

FT-ARM: a software tool for multiplexed peptide identification and quantification



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Overview:

- Multiplexed peptide identification and quantification
- Accurate mass fragment ion intensities
- Wideband, data independent MS/MS acquisition
- Open source software tool

Introduction:

In contrast to standard data-dependent acquisition (DDA) which targets the most abundant peptides in any precursor scan, multiplexed fragmentation strategies hold the promise of increasing identification rates. Multiplexed fragmentation entails the isolation and fragmentation of multiple peptides simultaneously.

The Fourier Transform All Reaction Monitoring (FT-ARM) tool was developed to identify and quantify peptides by searching peptides from a sequence database against multiplexed spectra acquired in data independent mode. Identifications rely on accurate fragment ion matches. And in contrast to selected reaction monitoring (SRM), quantification is performed sensitively on a large scale without the need for assay development.

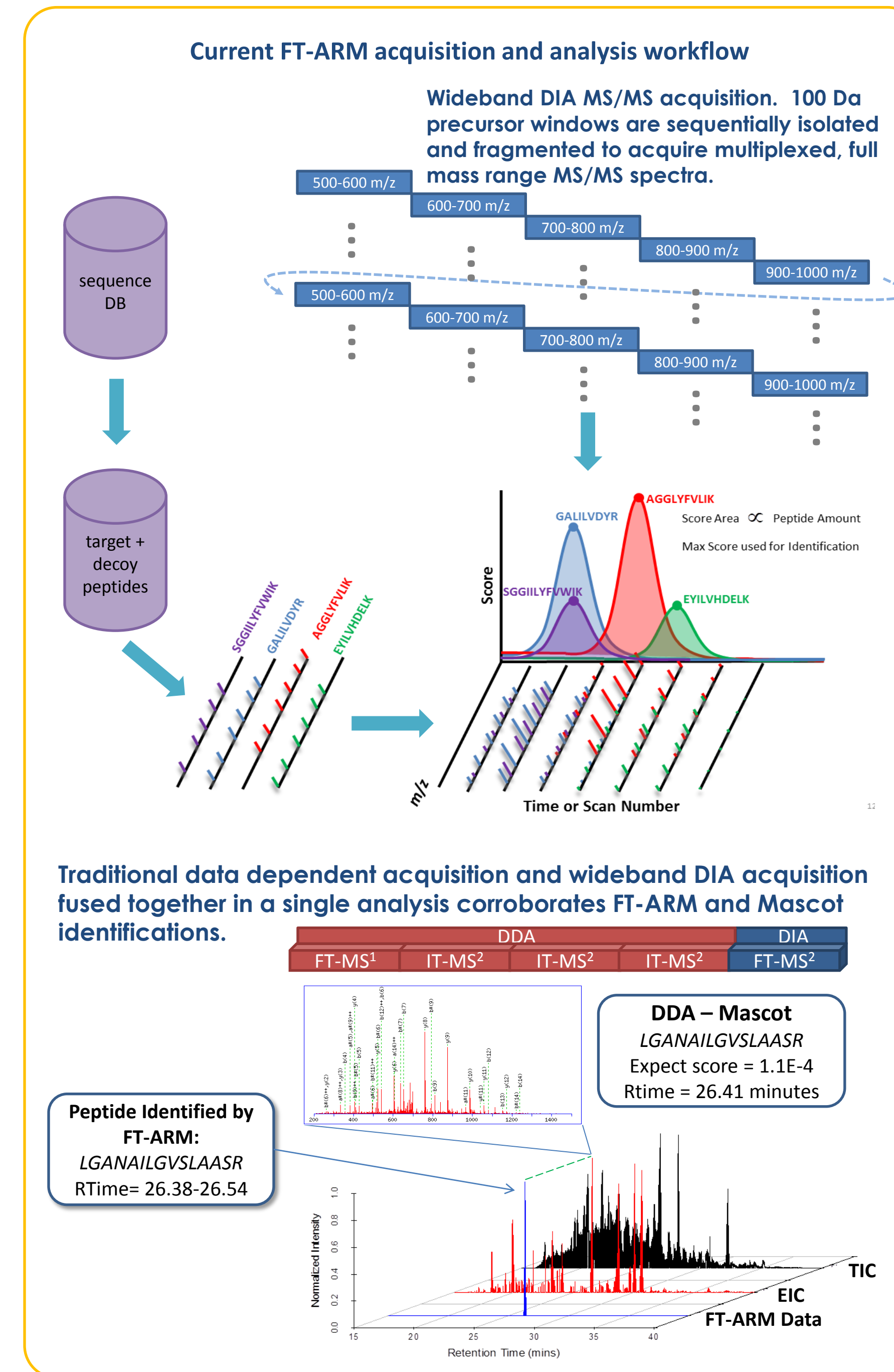
The FT-ARM software tool has been developed to perform both identification and quantification. Inputs are search parameters, a sequence database, and the wideband data independent (DIA) mass spectrometry acquisition file. Originally developed to target data acquired on Thermo FTICR and Orbitrap instruments, FT-ARM has been ported to interface with the ProteoWizard library to facilitate compatibility with data from other vendors.

