MSSTATS

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OUTLINE

- Methods: background
  - Linear mixed effects models

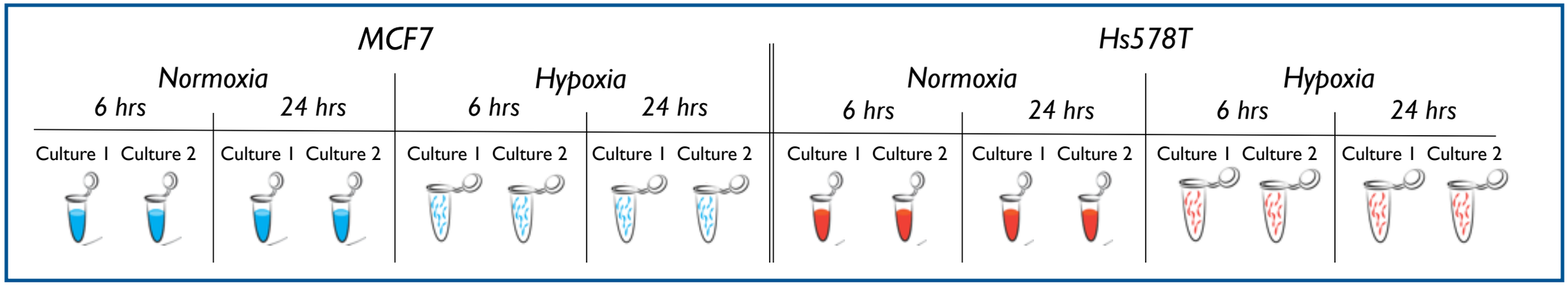
- Evaluation
  - Spike-in and experimental datasets

