Proteomics Standards Research Group (sPRG)

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www.abrf.org/sprg

Development and Characterization of a Proteomics Standard Consisting of 1000 Stable Isotope Labeled Peptides

Gordana Ivosev

Proteomics Standards Research Group (sPRG)

Proteomics Standards Research Group (sPRG) 2014-2015

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Brendan MacLean	University of Washington
Brian Searle	Proteome Software
Craig Dufresne	Thermo Fisher Scientific
David Hawke	UT MD Anderson Cancer Center
Gordana Ivosev	Sciex
Kristie Rose	Vanderbilt University
Paul Rudnick	National Institute of Standards and Technology
Scott A. Shaffer	University of Massachusetts Medical School
Toni Koller	Columbia University

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Study Timeline

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Three Year Study

- **Year 1**: Peptide synthesis and qualification by sPRG.
- **Year 2**: Initiate the study, collect and analyze the data, commercialization of the standard
- **Year 3:**
  - **Additional analysis conducted to put some light on unexpected intra-lab reproducibility**
  - **Characterization of the standard across different matrixes**
- (Future) Publication of Manuscript

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Continuing from 2014 results…
multi-lab experiment questions

- What is correct answer?
  - It is impractical to manually validate ~1000 peptides
- Are we averaging across too many different factors?
  - Or we have some ‘noise’, bias, that we did not account for?
- What can we learned about the standard
  - Useful guidelines for more accurate results

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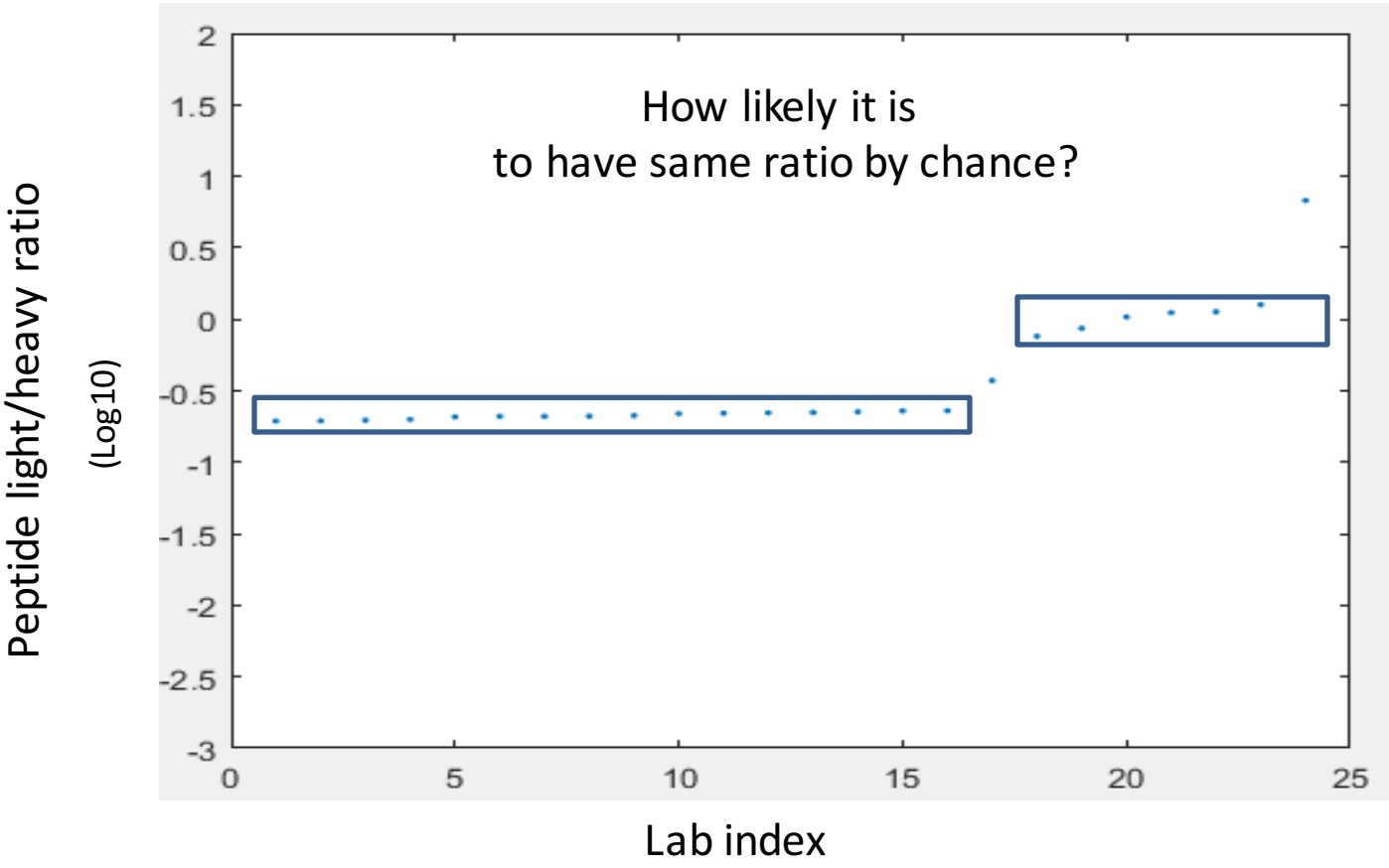
What is the correct ratio for this peptide?

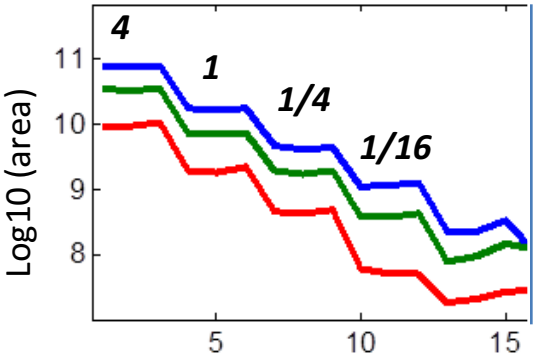
- Multi-Lab experiment –
  - what is reasonable estimate of true light/heavy ratio



Dilution experiment and the correct answer

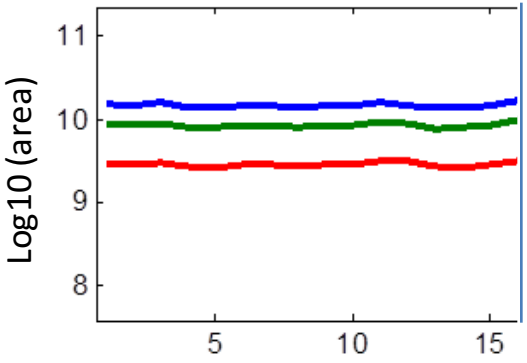
- Dilution experiment:
  - If we are integrating correct peak, we expect to see characteristic intensity patterns
    - We expect ratio of two dilutions to be 4X

Heavy – 3 isotopes



Sample replicate

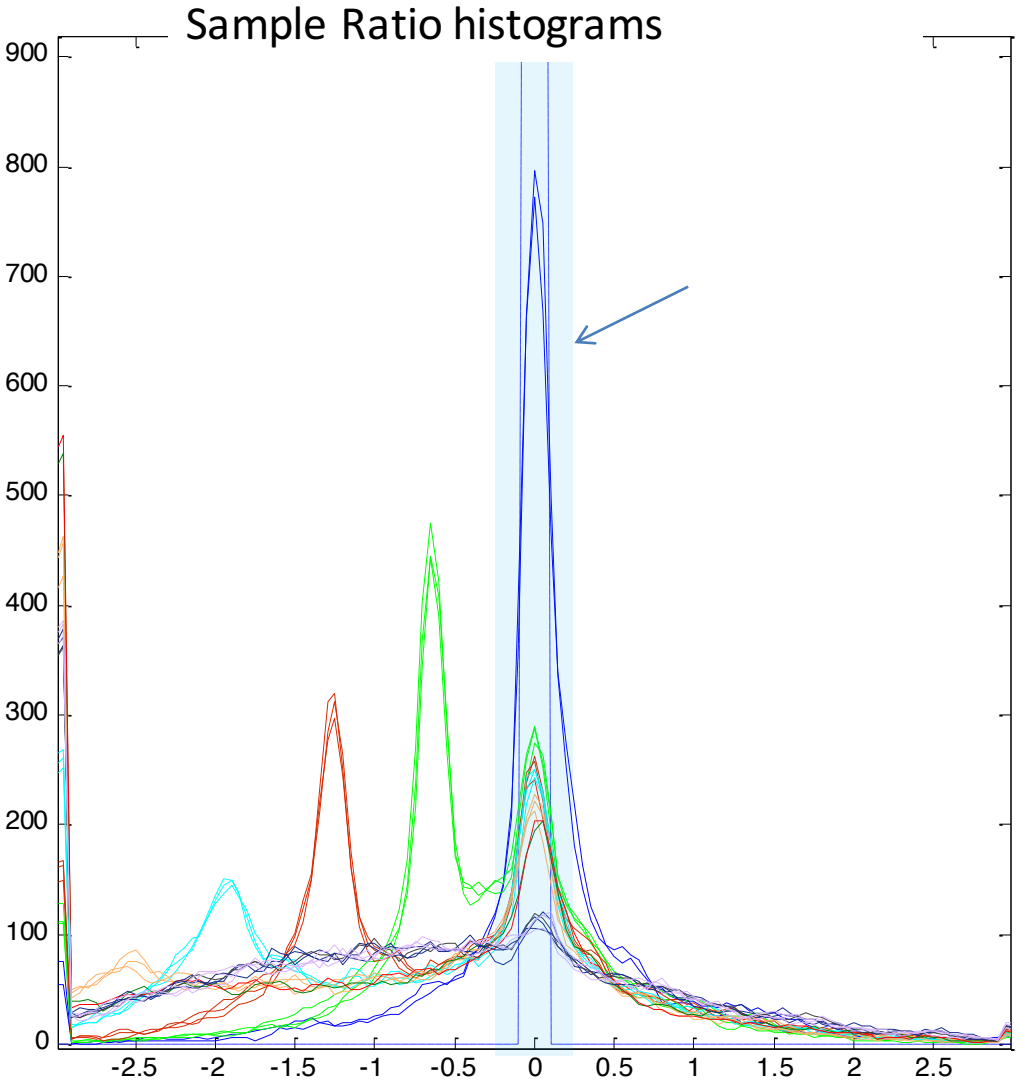
Light – 3 isotopes



Sample replicate

Proteomics Standards Research Group (sPRG)

Use known dilution ratio



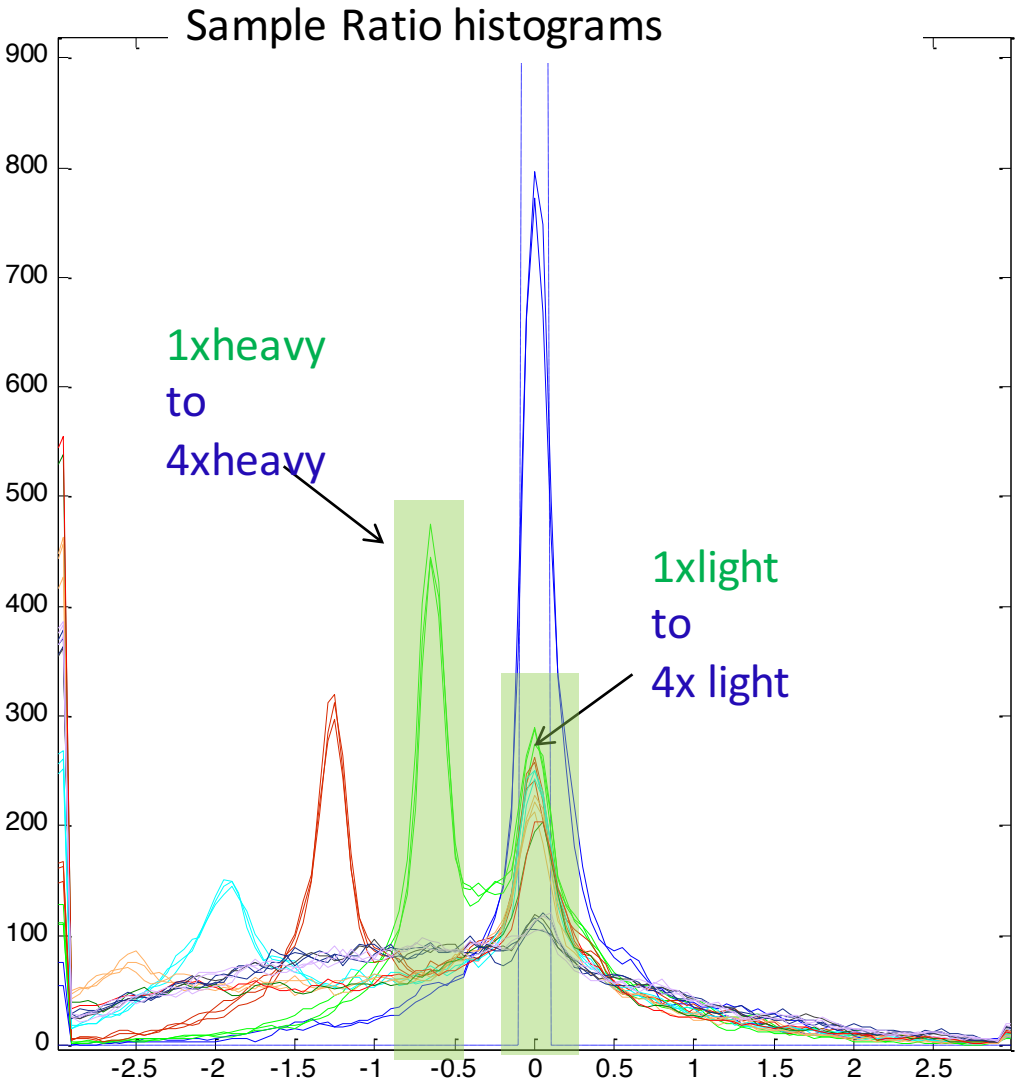
- - - - 4x tch.1 to tch.1

——— 4x tch.2 to tch.1

——— 4x tch.3 to tch.1

Proteomics Standards Research Group (sPRG)