Method:

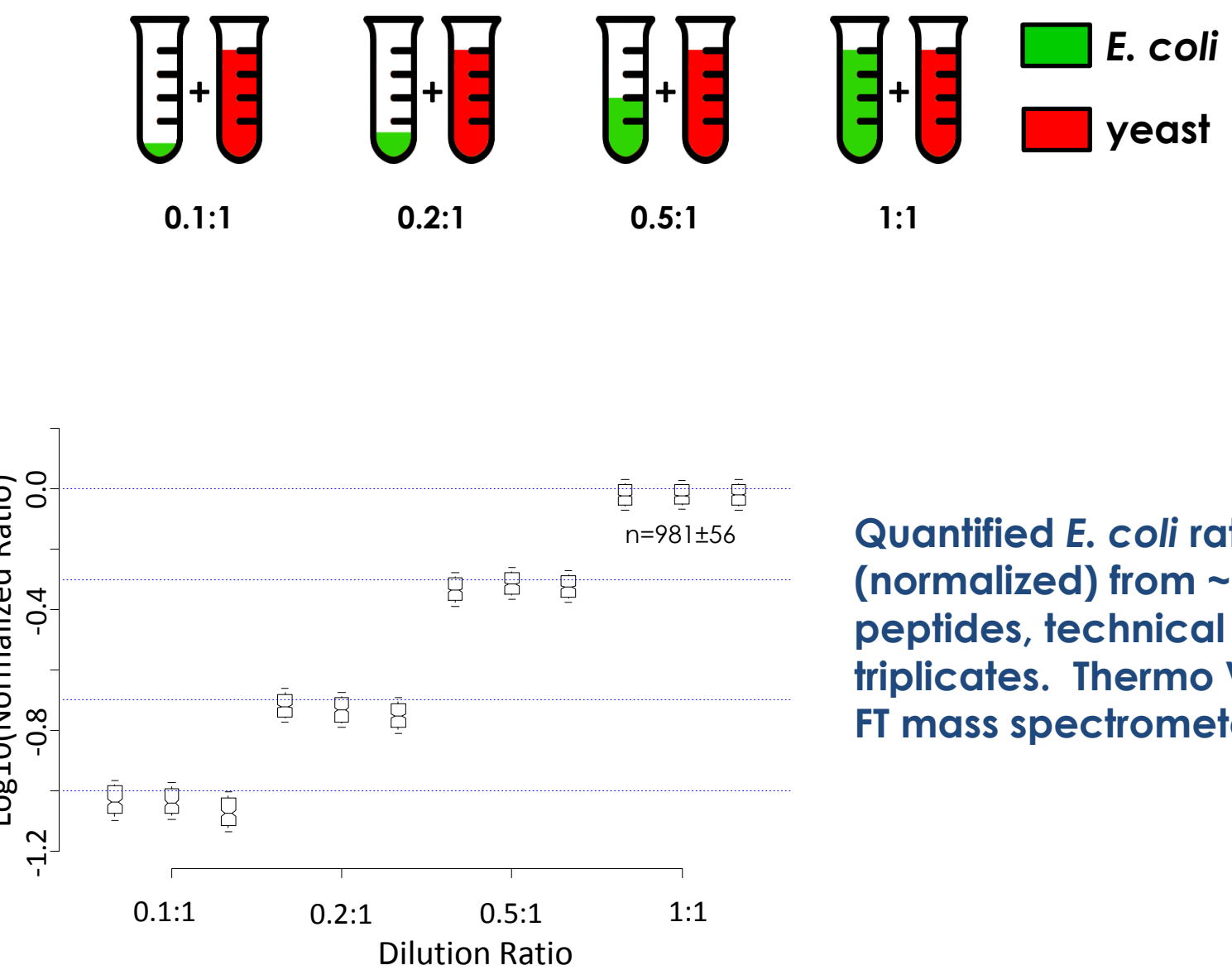
- FT-ARM analysis tool written in C++
- Graphical user interface written in C#
- Searches are performed by scoring peptides against every DIA MS/MS spectra using an accurate fragment mass dot product score
- Quantification abundance values are derived from the area of the dot product score chromatogram peak

