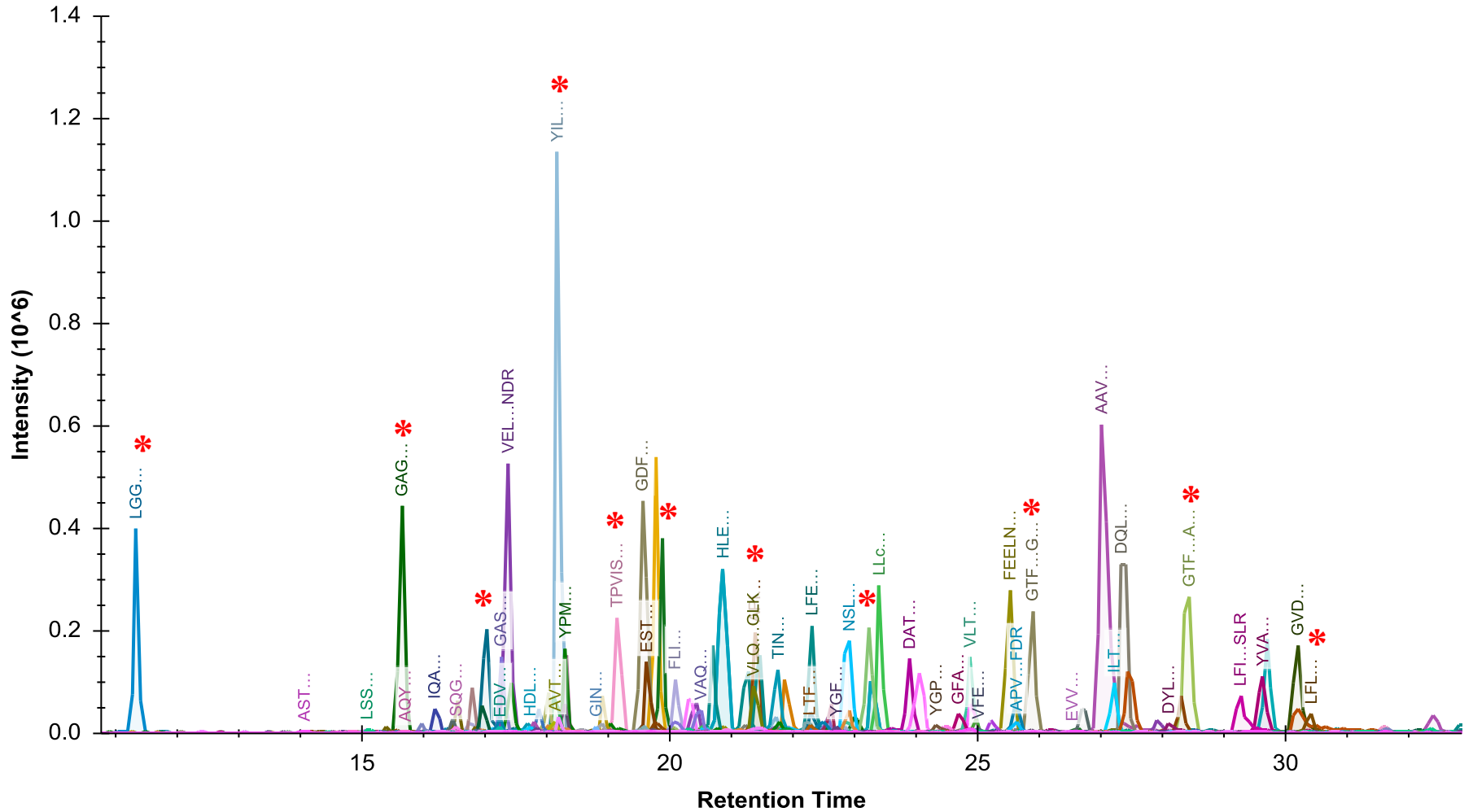


Defining an iRT Scale