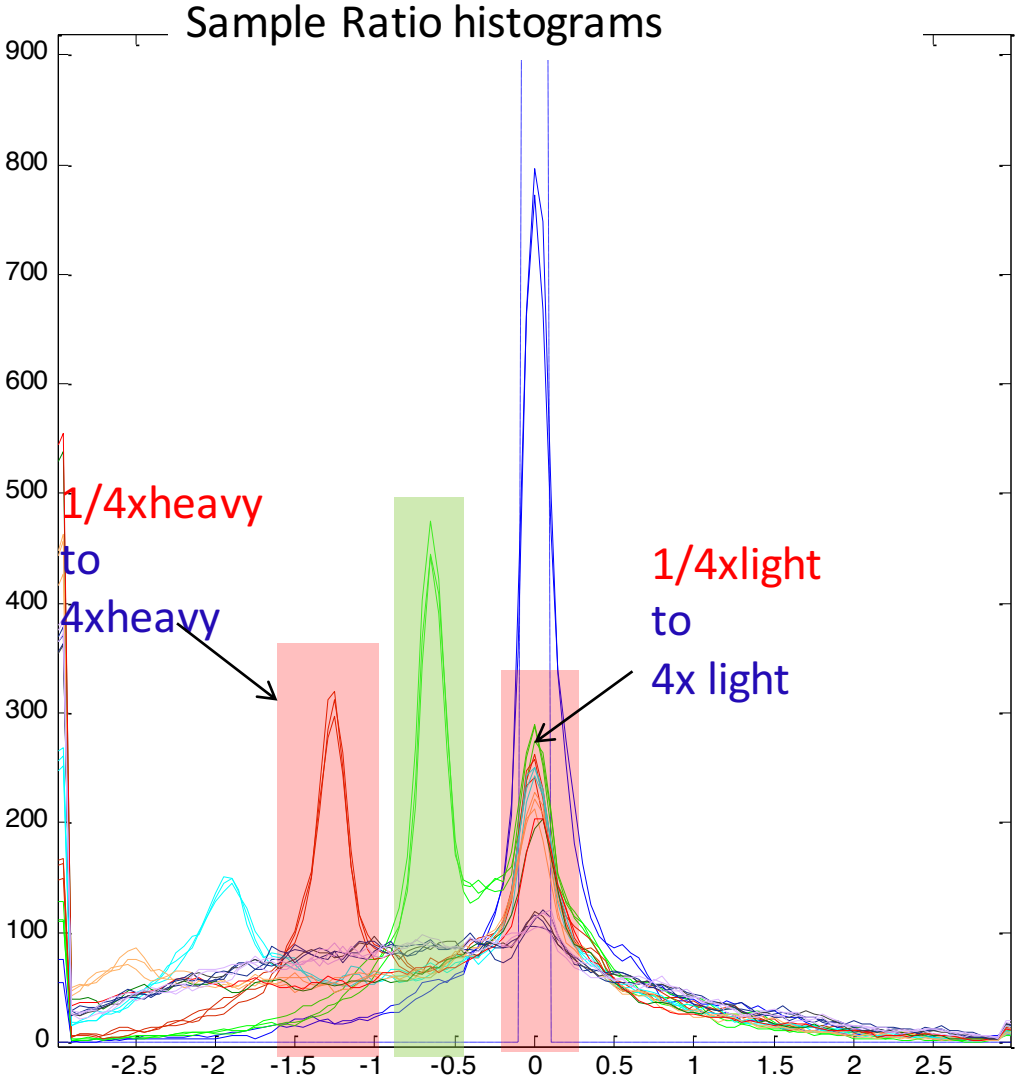
Use known dilution ratio



4x tch.1 to tch.1
4x tch.2 to tch.1
4x tch.3 to tch.1
1x tch.1 to 4x tch.1
1x tch.2 to 4x tch.1
1x tch.3 to 4x tch.1

Proteomics Standards Research Group (sPRG)

Use known dilution ratio



4x tch.1 to tch.1
4x tch.2 to tch.1
4x tch.3 to tch.1
1x tch.1 to 4x tch.1
1x tch.2 to 4x tch.1
1x tch.3 to 4x tch.1
1/4x tch.1 to 4x tch.1
1/4x tch.2 to 4x tch.1
1/4x tch.3 to 4x tch.1

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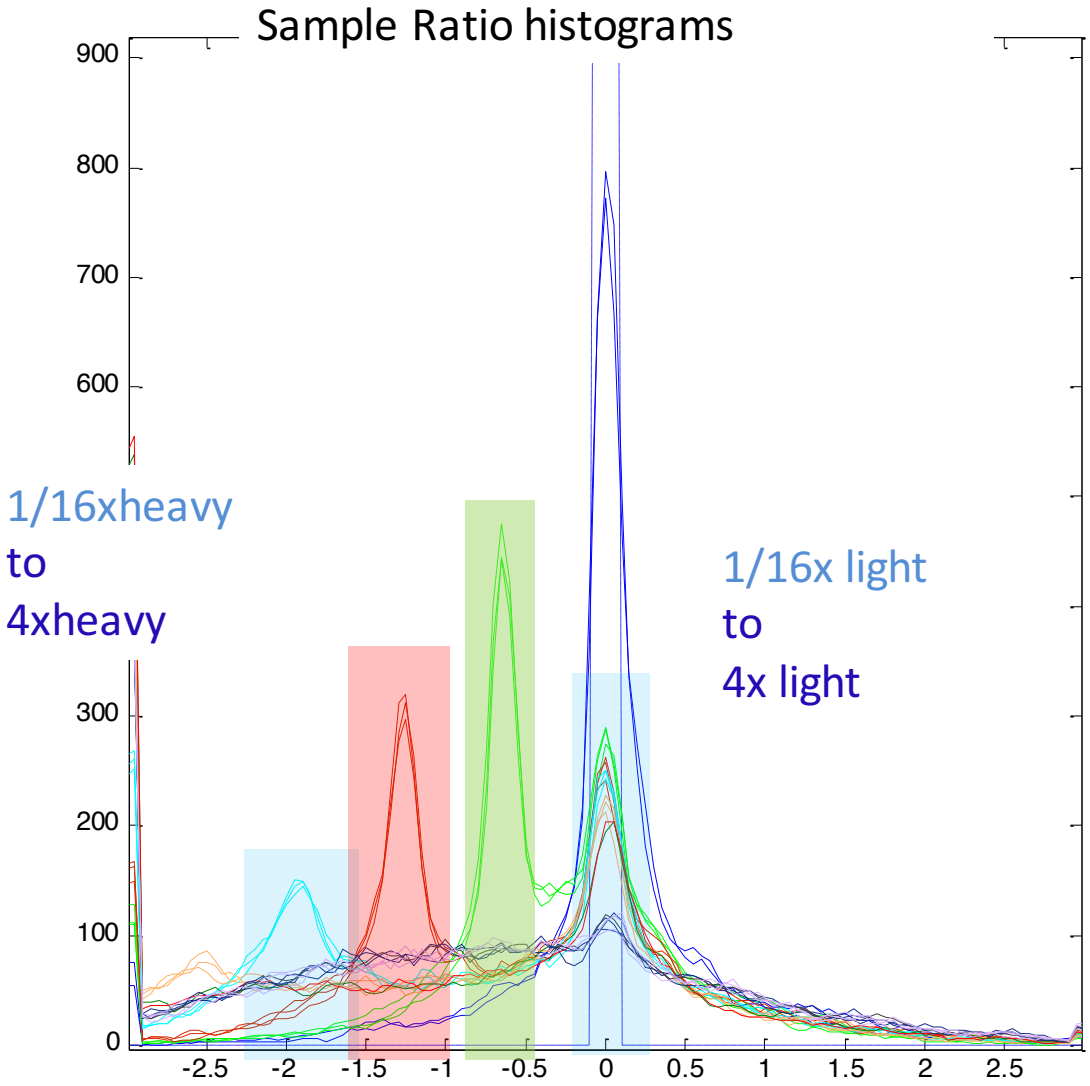
Use known dilution ratio



- 4x tch.1 to tch.1
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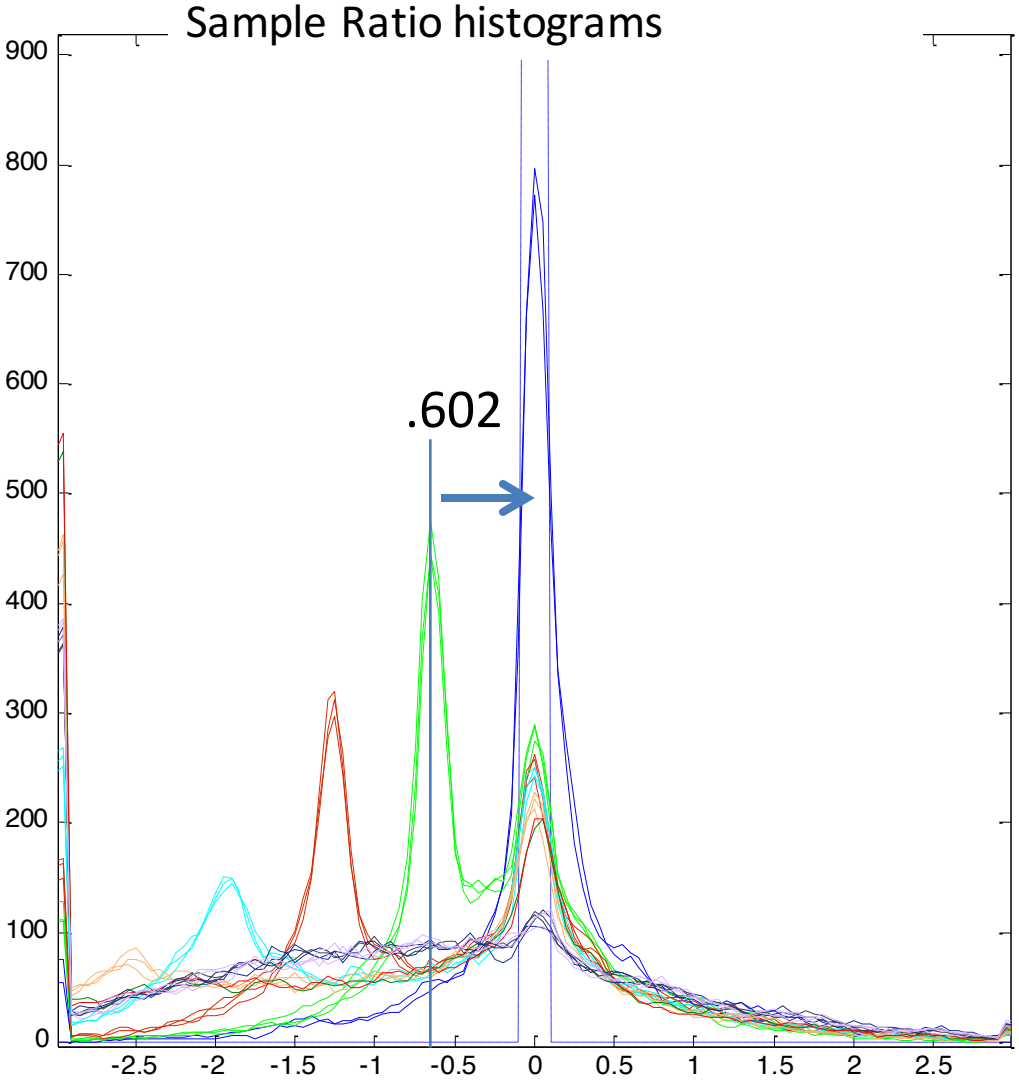
- 4x tch.3 to tch.1
- 1x tch.1 to 4x tch.1
- 1x tch.2 to 4x tch.1
- 1x tch.3 to 4x tch.1
- 1/4x tch.1 to 4x tch.1
- 1/4x tch.2 to 4x tch.1
- 1/4x tch.3 to 4x tch.1
- 1/16x tch.1 to 4x tch.1
- 1/16x tch.2 to 4x tch.1
- 1/16x tch.3 to 4x tch.1
- 1/64x tch.2 to 4x tch.1
- 1/64x tch.2 to 4x tch.1
- 1/64x tch.3 to 4x tch.1