Results:

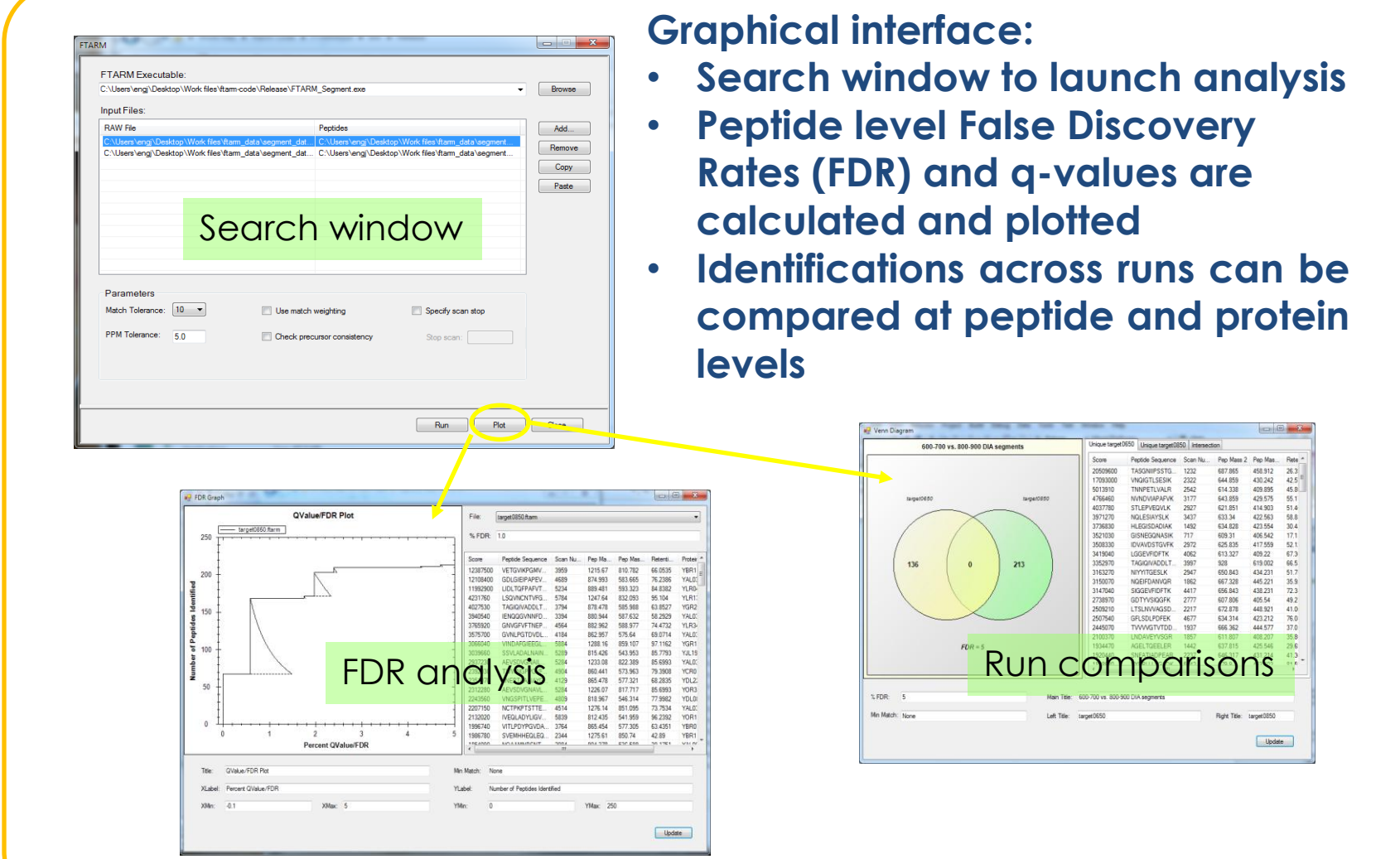
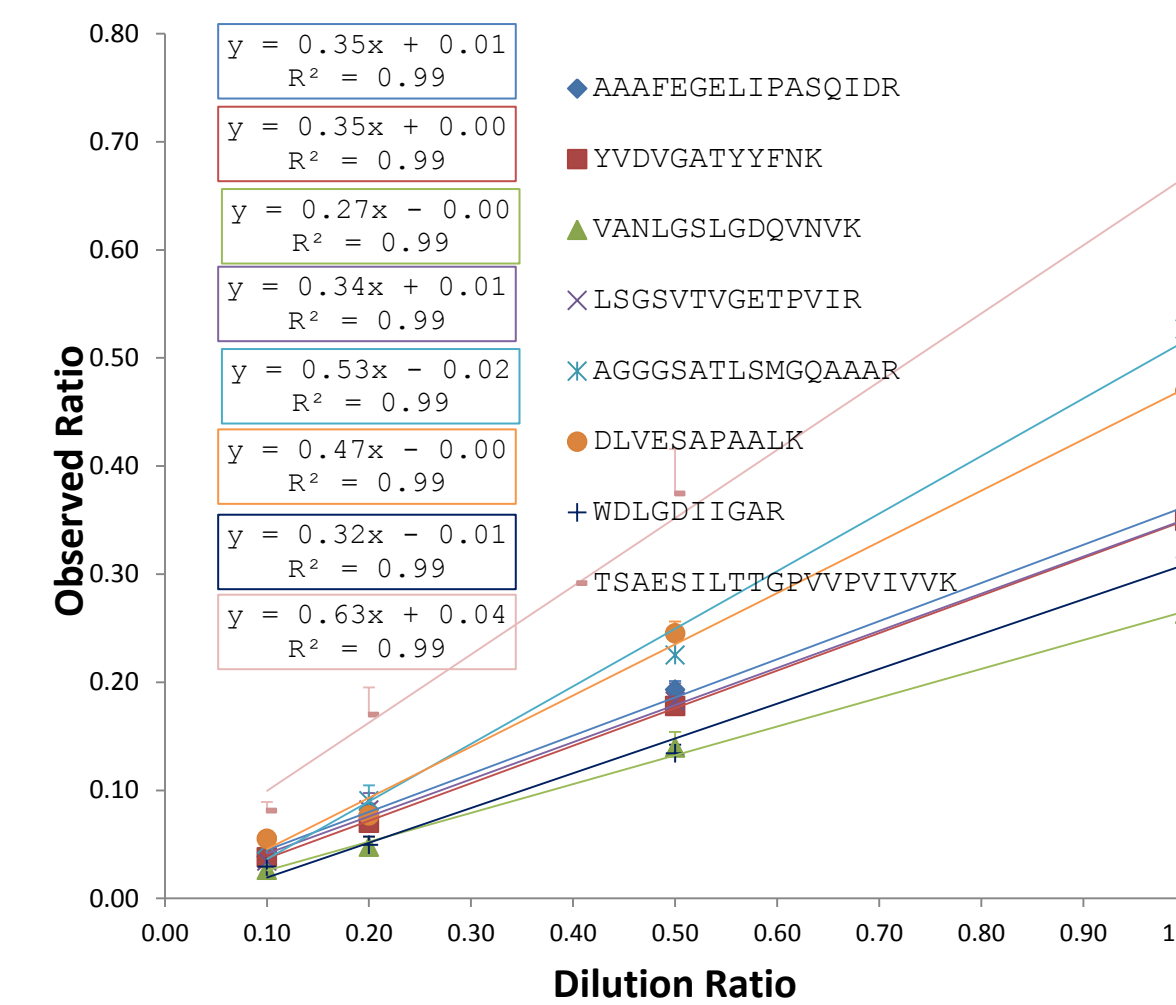
- The software port for file reading via the ProteoWizard library is complete; support for specific vendor data will be addressed when such data is acquired
- An automated linear regression of calculated vs. measured retention times has been implemented; this restricts searching peptides against spectra in an expected retention time range



