ABRF PRG STUDY TO EVALUATE DATA-INDEPENDENT ACQUISITION FOR PROTEIN QUANTIFICATION IN ACADEMIC AND CORE FACILITY SETTINGS

Yan Wang[1]; Joanna Kirkpatrick[2]; Benjamin Neely[3]; LeRoy Martin[4]; Allis Chien[5]; Laura E. Herring[6]; Mukul Midha[7]; Brett S. Phinney[8]; Baozhen Shan[9]; Paul M. Stemmer[10]; Pratik D Jagtap[11]

[1]University of Maryland, College Park, MD; [2]Leibniz Institute on Aging, Germany; [3]Chemical Sciences Division, National Institute of Standards and Technology, Charleston, SC; [4]Waters Corp., Beverly, MA; [5]Stanford University, Stanford, CA; [6]Department of Pharmacology, UNC-Chapel Hill, Chapel Hill, NC; ; [7]Institute for Systems Biology, [8]UC Davis, Davis, CA; [9]Bioinformatics Solutions Inc., Waterloo, ON; [10]Wayne State University, Detroit , MI; [11]Department of Biochemistry, Molecular Biology and Biophysics, University of Minnesota, Minneapolis, MN
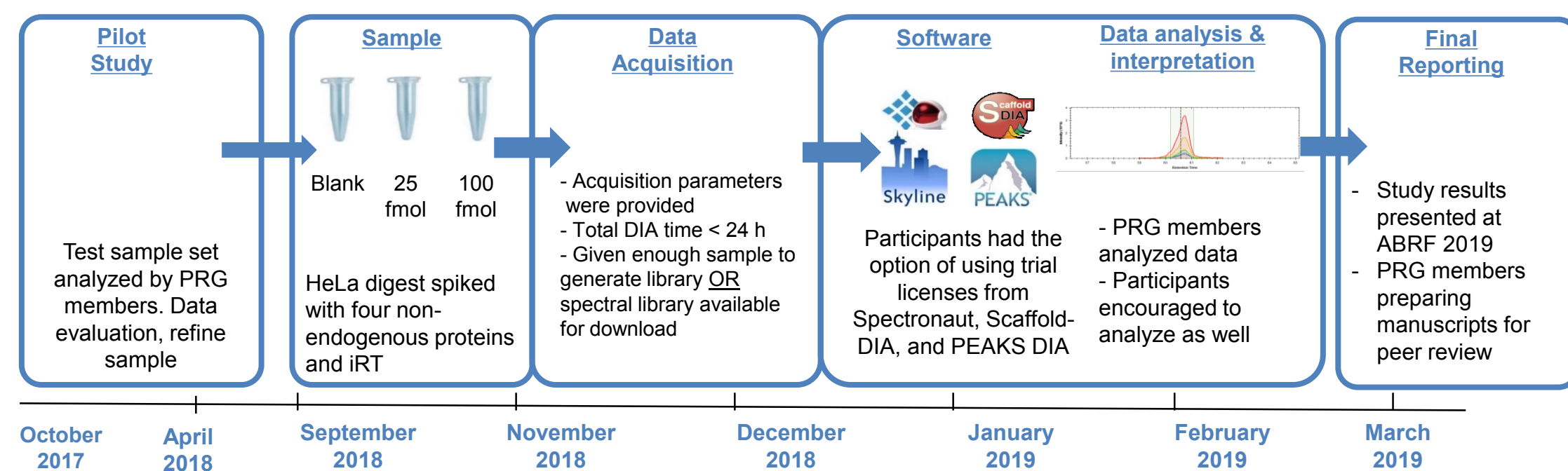
Study Highlights

- Study samples were send to 63 labs from 20 countries around the globe
- 40 labs uploaded data, 53 labs filled out survey, 35 data sets used for preliminary analysis
- Most participants were experienced LCMS users, but few had used DIA
- Data from most participants reflects expected fold change of spike-in proteins. Accuracy of quantitation was not correlated to previous DIA experience
- By providing prototype sample set and recommended methods for different instrument platforms, and extended trial licenses, the PRG provided an opportunity for labs to evaluate their readiness to carry out DIA analysis
- Samples were injection-ready, data was analyzed by PRG members, thus the results presented here reflects data quality