Proteomics Standards Research Group (sPRG)

Use known dilution ratio



4x tch.1 to tch.1
4x tch.2 to tch.1
4x tch.3 to tch.1

1x tch.1 to 4x tch.1
1x tch.2 to 4x tch.1
1x tch.3 to 4x tch.1

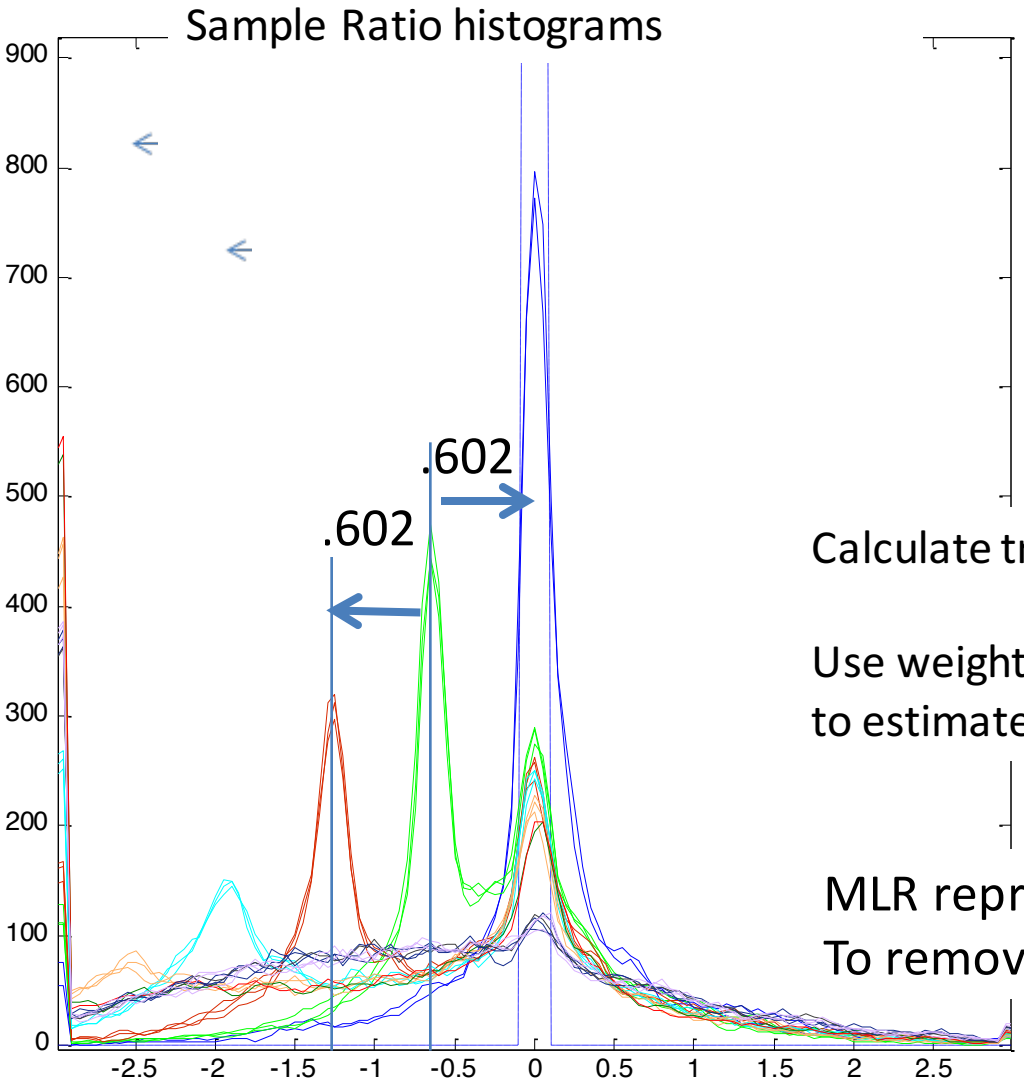
1/4x tch.1 to 4x tch.1
1/4x tch.2 to 4x tch.1

1/16x tch.1 to 4x tch.1
1/16x tch.2 to 4x tch.1

1/64x tch.2 to 4x tch.1
1/64x tch.2 to 4x tch.1
1/64x tch.3 to 4x tch.1

Proteomics Standards Research Group (sPRG)

Use known dilution ratio



4x tch.1 to tch.1

4x tch.2 to tch.1

4x tch.3 to tch.1

1x tch.1 to 4x tch.1

1x tch.2 to 4x tch.1

1x tch.3 to 4x tch.1

1/4x tch.1 to 4x tch.1

Calculate true ratio from the data!

Use weighted average of 4x, 1x and 1/4x to estimate expected 1x ratio

1/64x tch.2 to 4x tch.1

MLR reproducibility analysis
To remove bias and calculate weights

Mapping differential interactomes by affinity purification coupled with data-independent mass spectrometry acquisition

Jean-Philippe Lambert[1,13], Gordana Ivosev[2,13], Amber L Couzens[1], Brett Larsen[1], Mikko Taipale[3], Zhen-Yuan Lin[1], Quan Zhong[4–6], Susan Lindquist[3,7,8], Marc Vidal[4–6], Ruedi Aebersold[9,10], Tony Pawson[1,11,12], Ron Bonner[2], Stephen Tate[2] & Anne-Claude Gingras[1,11]

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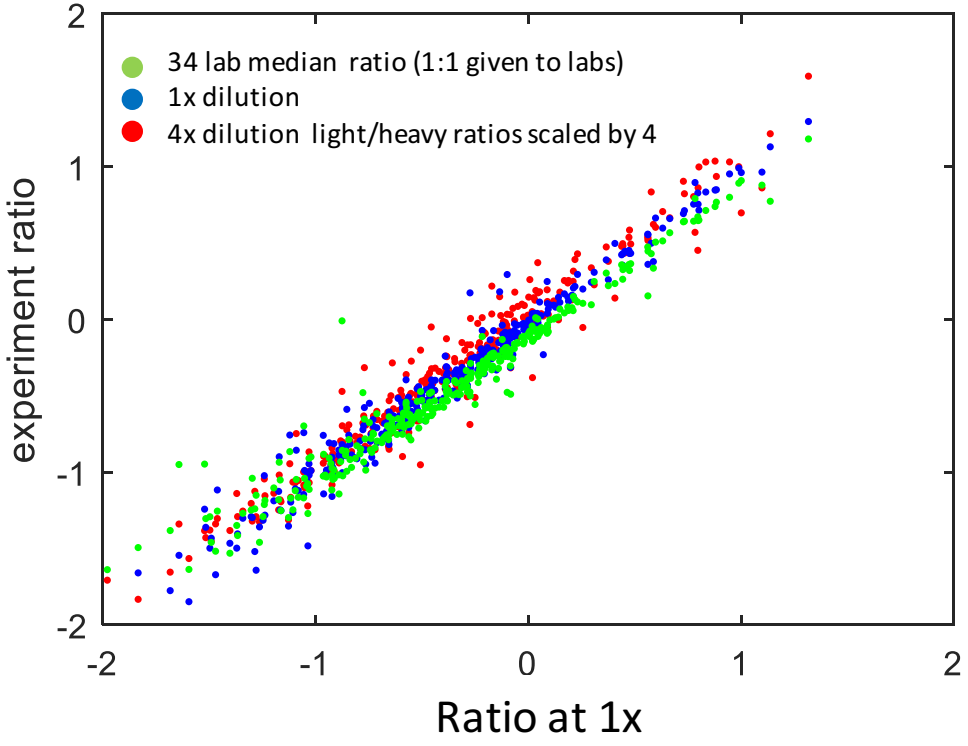
Check if all experiments are comparable



1x in dilution not in agreement with 1x in lab experiment
4x in dilution is not exactly 4x

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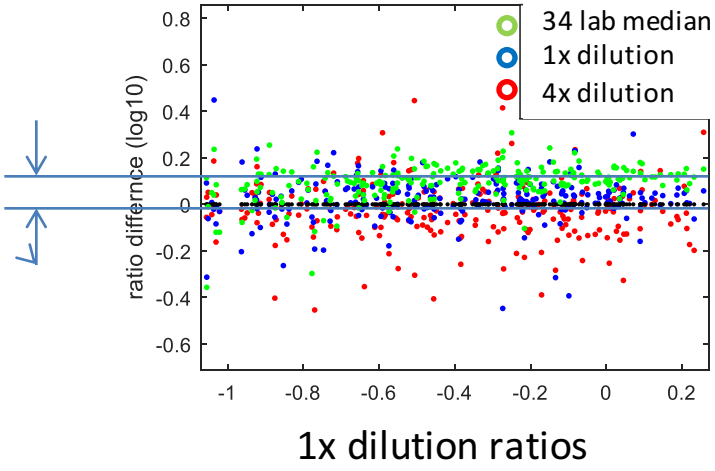
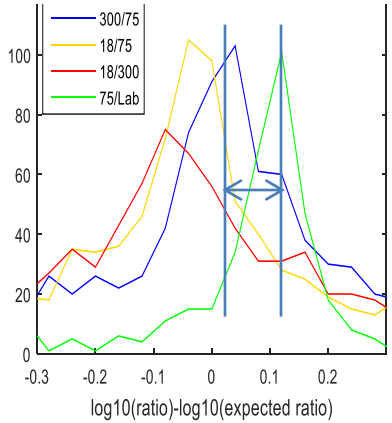
Correcting for experimental bias

.1 distance!





correction

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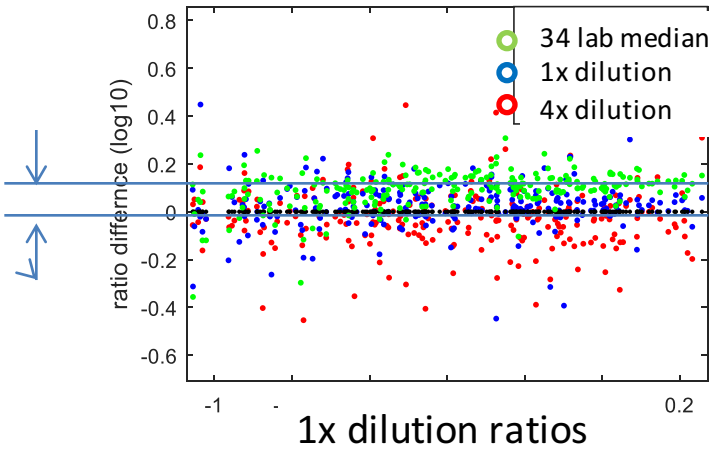
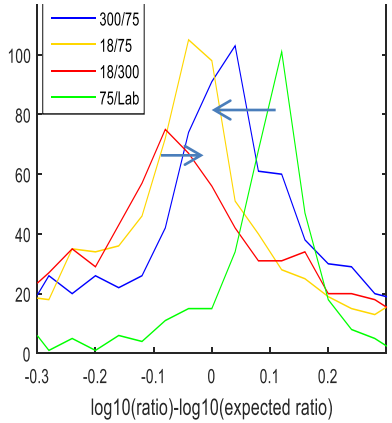
Calculate correction factor for dilutions

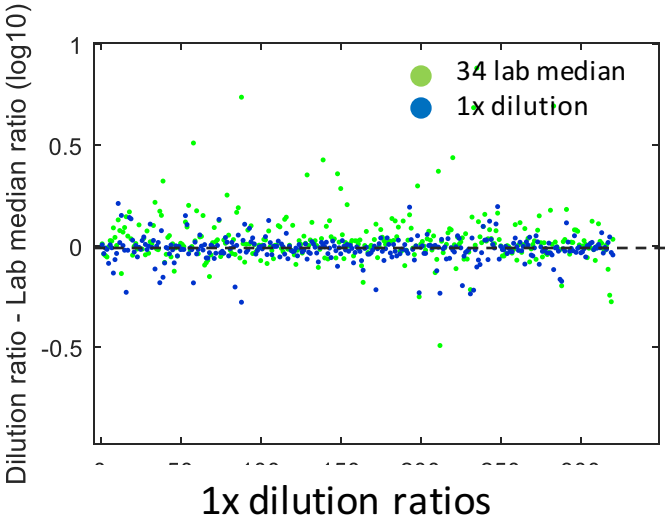
Originally:

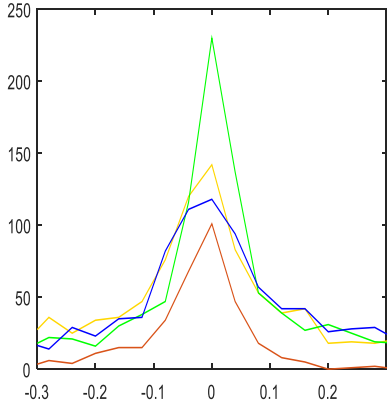
.1 distance!