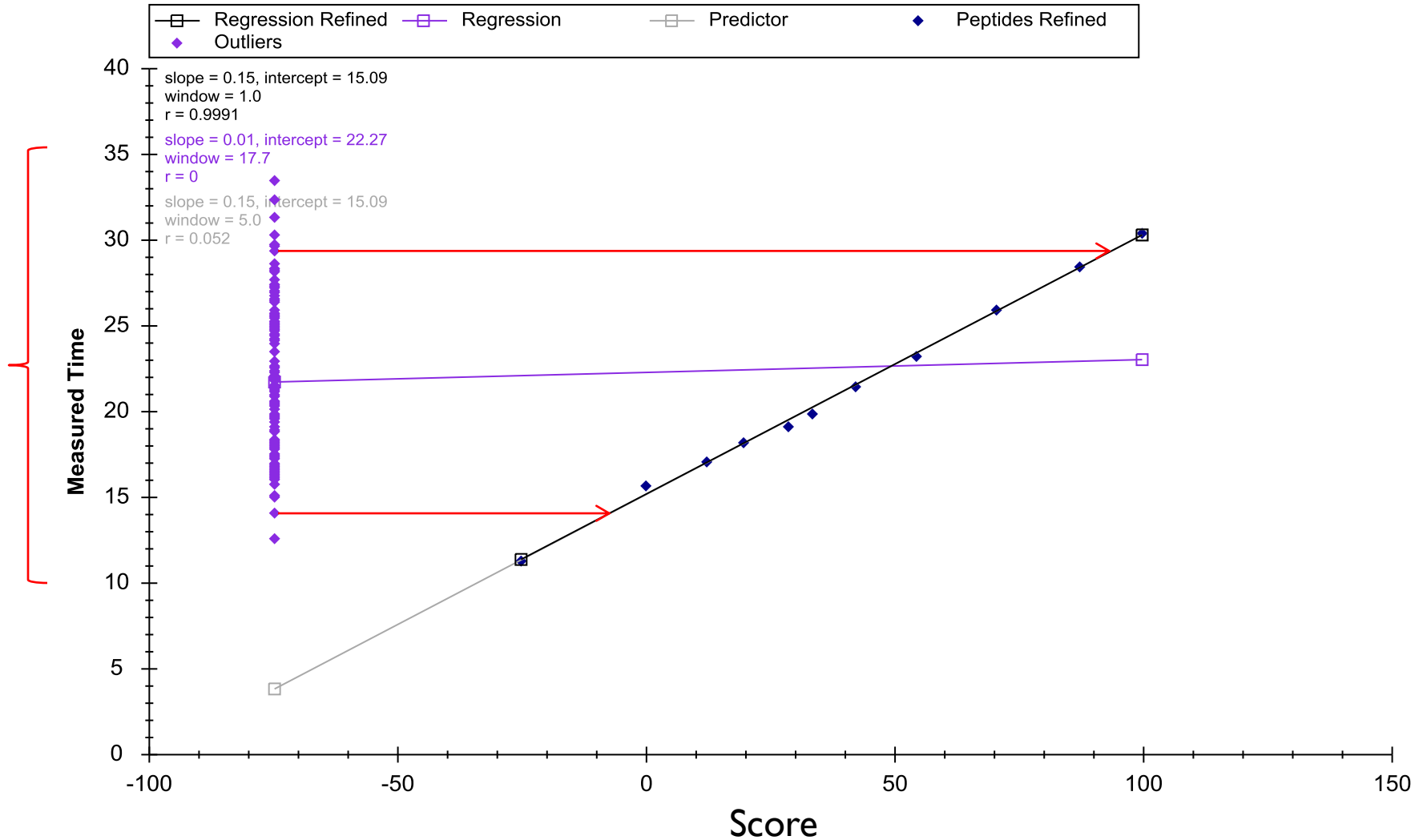
- ▶ Points on a line (score = time * slope + intercept)