Study Design

- Provided to participants
  - Predigested, C18 cleaned samples (3 in each set), ready for analysis, 25 µg total peptide each with iRT spiked in
  - Recommended mass spec methods for certain instruments, guidelines for method generation
  - Trial software license for DIA data analysis
  - Chromatogram and/or Spectral library
- Required from participants
  - Maximum DIA acquisition time is 24 hours – DO NOT EXCEED
  - Online survey on data acquisition and analysis (if self analyzed)
  - Raw data is required
  - All submissions are anonymous
- Optional for participants
  - Generation of in-house spectral library – if enough sample left after DIA analysis
  - Self analyze data
    - trial licenses provided with extra time period
    - Spectral library available
- Data analysis
  - Carried out by experienced PRG members as "expected" data interpret for participants
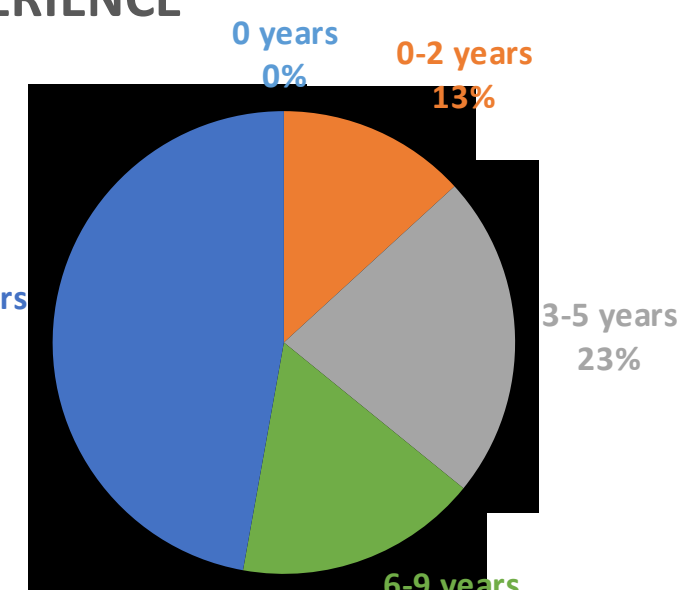
Study Timeline

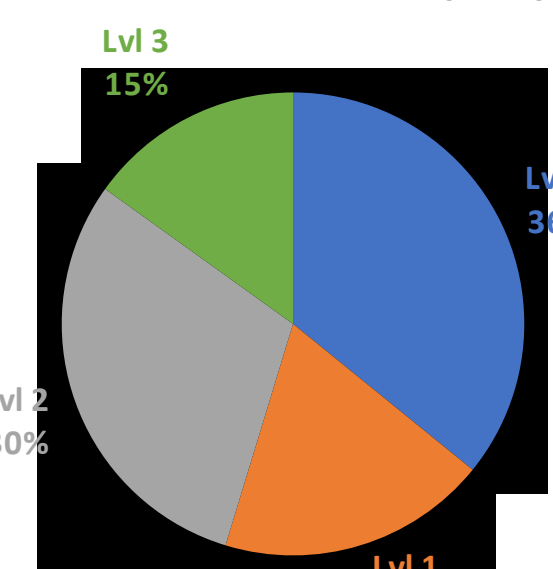| Pilot Study	Sample	Data Acquisition	Software	Data analysis & interpretation	Final Reporting
Test sample set analyzed by PRG members. Data evaluation, refine sample	Blank 25 fmol 100 fmol — HeLa digest spiked with four non-endogenous proteins and iRT	- Acquisition parameters were provided - Total DIA time < 24 h - Given enough sample to generate library OR spectral library available for download	Skyline PEAKS — Participants had the option of using trial licenses from Spectronaut, Scaffold-DIA, and PEAKS DIA	- PRG members analyzed data - Participants encouraged to analyze as well	- Study results presented at ABRF 2019 - PRG members preparing manuscripts for peer review

October 2017 — April 2018 — September 2018 — November 2018 — December 2018 — January 2019 — February 2019 — March 2019

Participant Matrices

MS INSTRUMENT

QEHFX 14%; QEHF 14%; QE+ 5%; QE 6%; Lumos 26%; Fusion 17%; tripleTOF 11%; VelosPro 3%; Xevo 3%

Study Participants: 63 labs, 20 countries, 16 US States

LC EXPERIENCE
0 years 0%; 0-2 years 13%; 3-5 years 23%; >10 years 47%; 6-9 years 17%