After alignment





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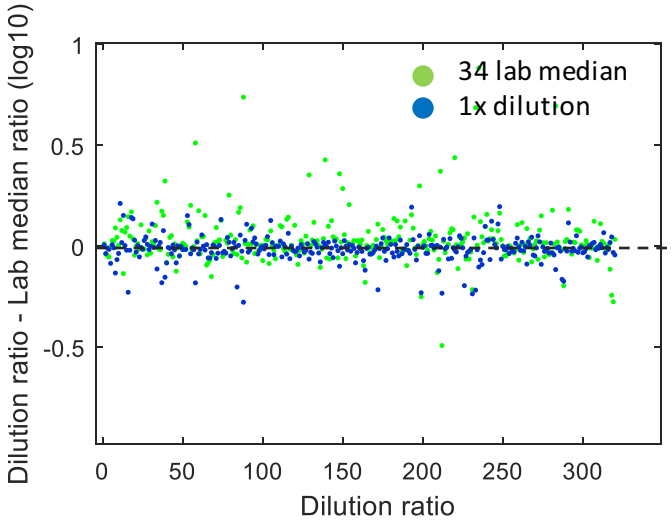
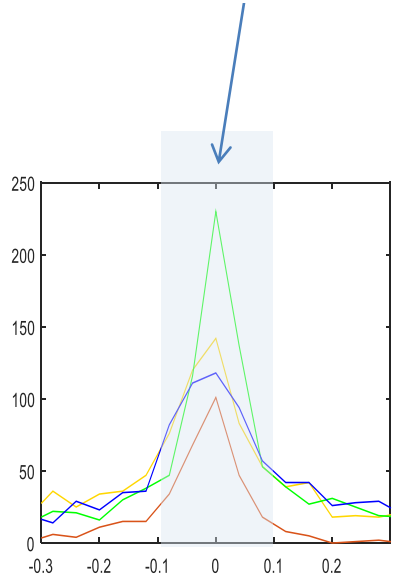
Recognizing reliable measurements

Peptides from this region
Are reproducible measurements

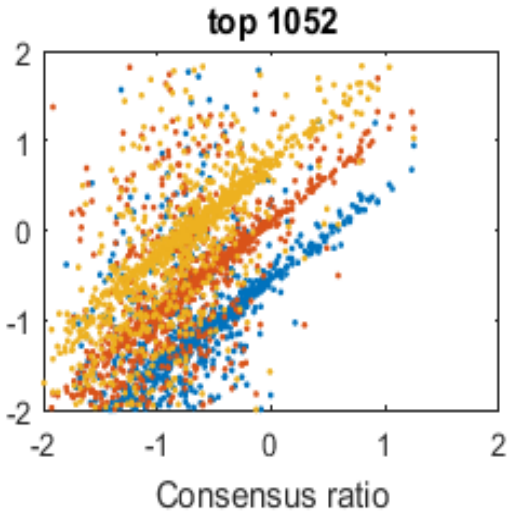
After alignment



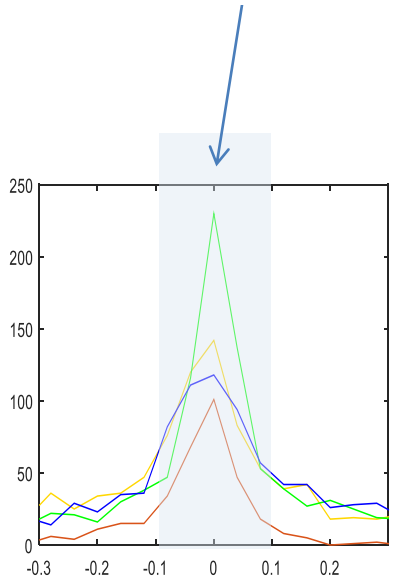


Proteomics Standards
Research Group (sPRG)

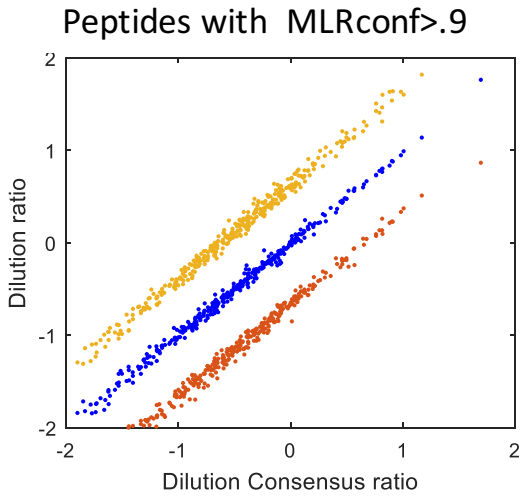
Recognizing reliable measurements



Peptides from this region
Are reproducible measurements



Correct Answer – how certain we are?



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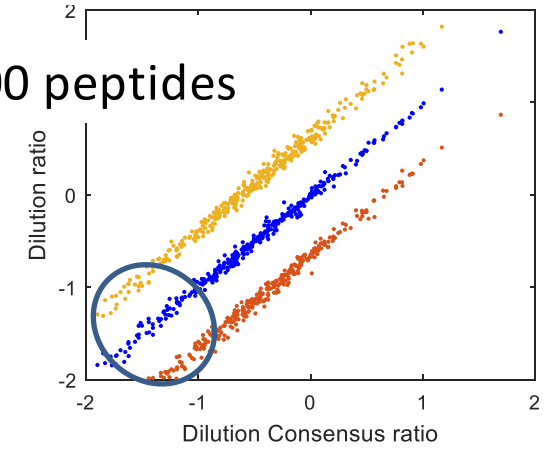
Correct Answer – how certain we are?

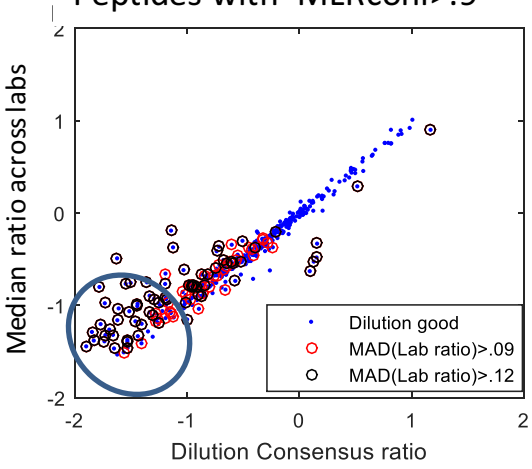
LAB median versus good from dilution

Peptides with MLRconf>.9

>300 peptides



Peptides with MLRconf>.9



Larger variance for low intensity light

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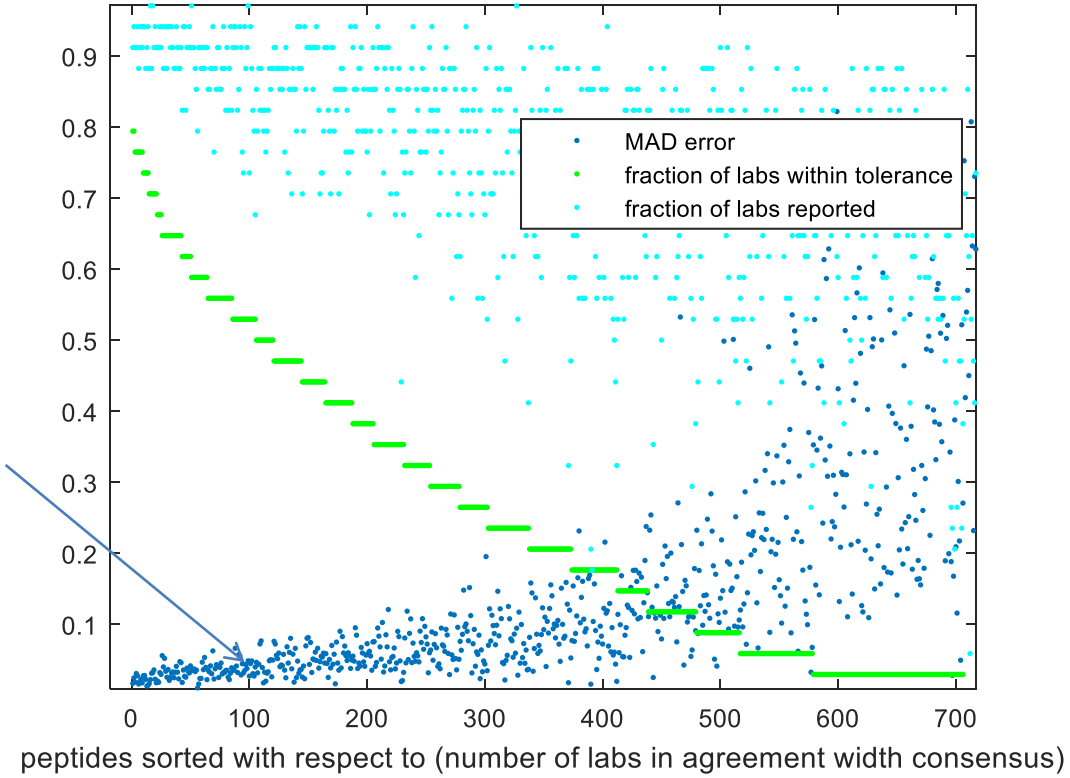
What is definition of '*Good peptide*'

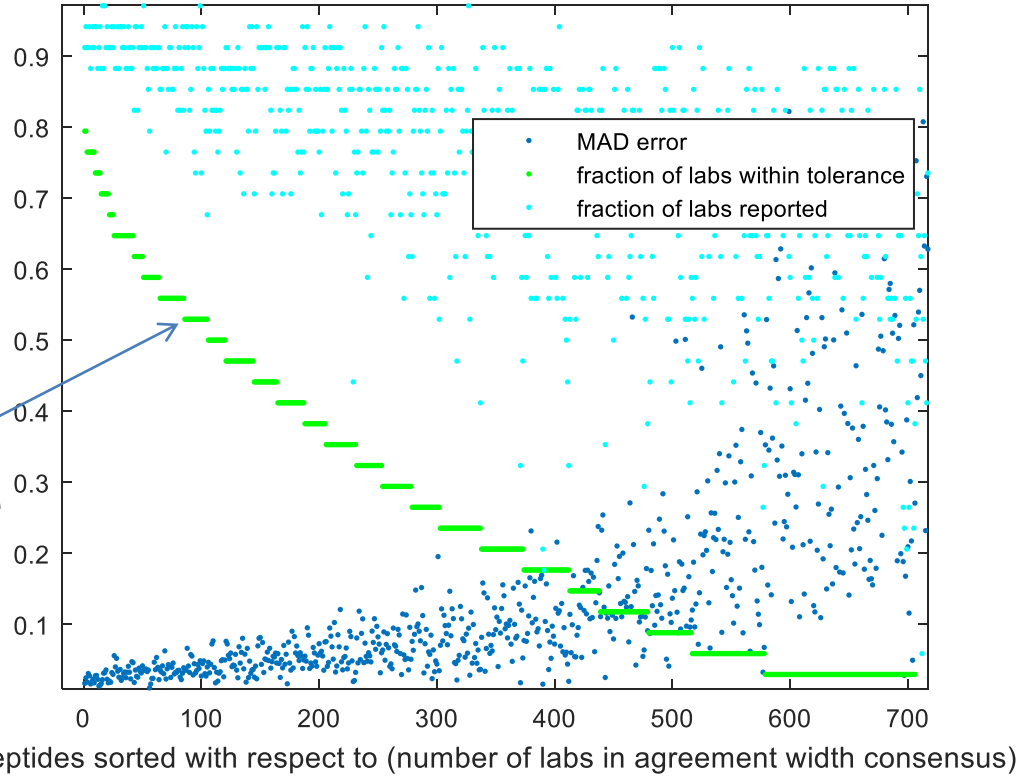
MAD error
Across labs
For a peptide



Proteomics Standards Research Group (sPRG)