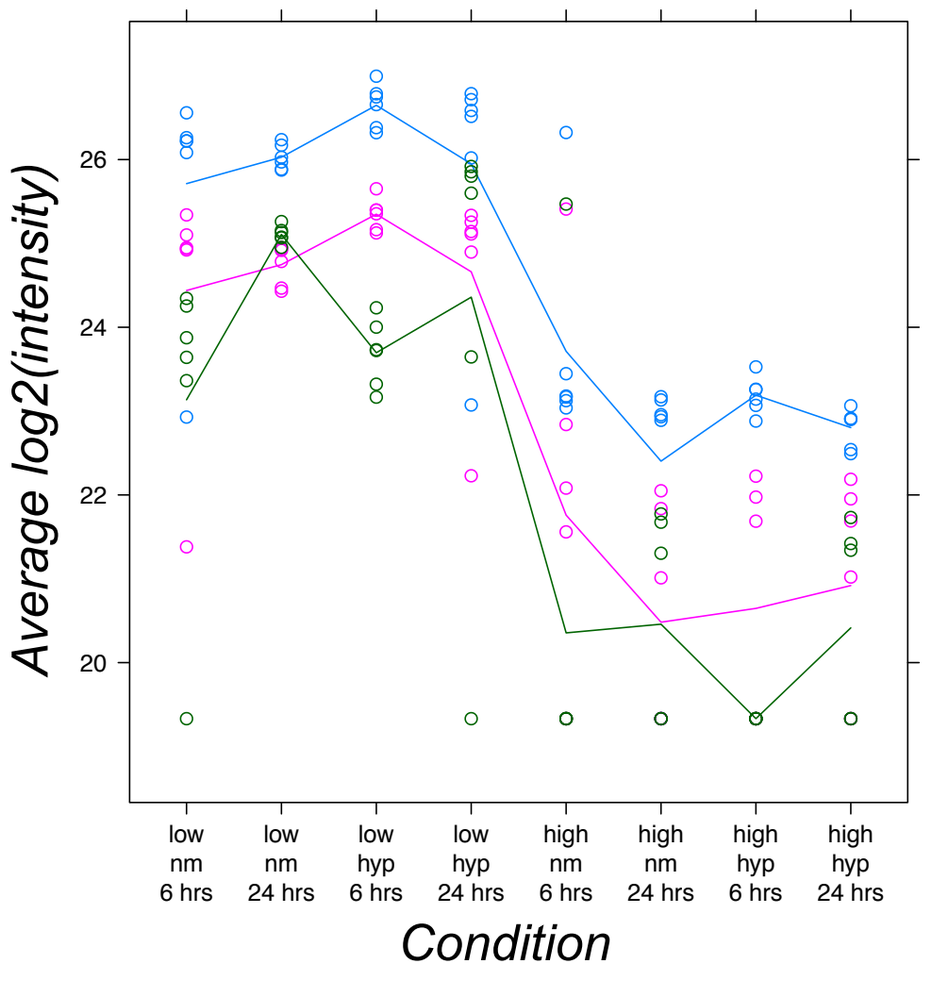
MSSTATS

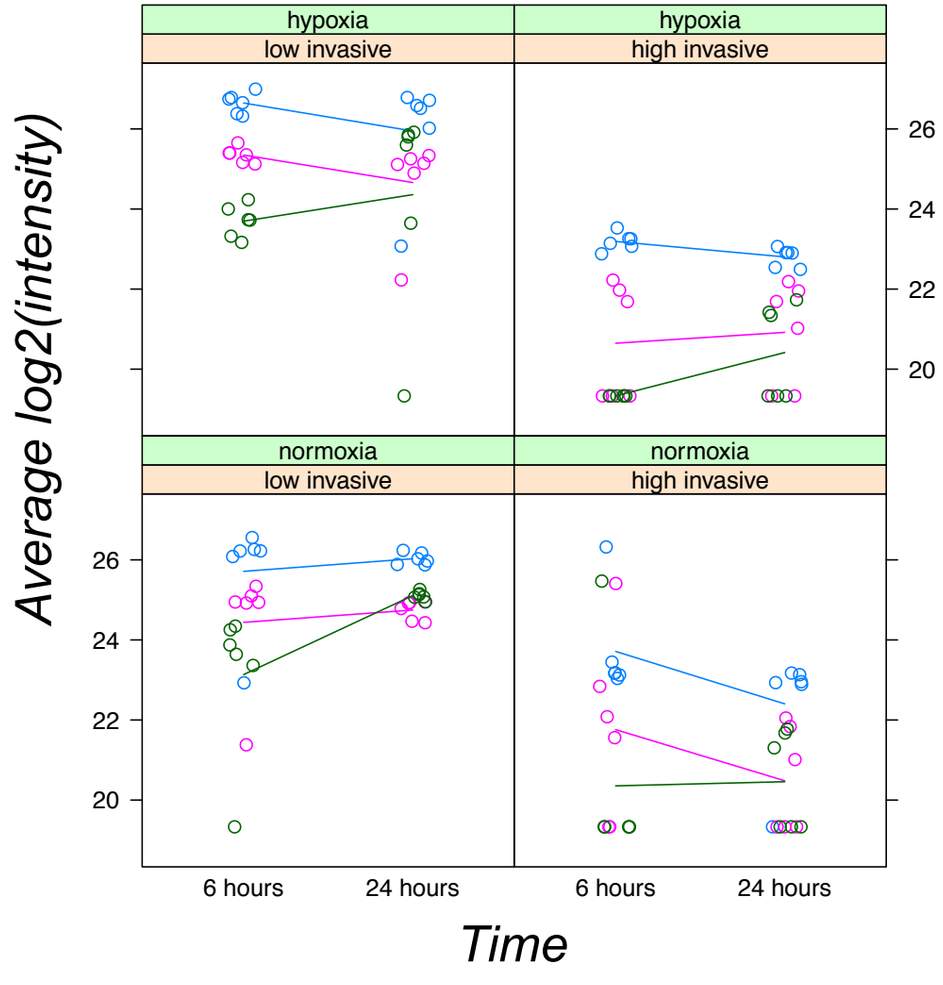
- Statistical relative quantification of proteins and peptides
  - Which protein changes in abundance?
- Complex experimental designs
  - Multiple conditions, factorial experiments, paired designs, time course
- Chromatography-based quantification
  - Shotgun DDA, targeted SRM, data independent DIA/SWATH
- Label-free or label-based
  - Simple summaries and models
- Multiple functionalities
  - Data visualization, statistical modeling and inference, sample size
- Free, open-source and inter-operable with other tools
  - External tool for Skyline, converter from MaxQuant

EXAMPLE: A LABEL-FREE EXPERIMENT

Question: which proteins change in abundance?