DIA EXPERIENCE (0-3)
Lvl 3 15%; Lvl 0 36%; Lvl 2 30%; Lvl 1 19%

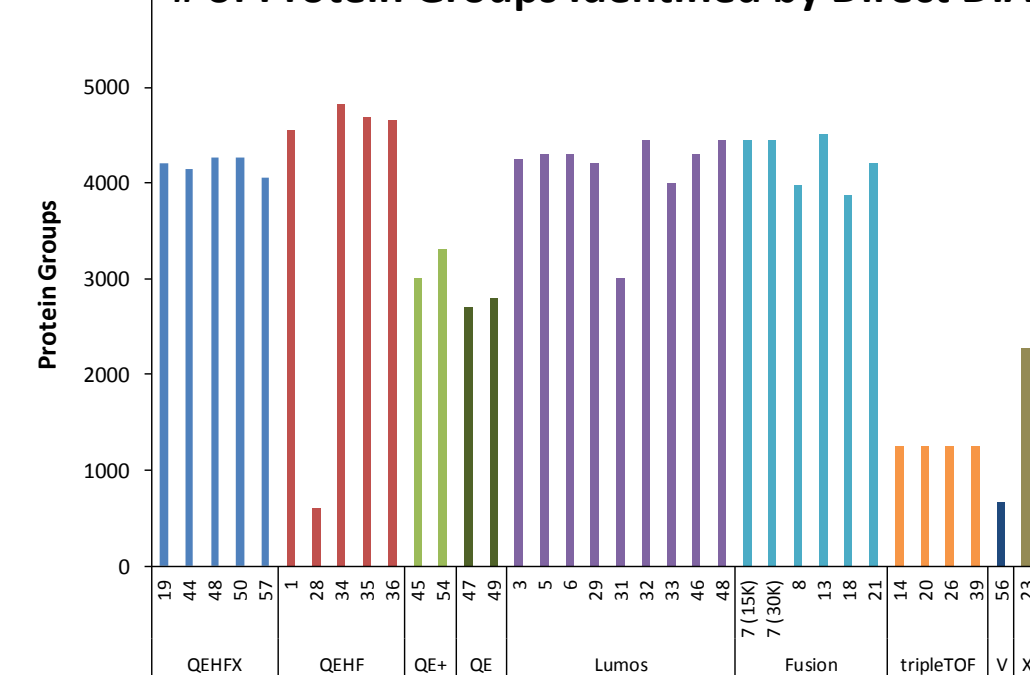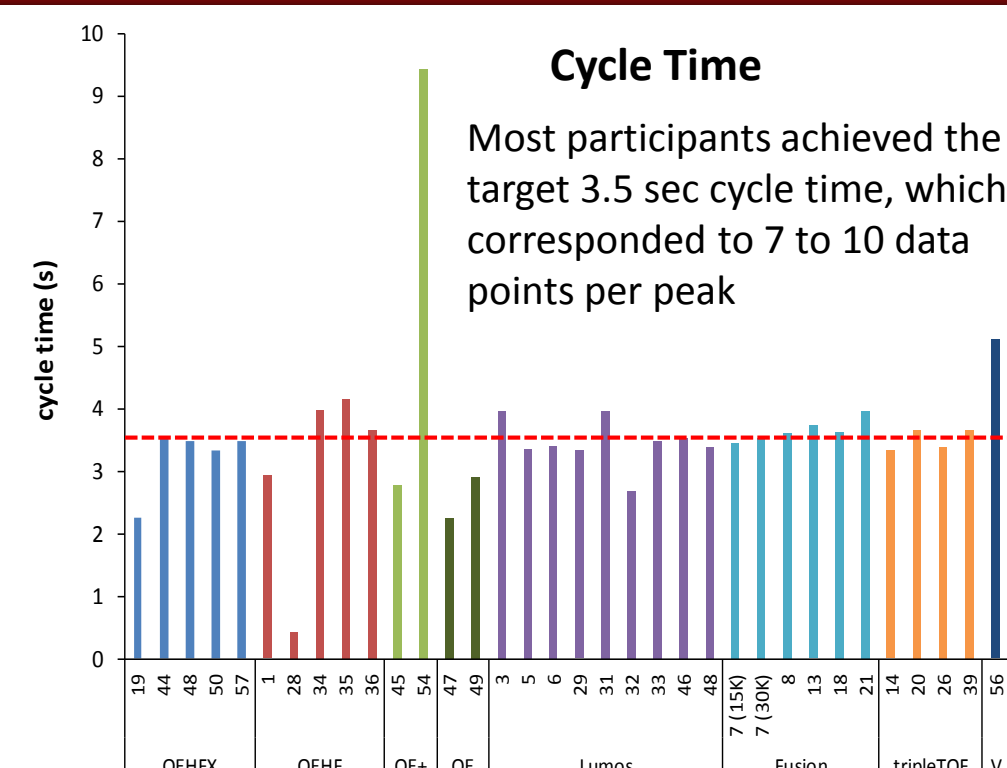### PRG SUGGESTED MS METHOD
No 40%; Yes 60%
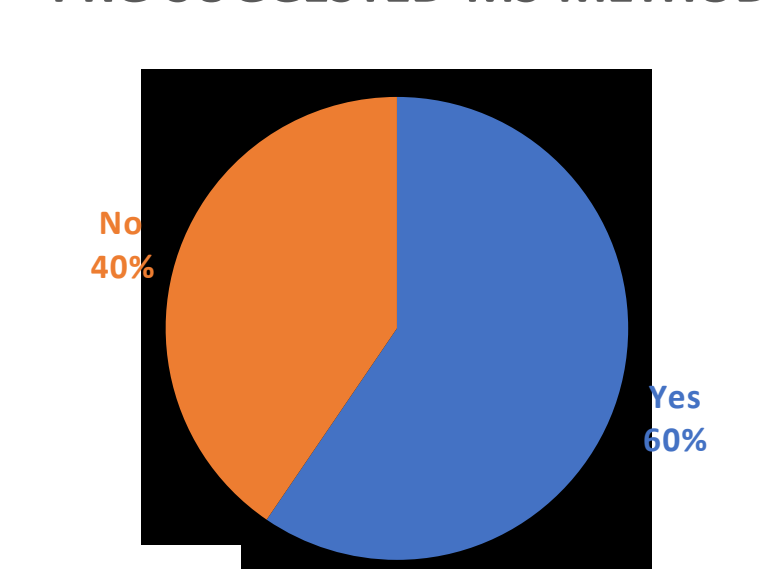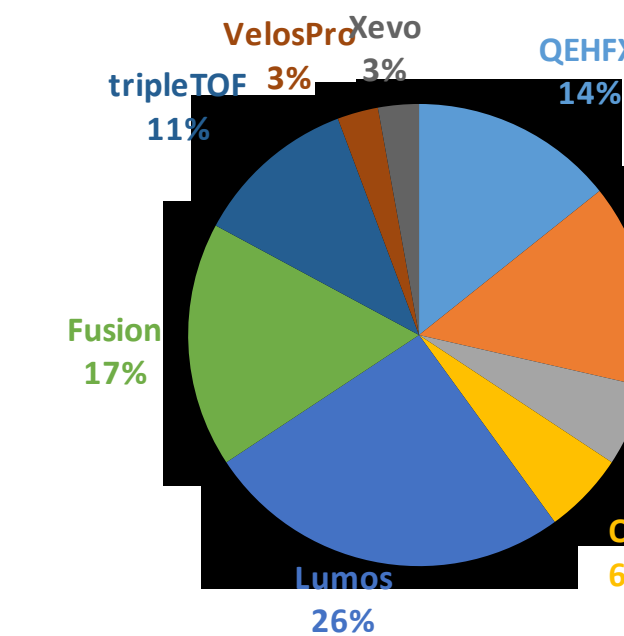
Overall Data Quality