What is definition of '*Good peptide*'

Fraction of labs Within tolerance For a peptide



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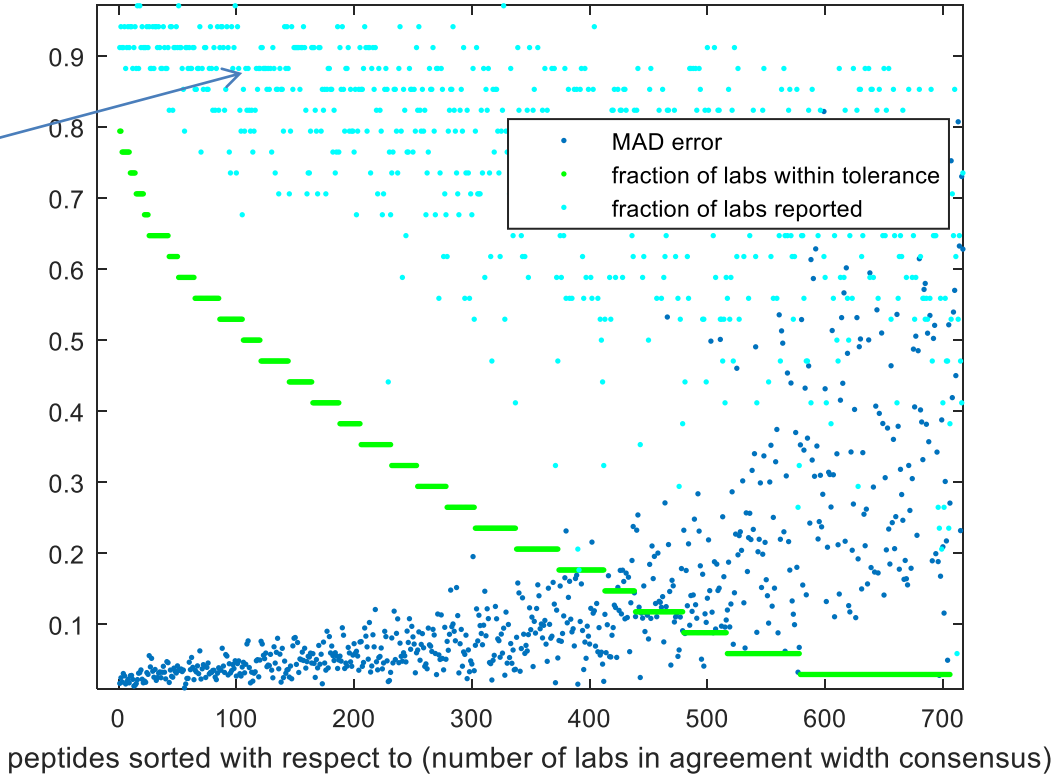
What is definition of '*Good peptide*'

Fraction of labs reported a peptide



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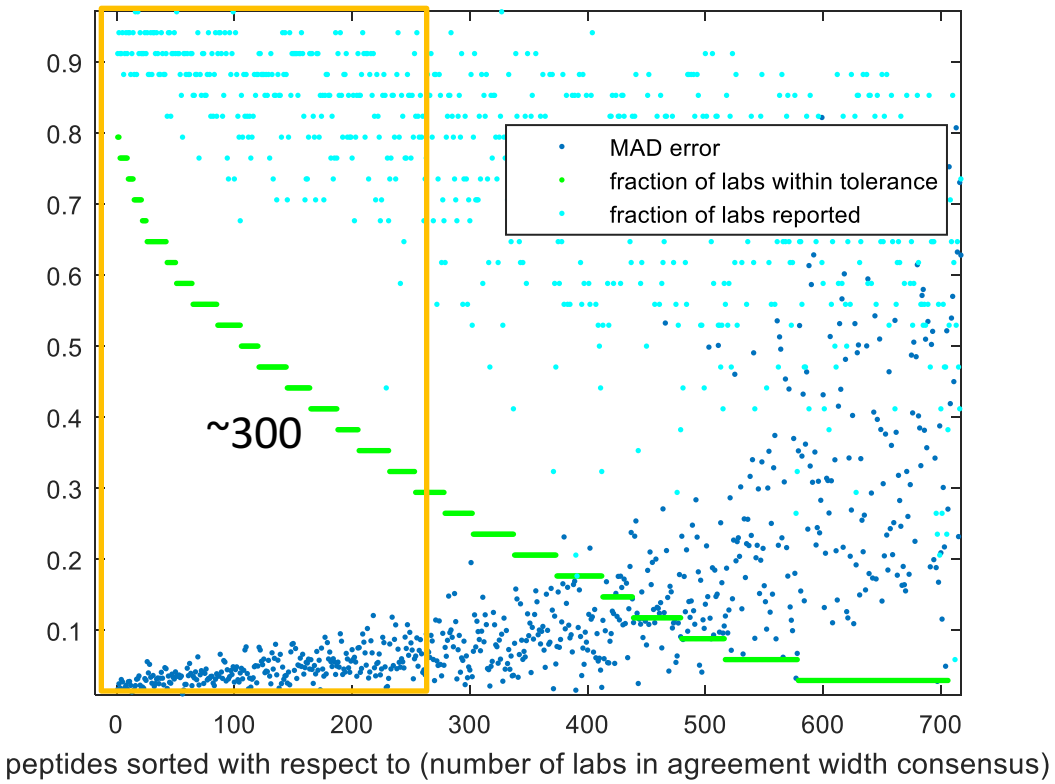
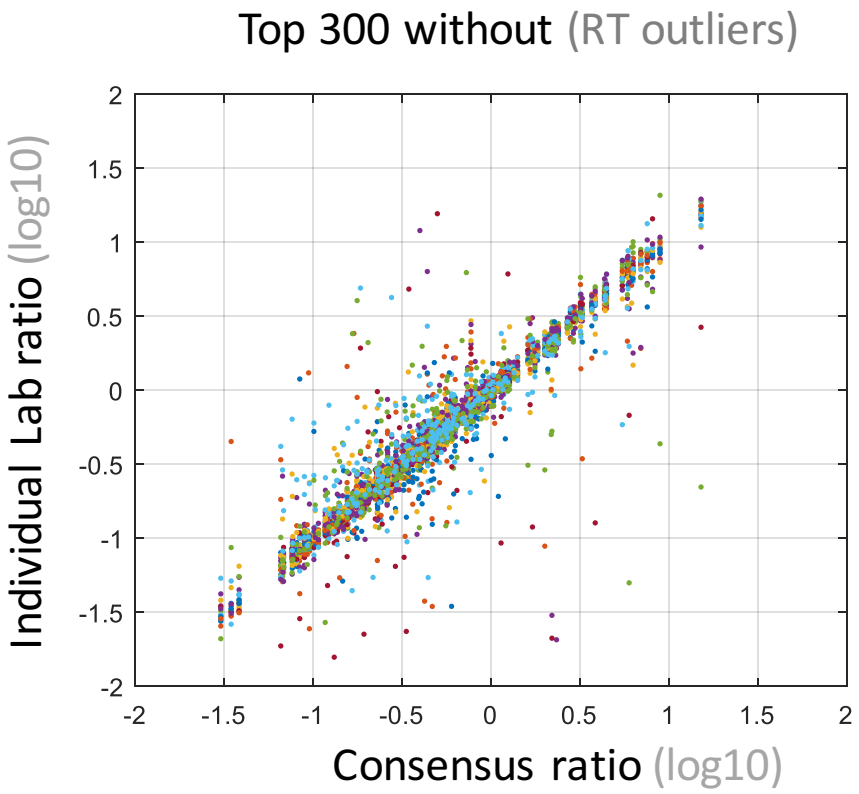
What is definition of '*Good peptide*'



Take the best out of each experiment!

Lab ratio reproducibility



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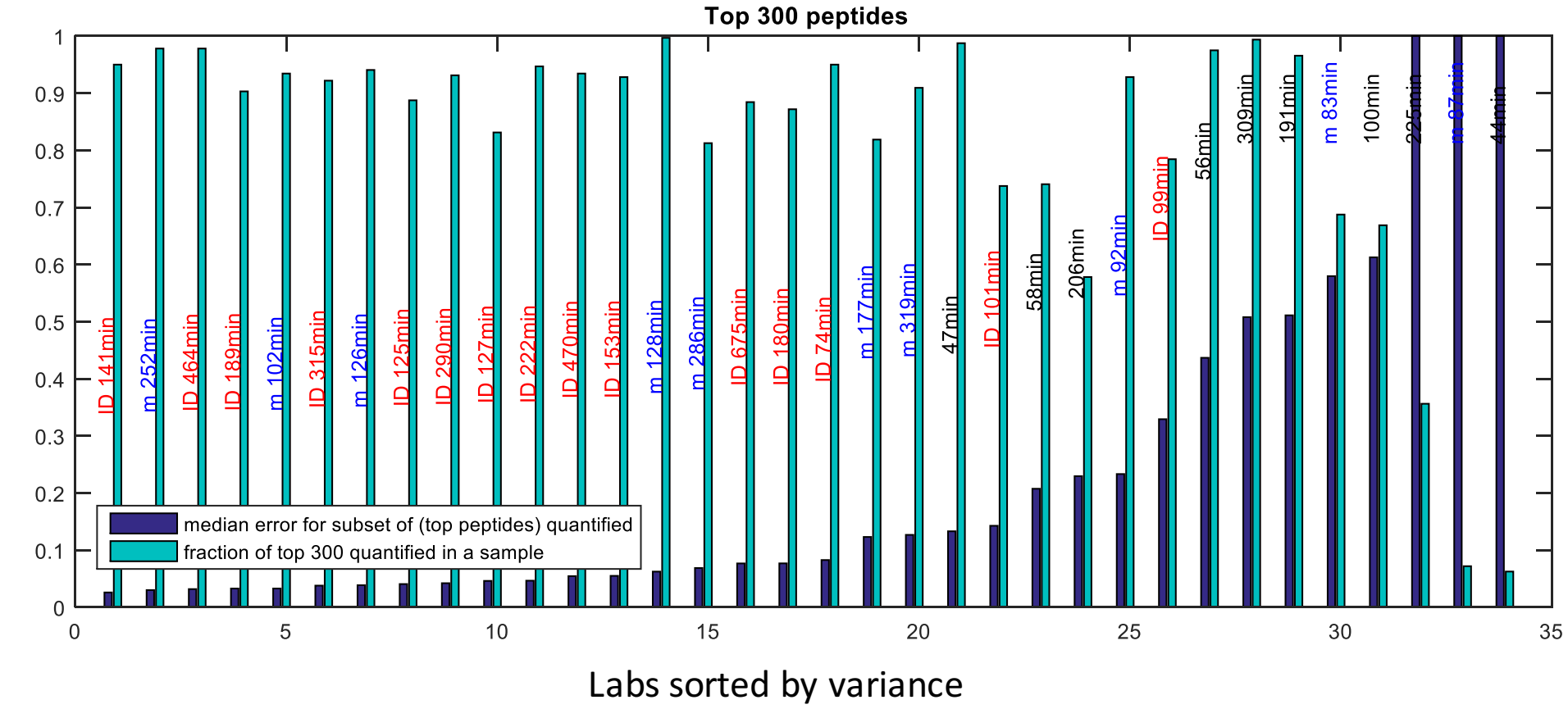
34 lab summary results – ratio accuracy