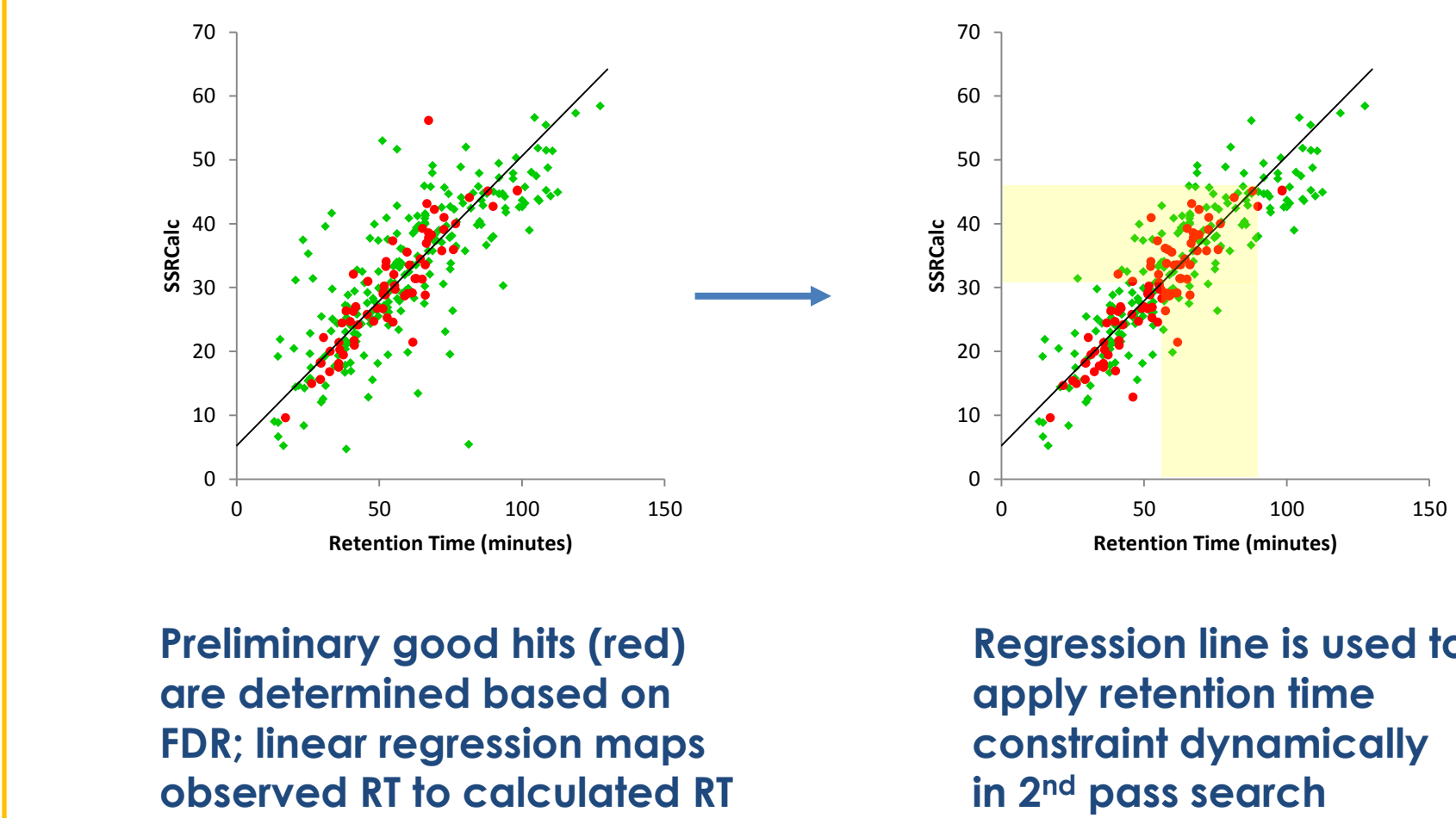
To demonstrate robustness, reproducibility, and linearity in quantification on a complex sample, *E. coli* is spiked into a yeast background at 1:1, 0.5:1, 0.2:1, and 0.1:1 dilution ratios.



A selection of *E. coli* peptides displaying reproducibility and linearity of quantification.



FT-ARM has been extended to perform automated linear regression to apply retention time filtering to the search. Initial pass performs target/decoy searches against all spectra. Confident peptide IDs are extracted, a linear correlation of SSRCalc predicted vs. measured retention times is calculated. A second pass, retention time restricted search is then performed.



References:

- FT-ARM: doi 10.1021/pr2008175
- ProteoWizard: doi 10.1093/bioinformatics/btn323
- SSRCalc: doi 10.1021/ac060777w

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Conclusions:

- First demonstrated method of peptide identification and quantification in the absence of precursor mass
- A free software tool that performs both identification and quantification on wideband, DIA MS/MS spectra
- Poster reprint & software are available at <http://brucelab.gs.washington.edu>