of Protein Groups Identified by Direct DIA

Cycle Time
Most participants achieved the target 3.5 sec cycle time, which corresponded to 7 to 10 data points per peak
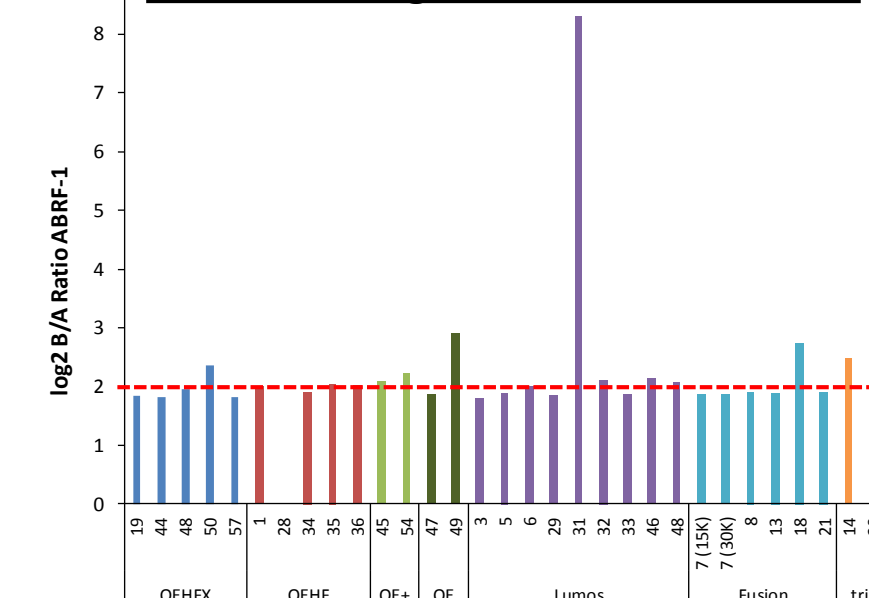
Acknowledgements