Knowledge of expected RT helps to integrate correct peak

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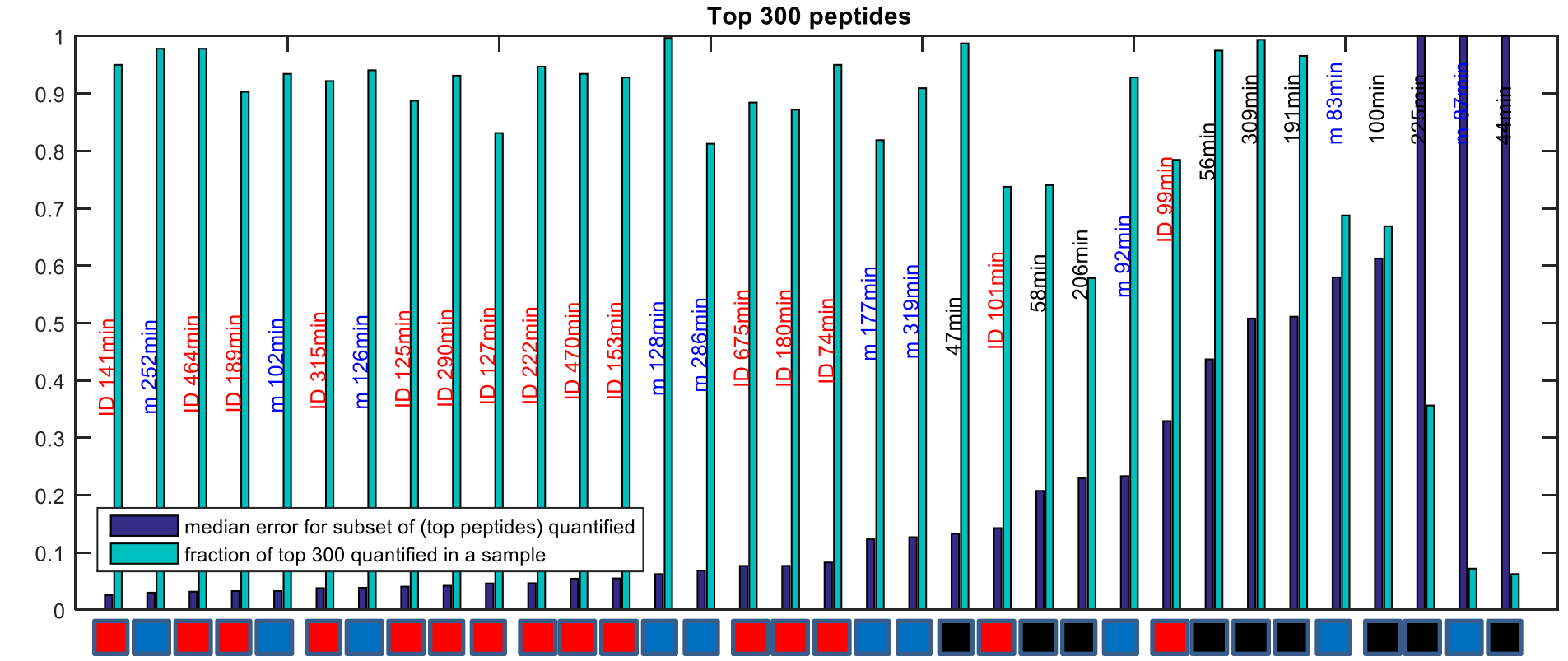
34 lab summary results – ratio accuracy



ID aligned
Use manual integrated
No alignment
Or no info

Knowledge of expected RT helps to integrate correct peak

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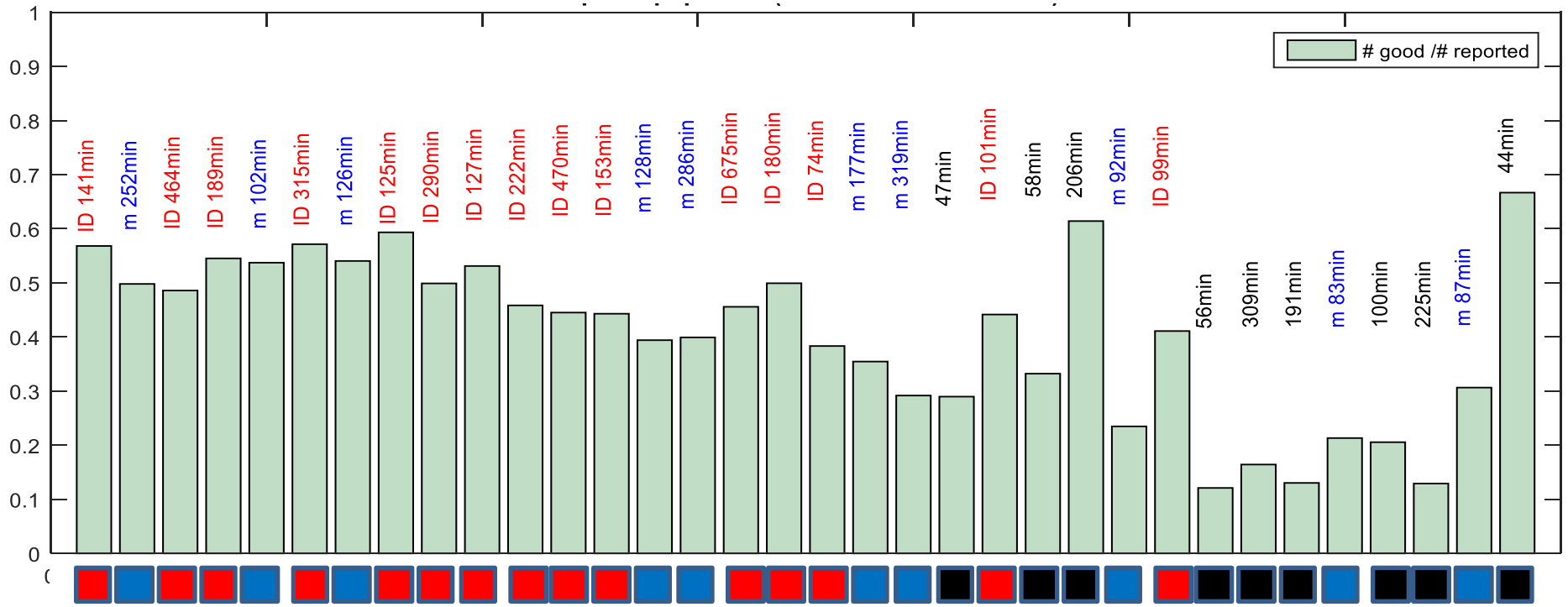
Quant specificity

(subset in agreement with the consensus ratio)/ (number of peptides reported)



Even labs that reported most of the peaks within Standard ERT window, have ~ 50% ratio agreement with consensus

What is happening with the other half?

Why are we over reporting?

- Are we integrating wrong peak?

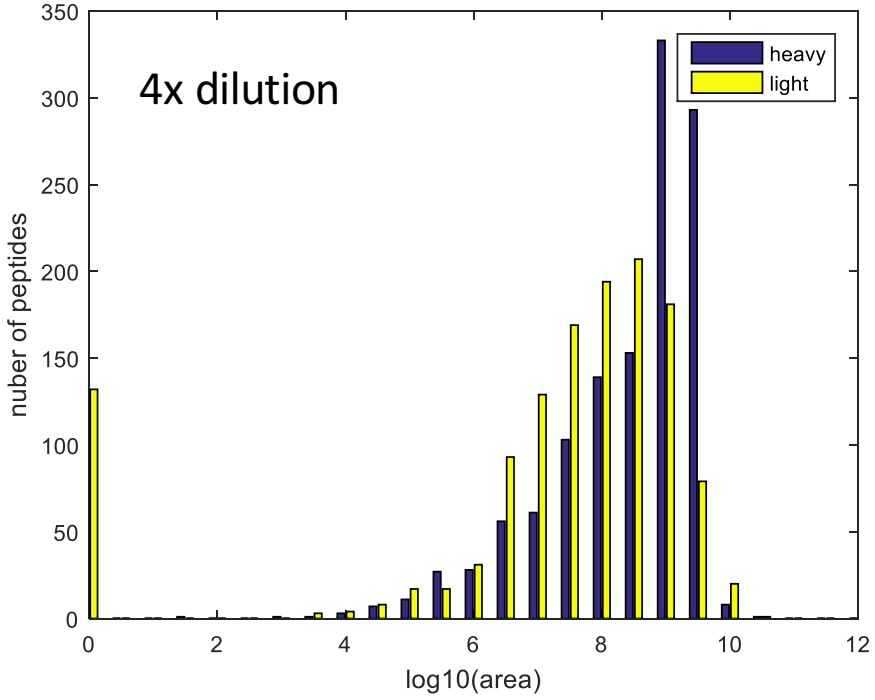
- Could we use attributes such as RT variance, IDP/LDP, technical replica variance, ….

  to explain or filter those incorrectly reported values?

- Few observations might help answer this question

1st important attribute - Intensity

All peptides



Important to mix heavy and light at optimal ratio to maximize coverage of lights

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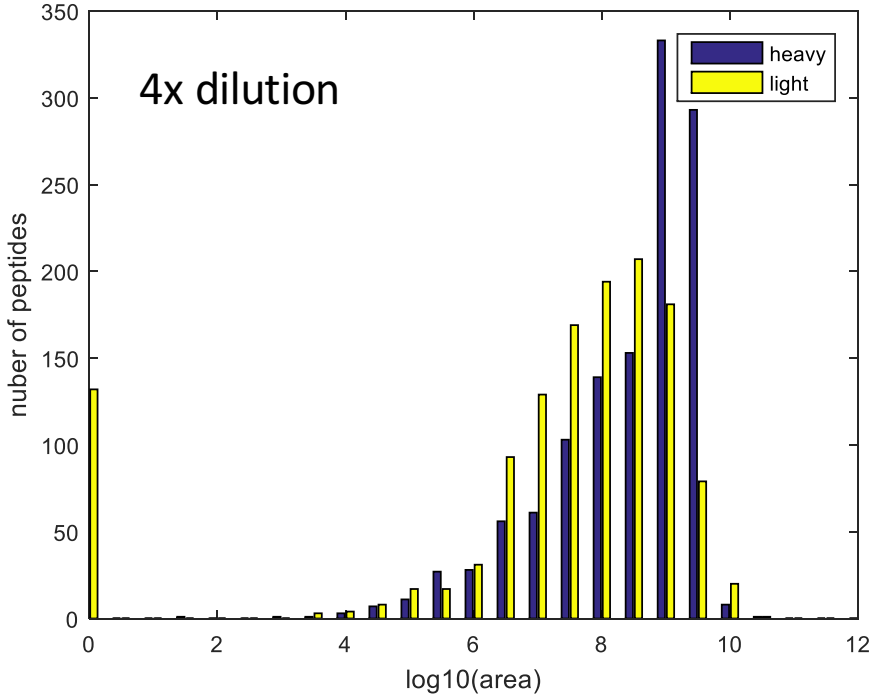
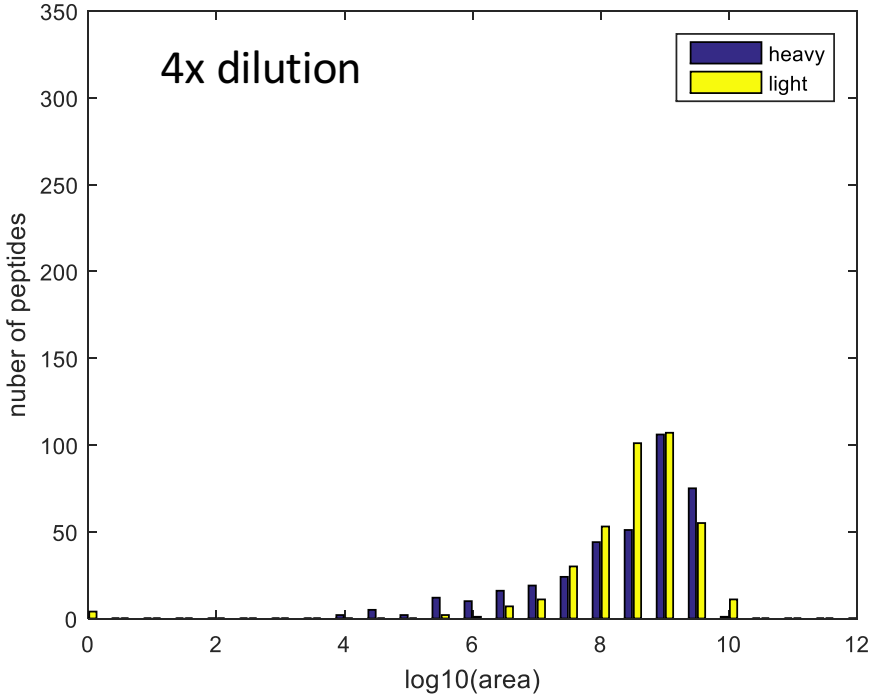
1st important attribute - Intensity