T. Clough et al. *BMC Bioinformatics., 2012*

INPUT DATA

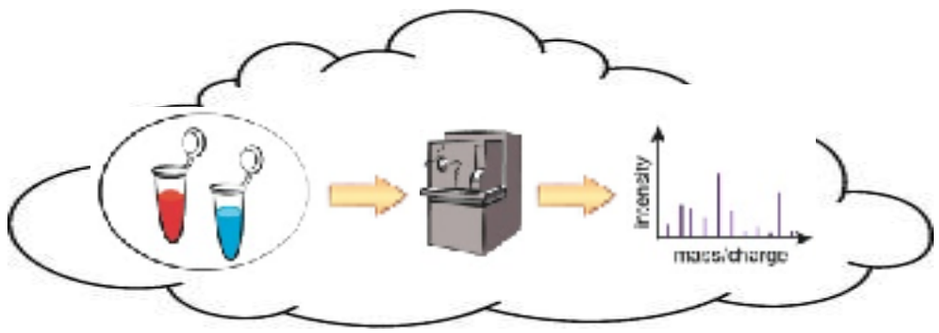
	A	B	C	D	E	F	G	H	I	J
1	ProteinName	PeptideSequence	PrecursorCharge	FragmentIon	ProductCharge	IsotopeLabelType	Condition	BioReplicate	Run	Intensity
2	ACEA	EILGHEIFFDWELP	3	y3	0	H	1	Repl A	1	66472.3847
3	ACEA	EILGHEIFFDWELP	3	y3	0	L	1	Repl A	1	5764.16228
4	ACEA	EILGHEIFFDWELP	3	y4	0	H	1	Repl A	1	101005.166
5	ACEA	EILGHEIFFDWELP	3	y4	0	L	1	Repl A	1	61.65238
6	ACEA	EILGHEIFFDWELP	3	y5	0	H	1	Repl A	1	90055.4993
7	ACEA	EILGHEIFFDWELP	3	y5	0	L	1	Repl A	1	472.691803
8	ACEA	TDSEAATLISSTID	2	y10	0	H	1	Repl A	1	43506.5425
9	ACEA	TDSEAATLISSTID	2	y10	0	L	1	Repl A	1	217.203553
10	ACEA	TDSEAATLISSTID	2	y7	0	H	1	Repl A	1	68023.0377
11	ACEA	TDSEAATLISSTID	2	y7	0	L	1	Repl A	1	725.284308
12	ACEA	TDSEAATLISSTID	2	y8	0	H	1	Repl A	1	68276.0489
13	ACEA	TDSEAATLISSTID	2	y8	0	L	1	Repl A	1	243.658527