Software support
- Biognosys
- Bioinformatic Solutions, inc
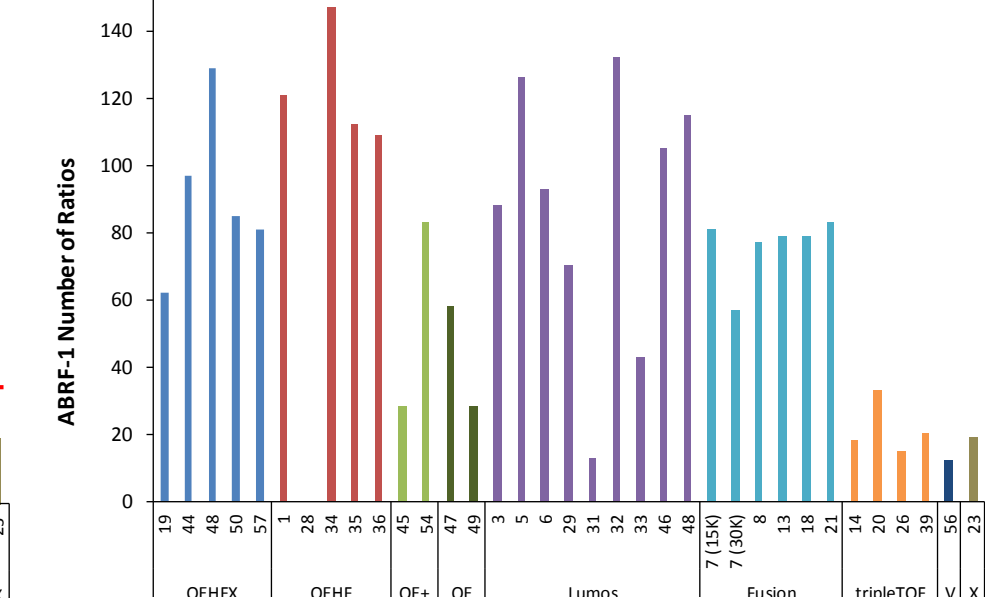- Proteome Software

Technical support
- Waters Corporation
- Thermo Scientific
- Sciex
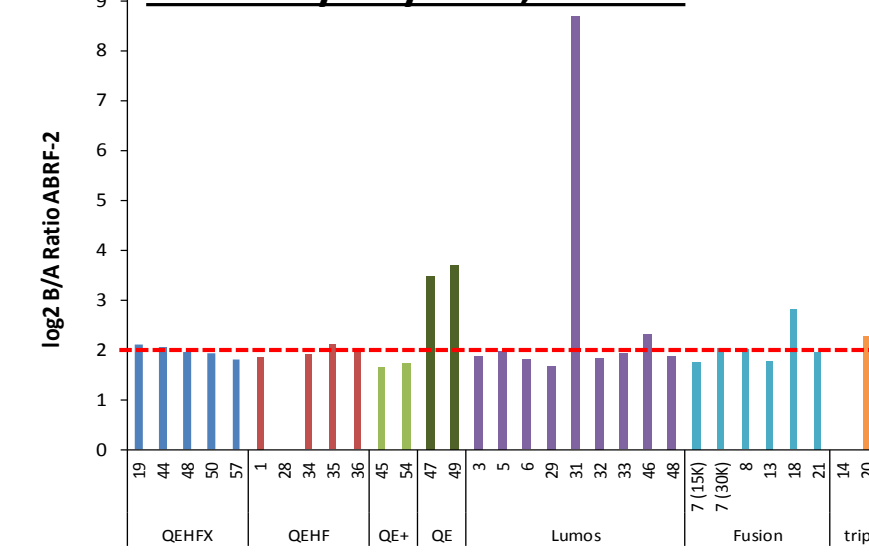
Quantification of Spike-in Proteins

ABRF-1 beta-galactosidase, 1024 AA

ABRF-2 lysozyme C, 147 AA

ABRF-3 glucoamylaseC, 640 AA

ABRF-4 protein G, 185 AA