All peptides



Top 300



Important to mix heavy and light at optimal ratio to maximize coverage of lights

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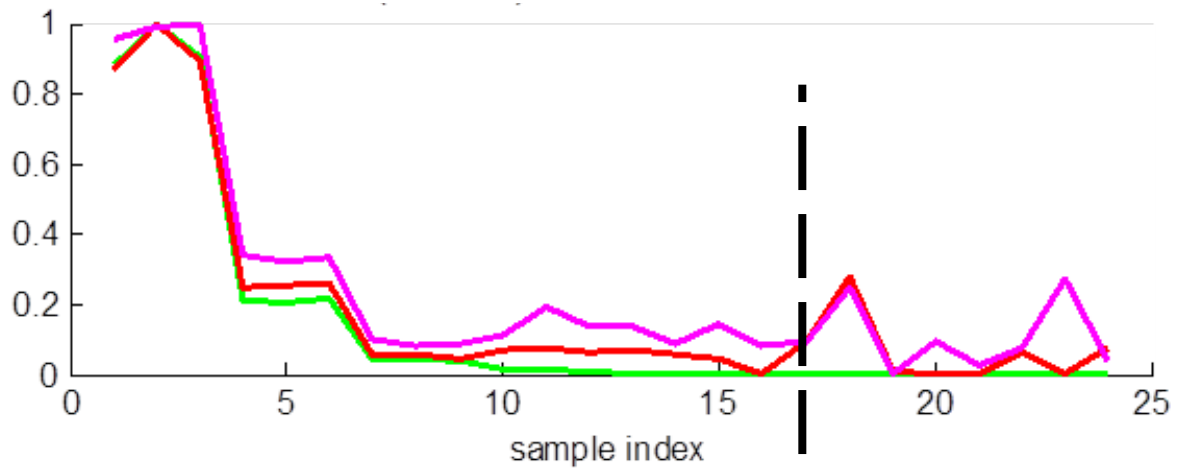
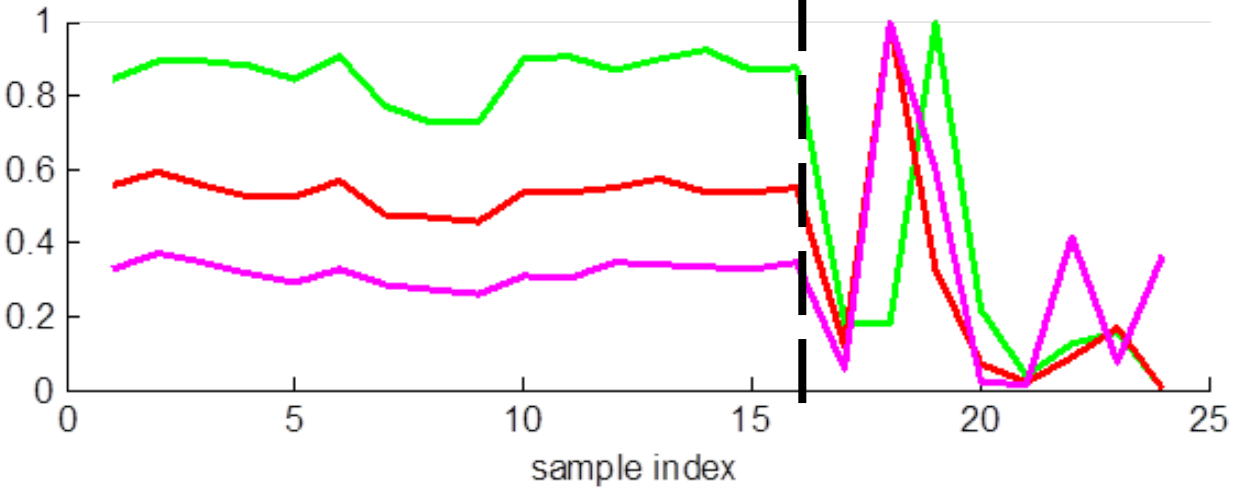
2nd important attribute
heavy peptide intensity affects light detection

heavy



light



When heavy peptide low intensity our ability to integrate light is reduced even when light is of significant intensity

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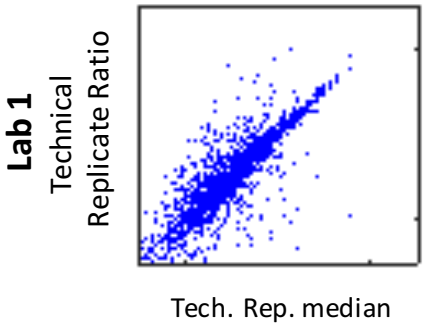
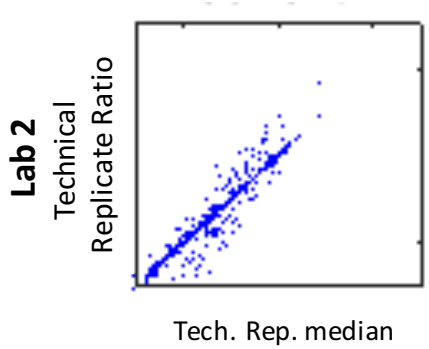
3rd Attribute– know ERT

Ratio agreement across tch. Rep.





Software will integrate at same retention time in replicates

How often we reproducibly integrate wrong peak?

Does ID RT help?

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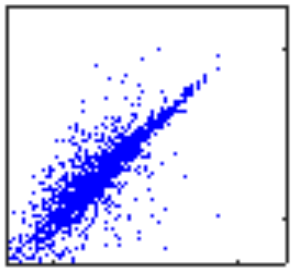
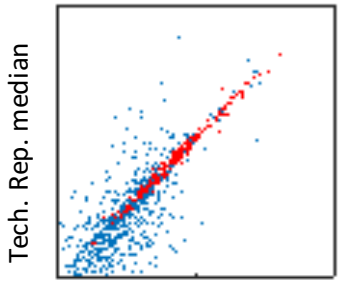
3rd Attribute– know ERT

Ratio agreement across tch. Rep.

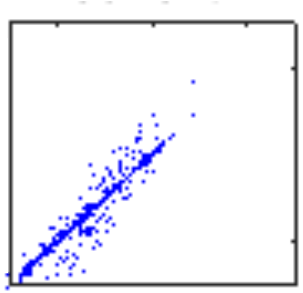
Ratio agreement with Consensus

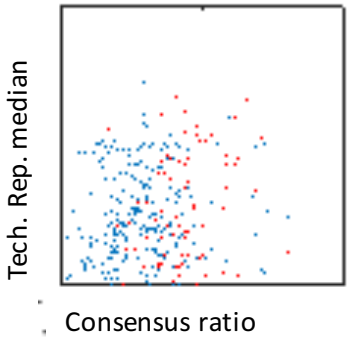
Lab 1





Lab 2



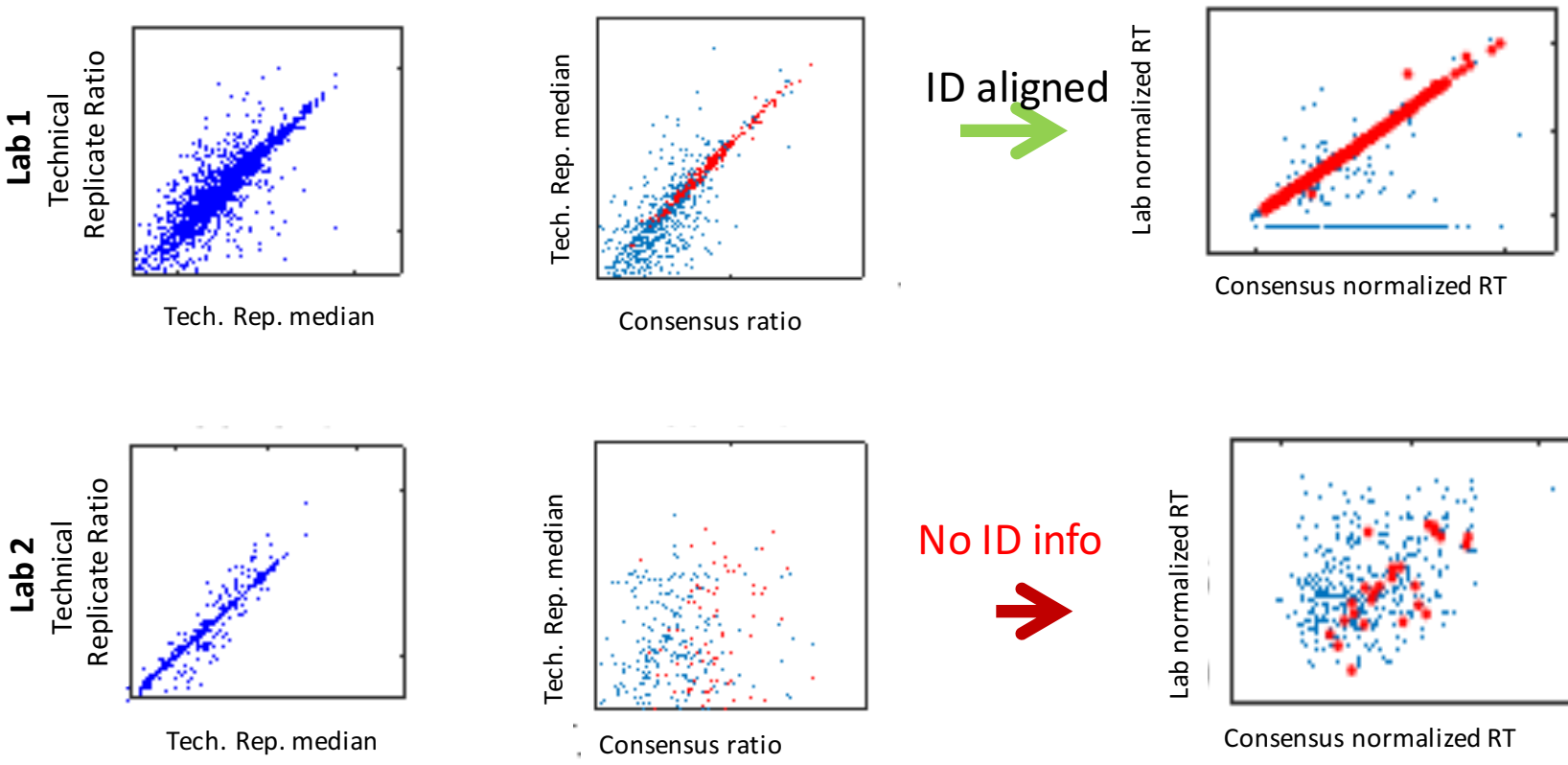


3rd Attribute– know ERT

Ratio agreement across tch. Rep.