A TYPICAL ANALYSIS WORKFLOW

Experimental design

Stresses or conditions

- Time course
- Multiple groups
- Paired design

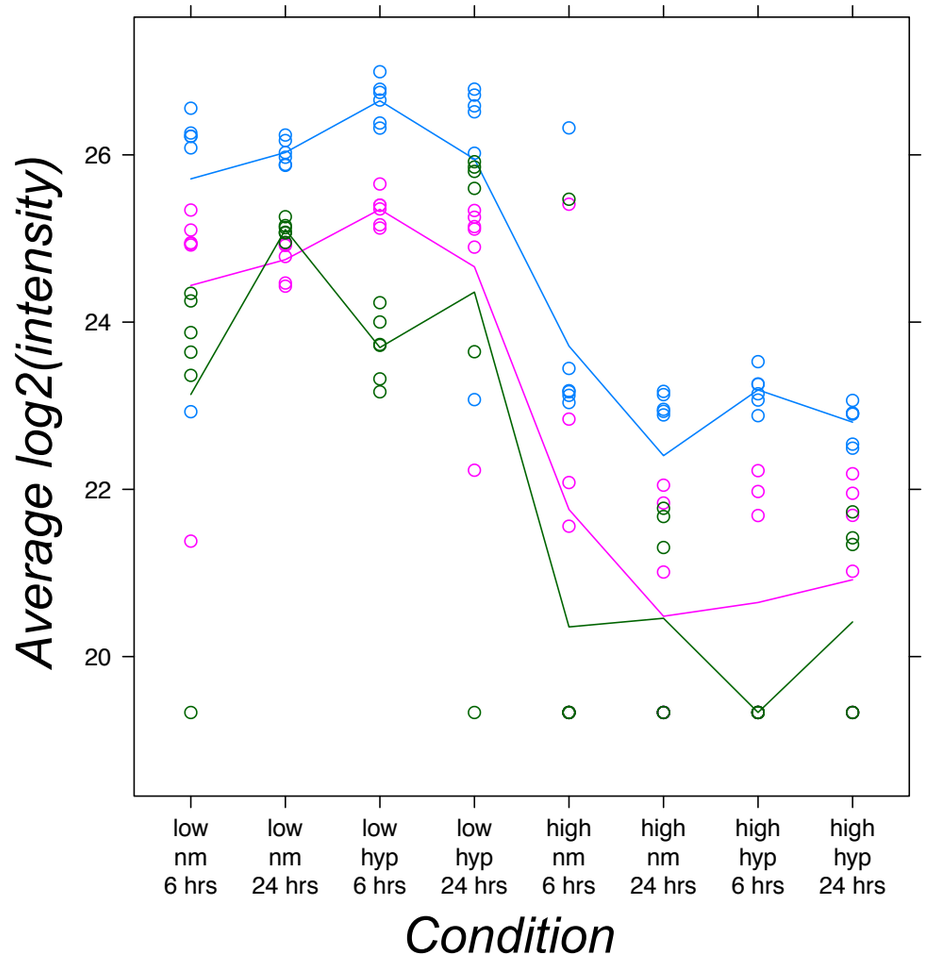


QC and normalization

Statistical modeling

Model-based conclusions

Experimental design



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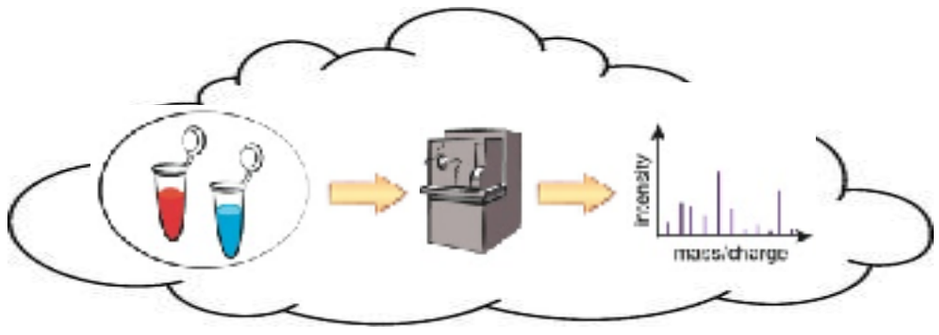

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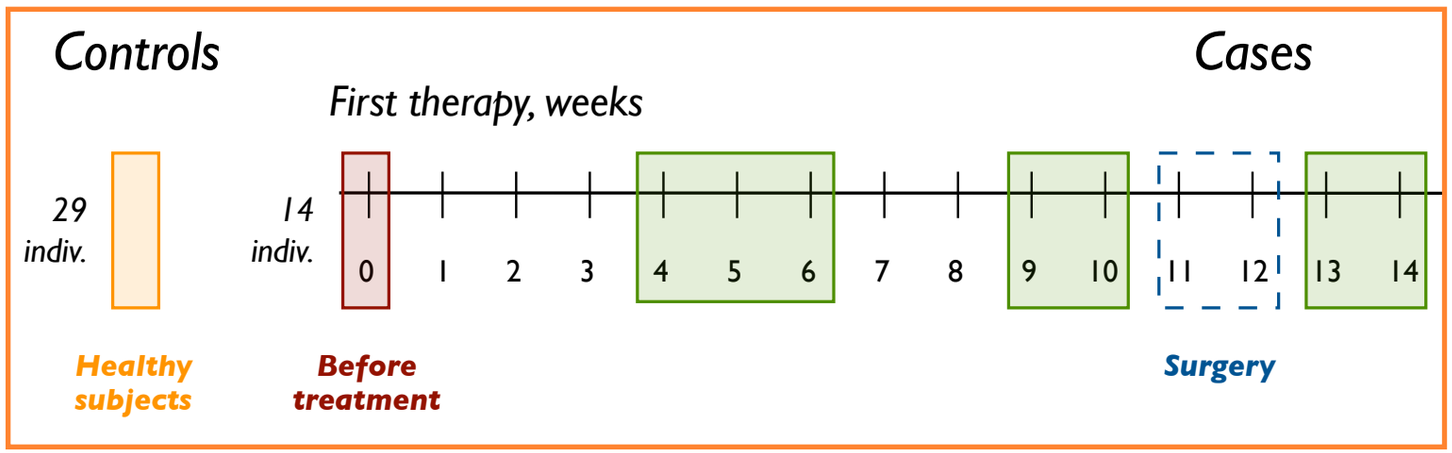