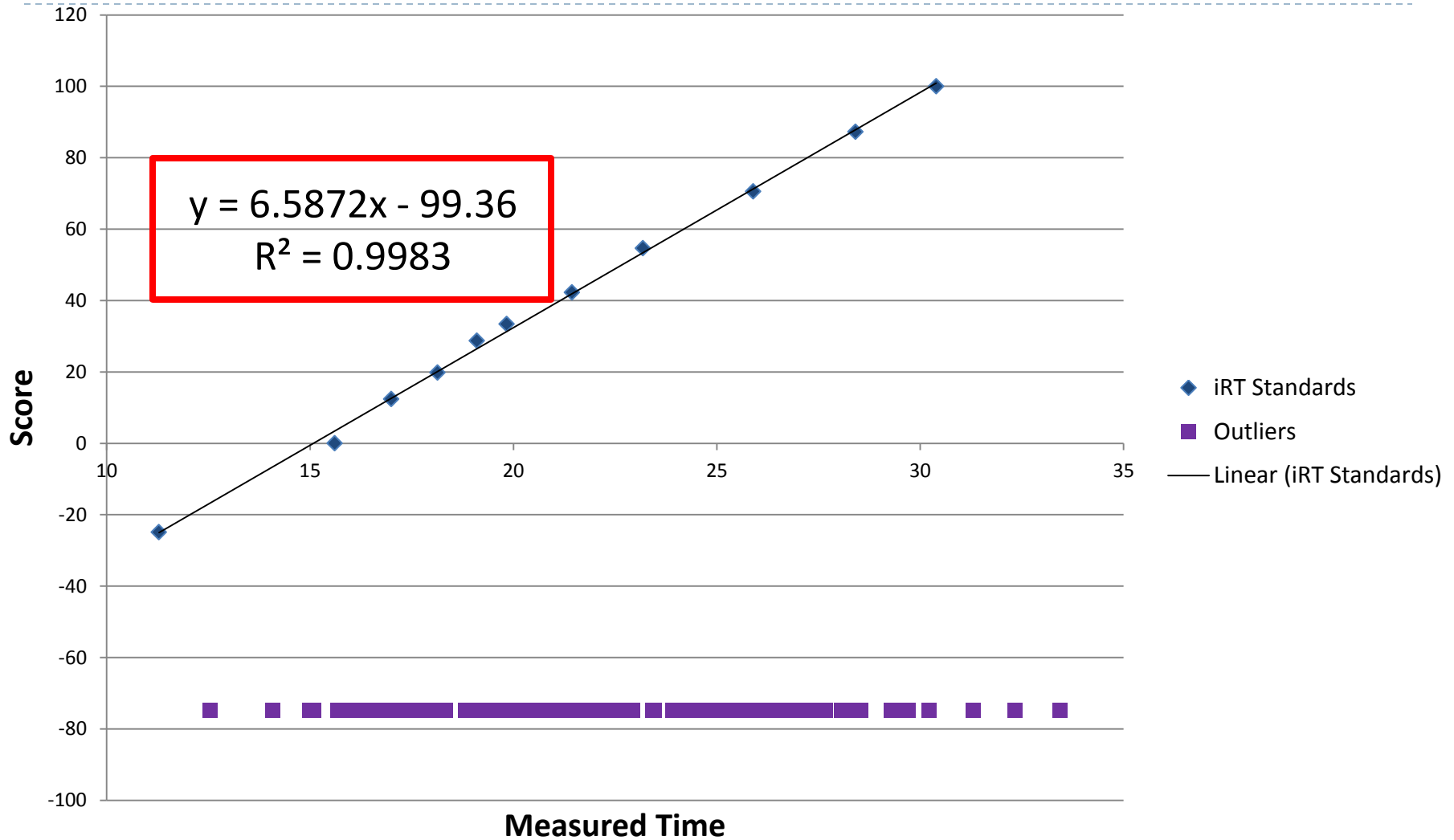
Building an iRT Library



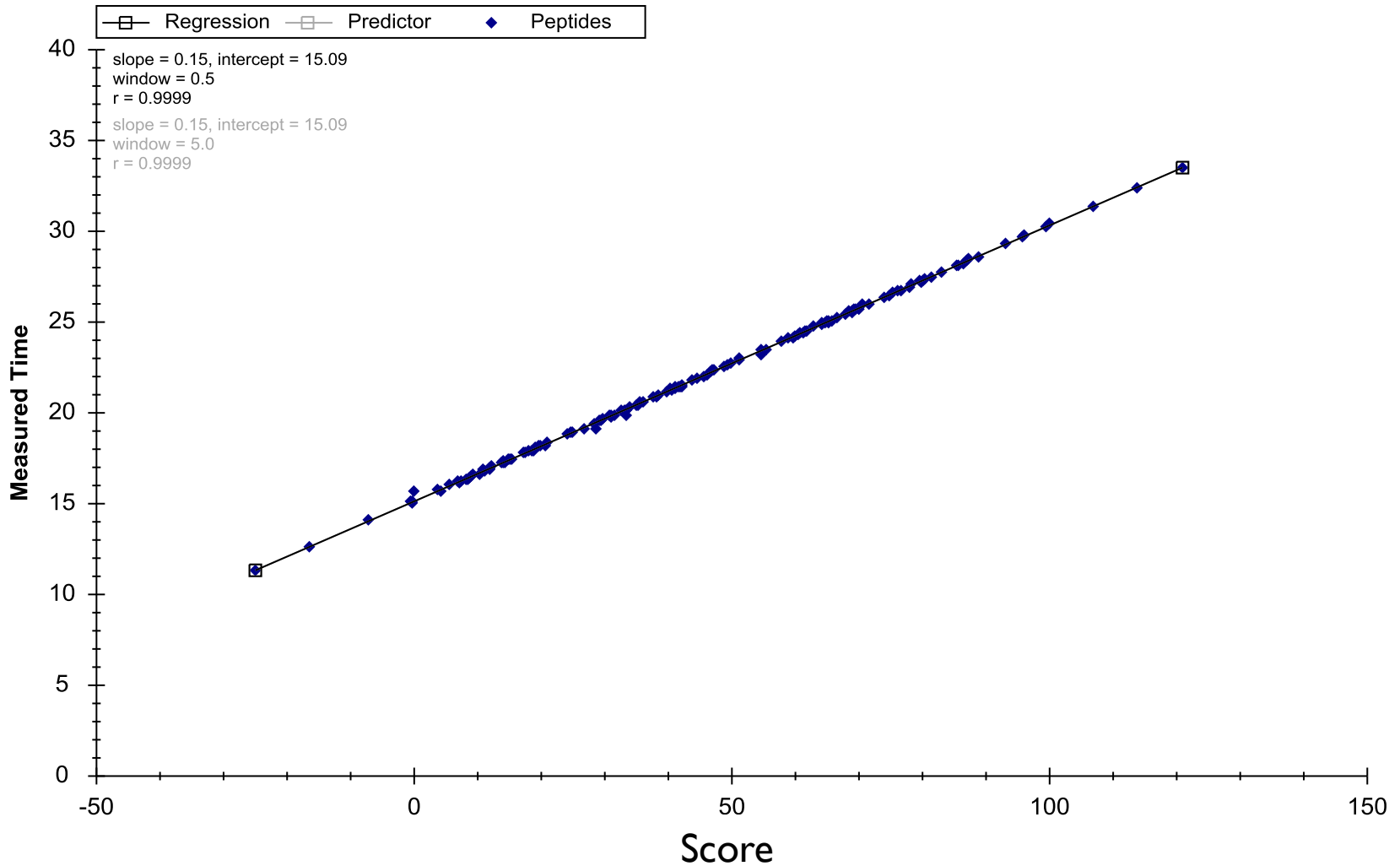
Building an iRT Library



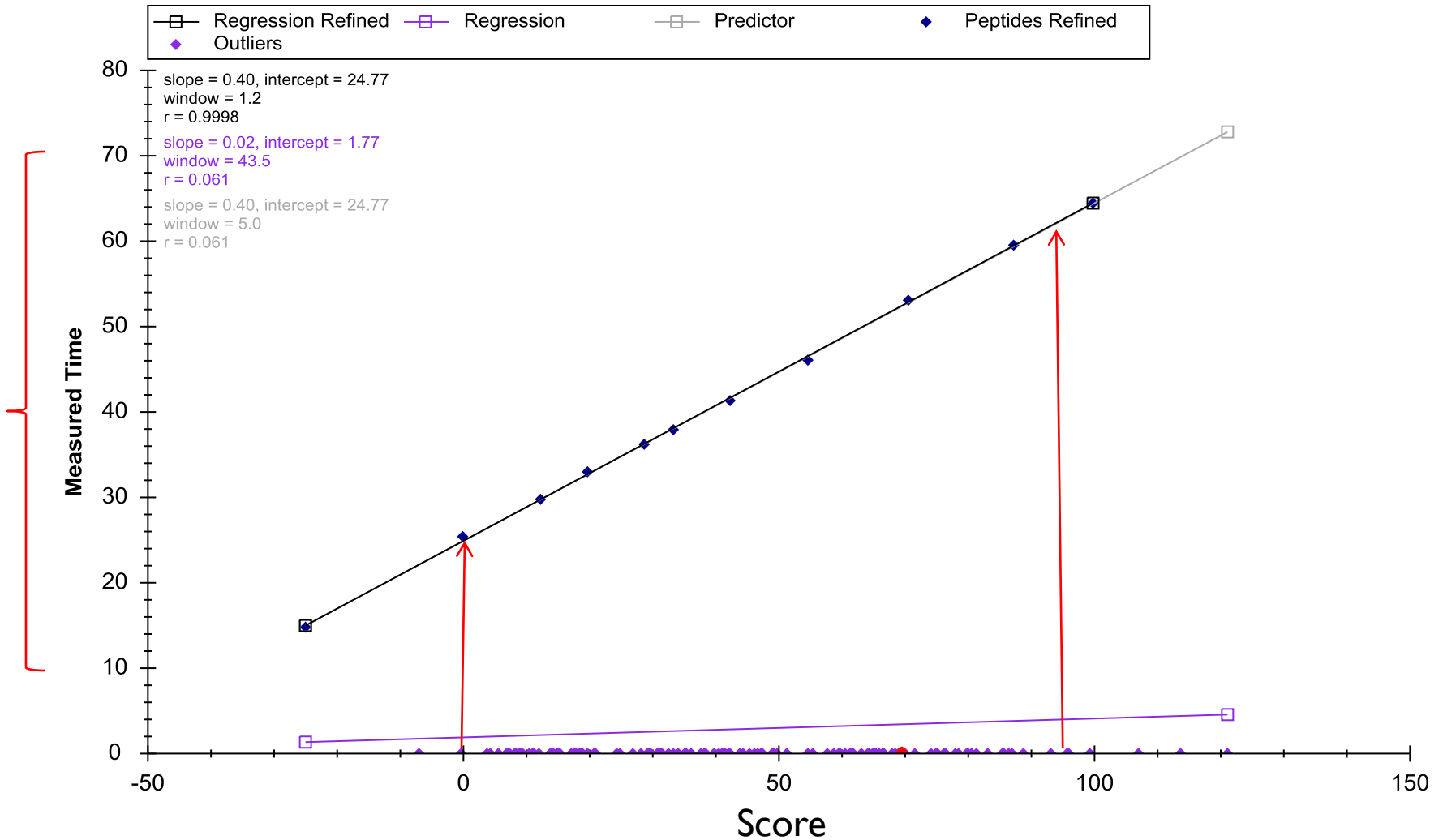
Building an iRT Library



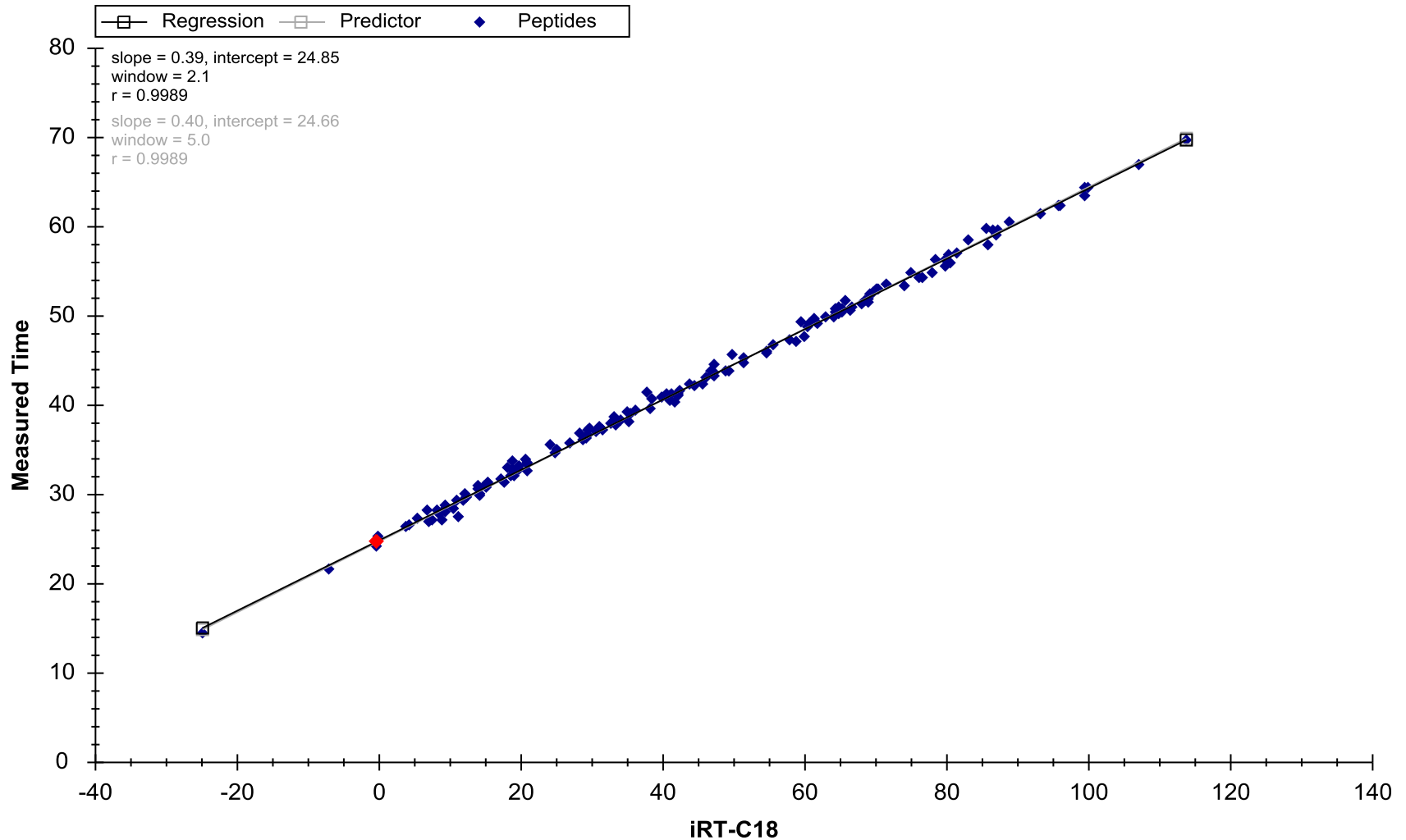
Building an iRT Library