Ratio agreement with Consensus

RT agreement with consensus



Software will integrate at same retention time in replicates but without ID alignment we mots often get the wrong answer

Proteomics Standards
Research Group (sPRG)

300 good peptides for HEK 293 matrix

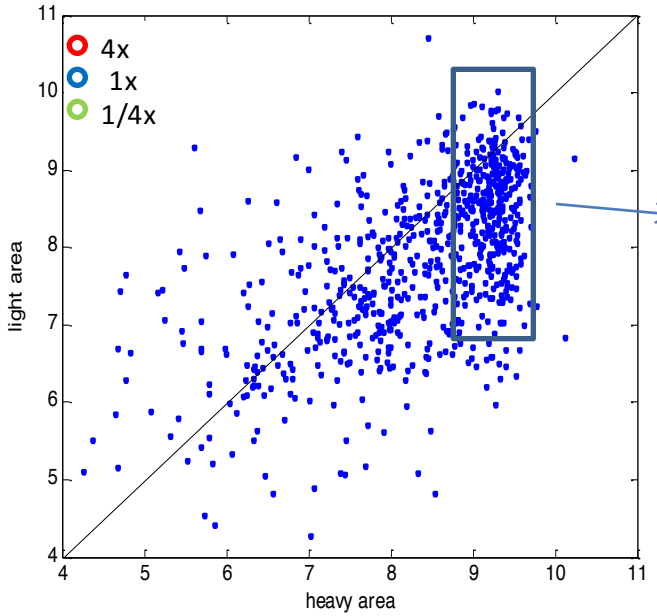
- Intra lab retention time reproducibility is outstanding
  - within 5% of the gradient
- True answer is not always straight-forward to calculate
  - Sample handling bias
  - Outliers due to large ID uncertainty
- Need to estimate reported number uncertainty
  - By evaluating the uncertainty in your experiment and alignment between measured and expected Standard features, possible to reduce error rate

Few conclusions about
Standard for use in intra-lab study normalization

Standard for intra-lab study normalization

Proteomics Standards Research Group (sPRG)

Standard provides good Internal Standard
(narrow range of heavy peptide responses)

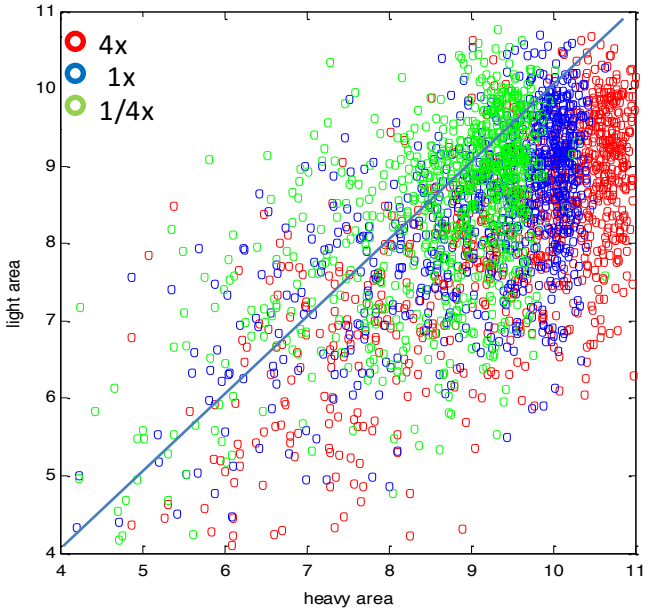


Heavies (>60%) have narrow intensity distribution

Standard for intra-lab study normalization

Proteomics Standards Research Group (sPRG)

Standard provides good Internal Standard
(narrow range of heavy peptide responses)

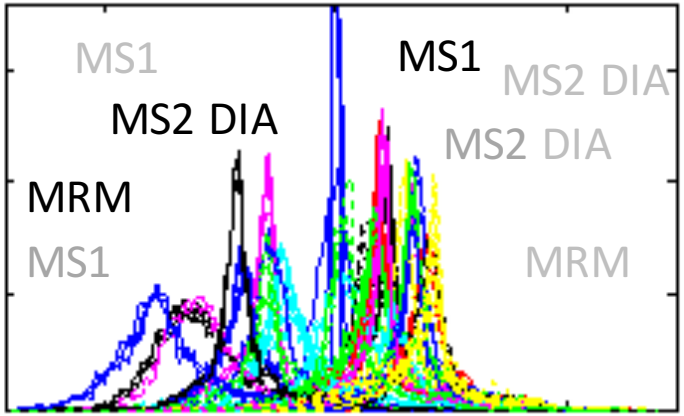


1:1 amount of sample and standers is ~1/4x ratio

Standard for intra-lab study normalization

Proteomics Standards Research Group (sPRG)

Enables comparison of data from variety of platforms, LC conditions and experiment of choice

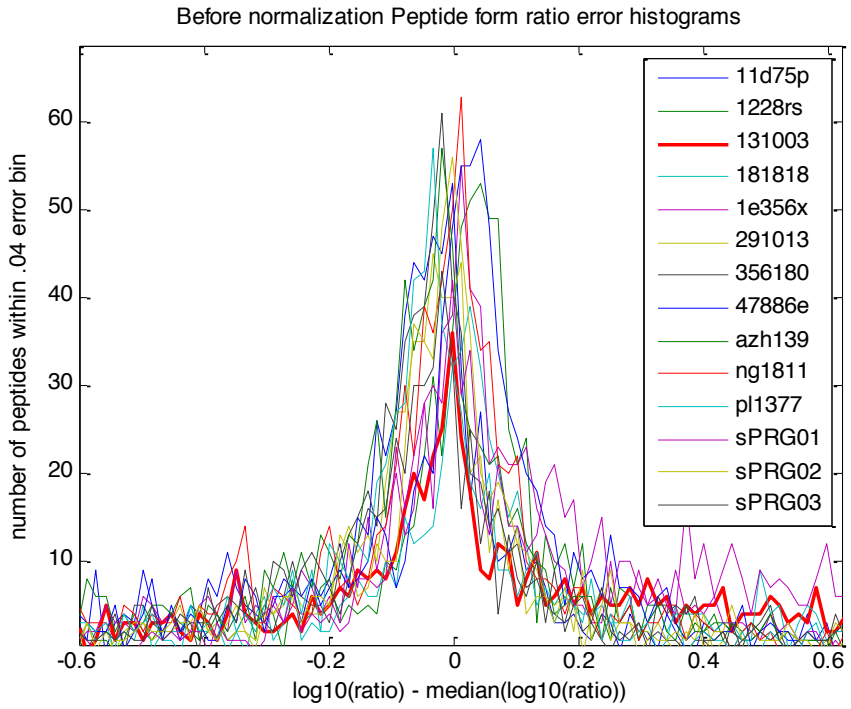


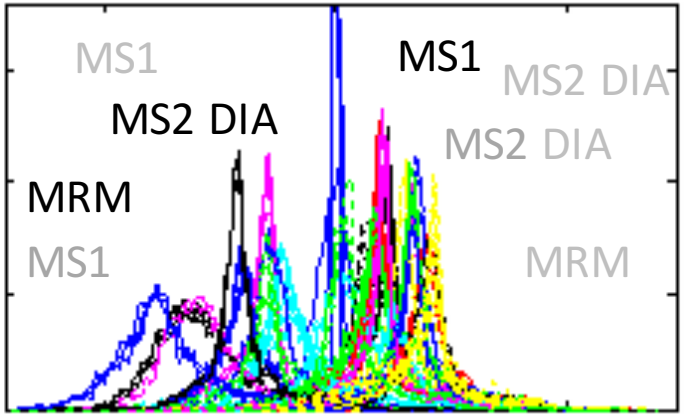
Lab peptide area versus selected lab peptide area

Standard for intra-lab study normalization

Proteomics Standards Research Group (sPRG)

Enables comparison of data from variety of platforms, LC conditions and experiment of choice





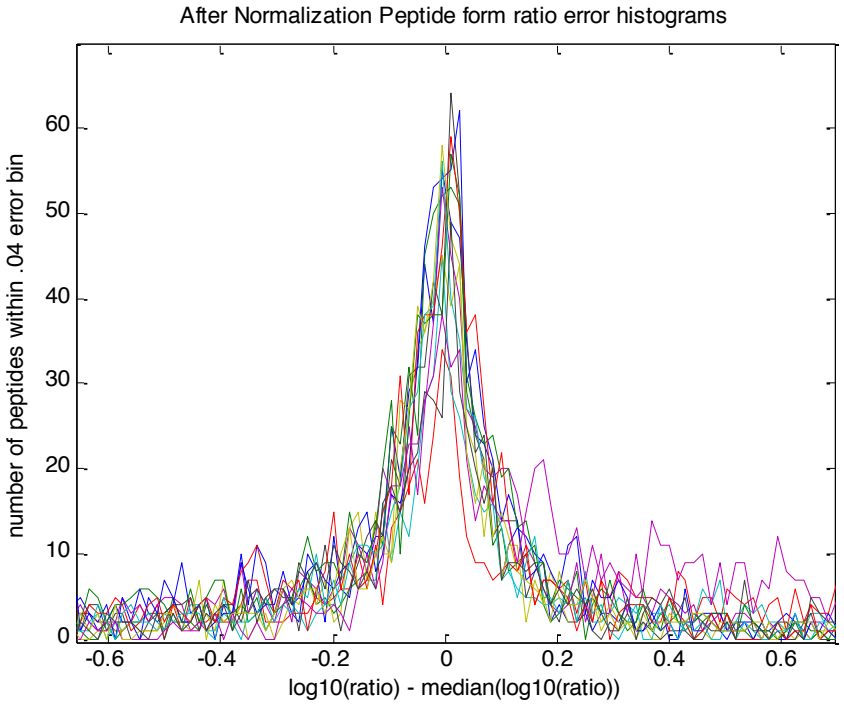
Lab peptide area versus selected lab peptide area

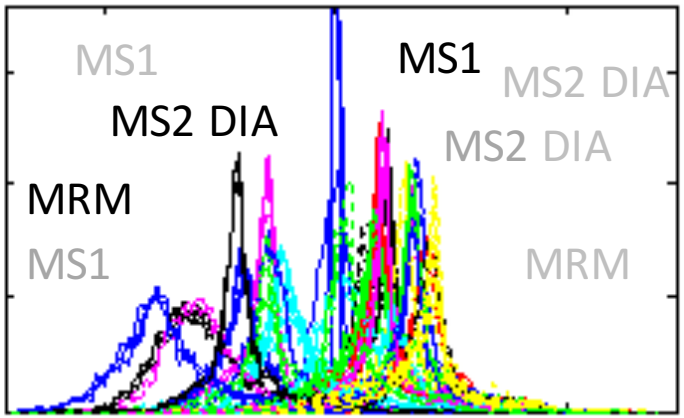
Standard for intra-lab study normalization

Proteomics Standards Research Group (sPRG)

After Normalization in ratio space minimized unwanted variance

Higher differential expression detection sensitivity





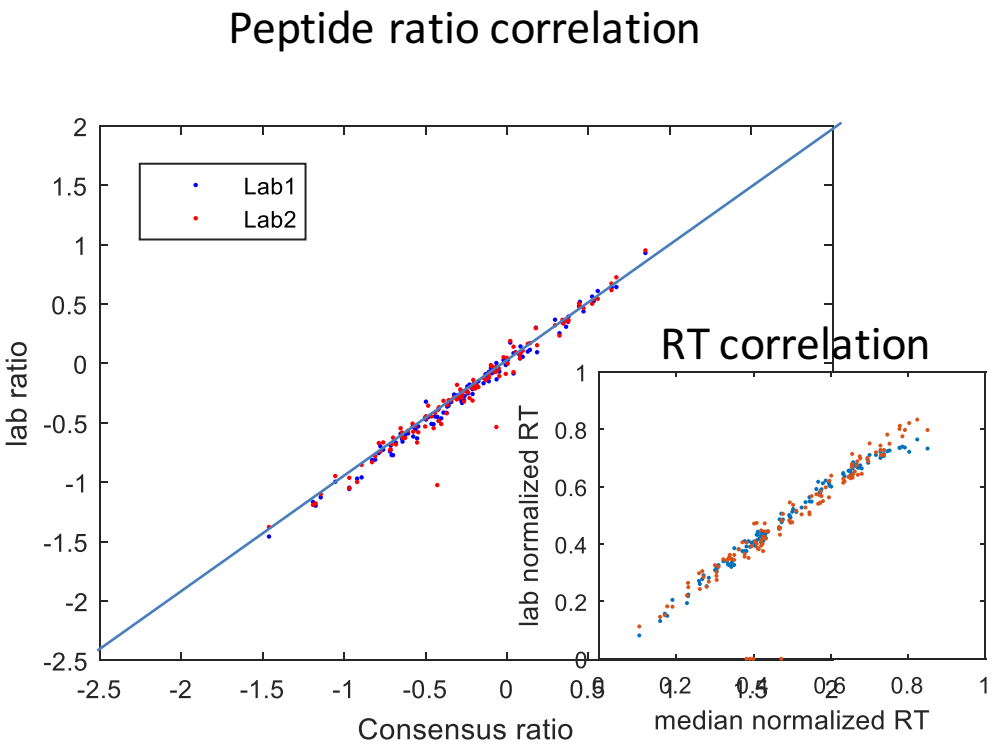
Lab peptide area versus selected lab peptide area

Proteomics Standards Research Group (sPRG)

Normalization across variable lab - factors

High reproducibility and agreement with dilution consensus regardless of difference in LC condition, platform and experiment of choice

- all different between Lab1 and Lab2, Same amount loaded
- peptide ratios within very tight tolerance



Proteomics Standards Research Group (sPRG)

Summary results

- Correct answer –
  - obscured by large amount of outliers and presence of bias
  - characterized peptide ratios, variance and RT
- Recognized attributes that can detect outliers and define confidence of the result in an experiment
  - Based on individual experiment features
  - Based on Standard characterization results
- Standard provides opportunity and method for alignment and quant analysis across many variable Mass-spec factors

Proteomics Standards
Research Group (sPRG)

THANK YOU!!!

PARTICIPANTS

Please visit our poster

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Questions, Ideas or Interested in Joining Us?

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