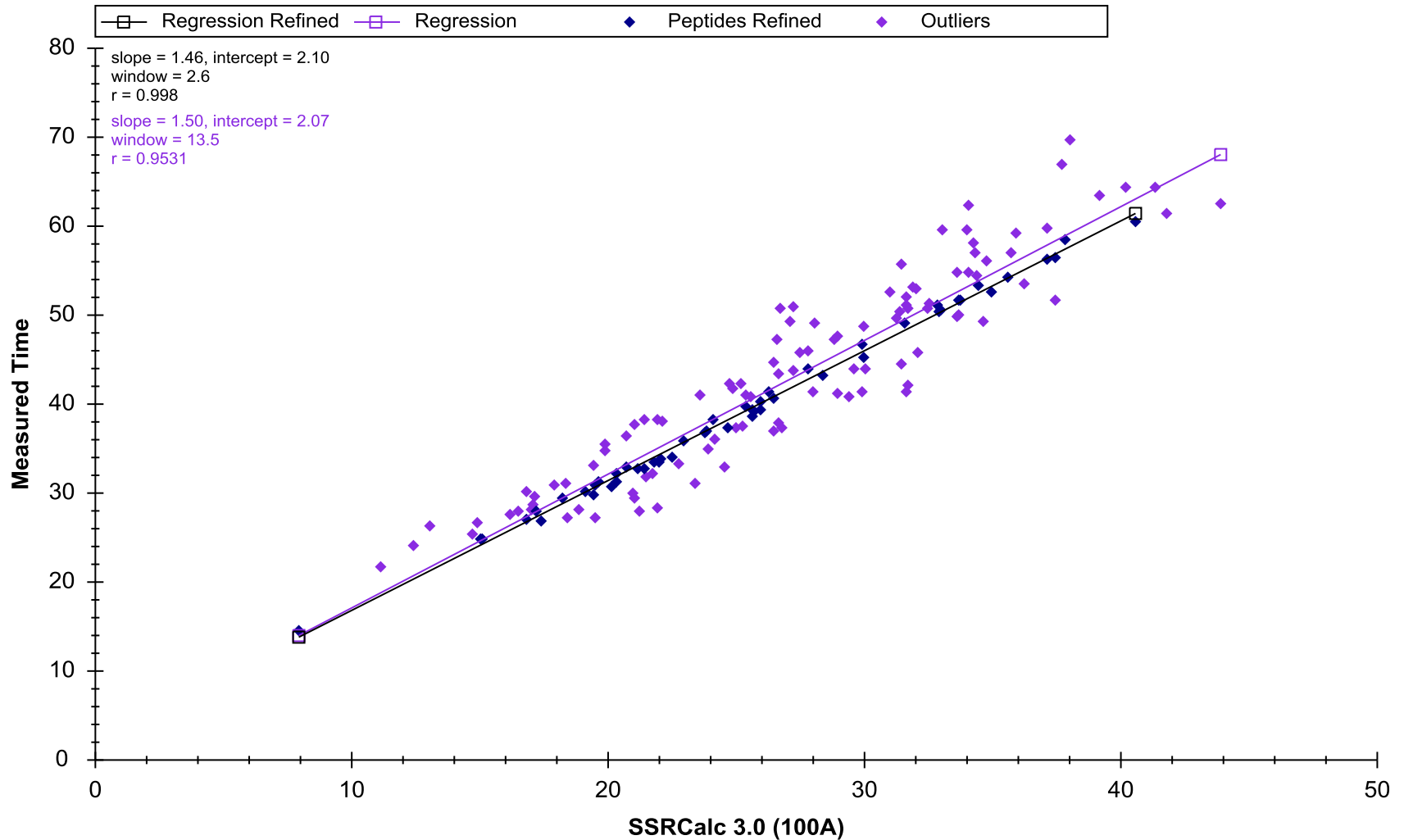
Using the iRT Library (Prediction)



Using the iRT Library to Measure



SSRCalc Predictor Correlation



Tutorial

- ▶ Calibrating, building and using an iRT library



Learn More

- ▶ iRT Retention Time Prediction Tutorial
- ▶ Webinar #8: TBD
 - ▶ Tuesday, June 16
- ▶ Workshop and ASMS
- ▶ Skyline User Group Meeting at ASMS
 - ▶ May 31 at Old Post Office, St. Louis, MO
- ▶ Workshop in Rio de Janeiro, August 31-September 2
- ▶ Workshop in Puerto Vallarta, November
- ▶ Weeklong Course at PRBB, Barcelona,
 - ▶ November 15-20



Questions?

- ▶ Ask any questions you have on iRT at the following form:

<https://skyline.gs.washington.edu/labkey/qa4skyline.url>

- ▶ Take the post-webinar survey:

<https://skyline.gs.washington.edu/labkey/survey4webinar.url>





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This ends this Skyline Tutorial Webinar.

Please give us feedback on the webinar at the following survey:

<https://skyline.gs.washington.edu/labkey/survey4webinar.url>

A recording of today's meeting will be available shortly at the Skyline website.

We look forward to seeing you at a future Skyline Tutorial Webinar.