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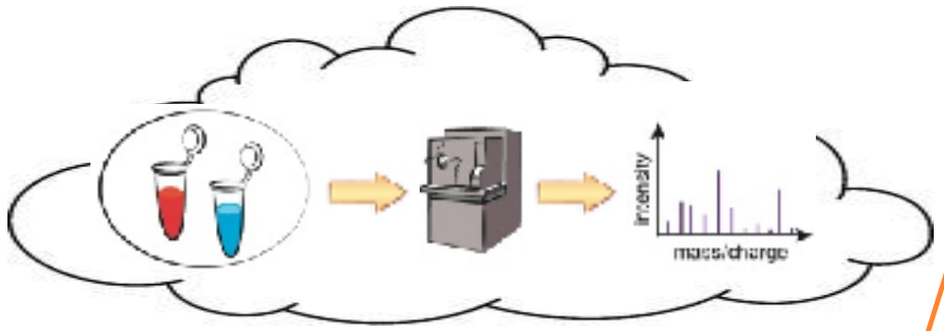

QC and normalization

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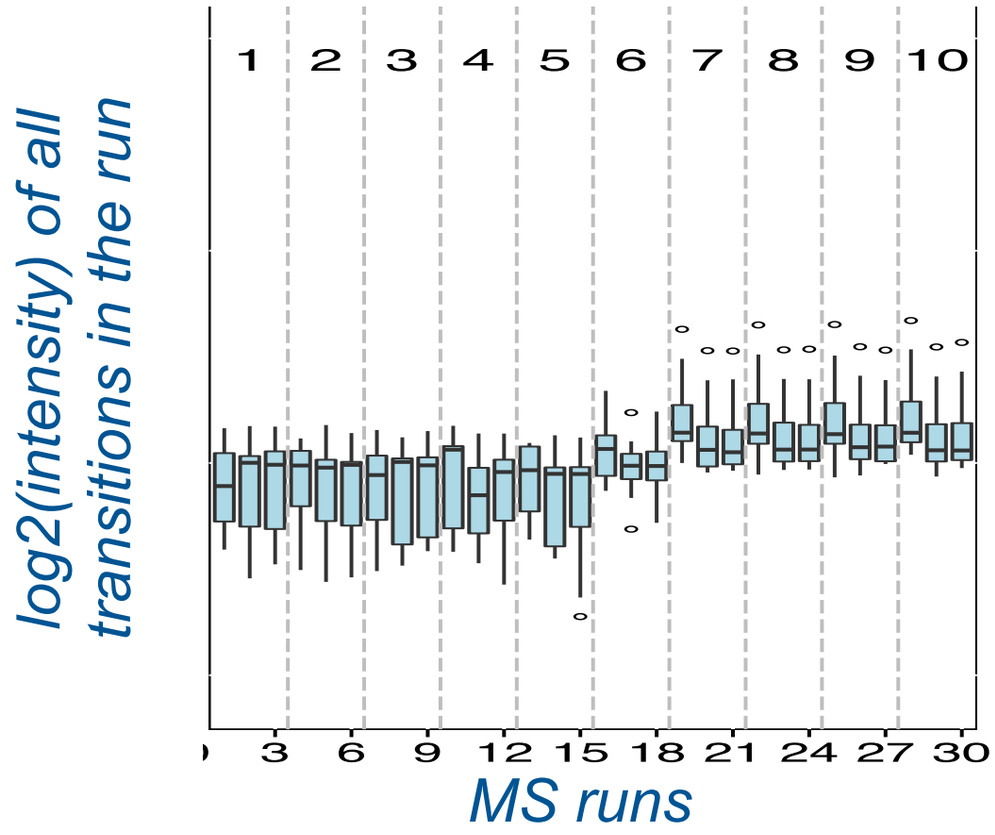
Data preparation

- Formatting
- Visualization
- Remove artifacts
- Normalization

Endogenous



C.-Y. Chang et al. *MCP*, 2012

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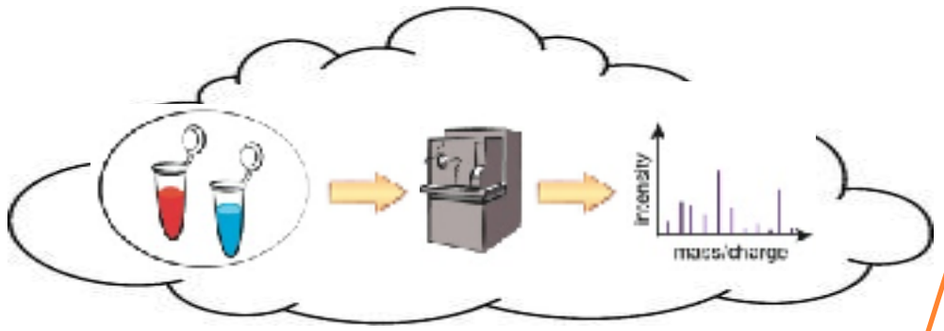

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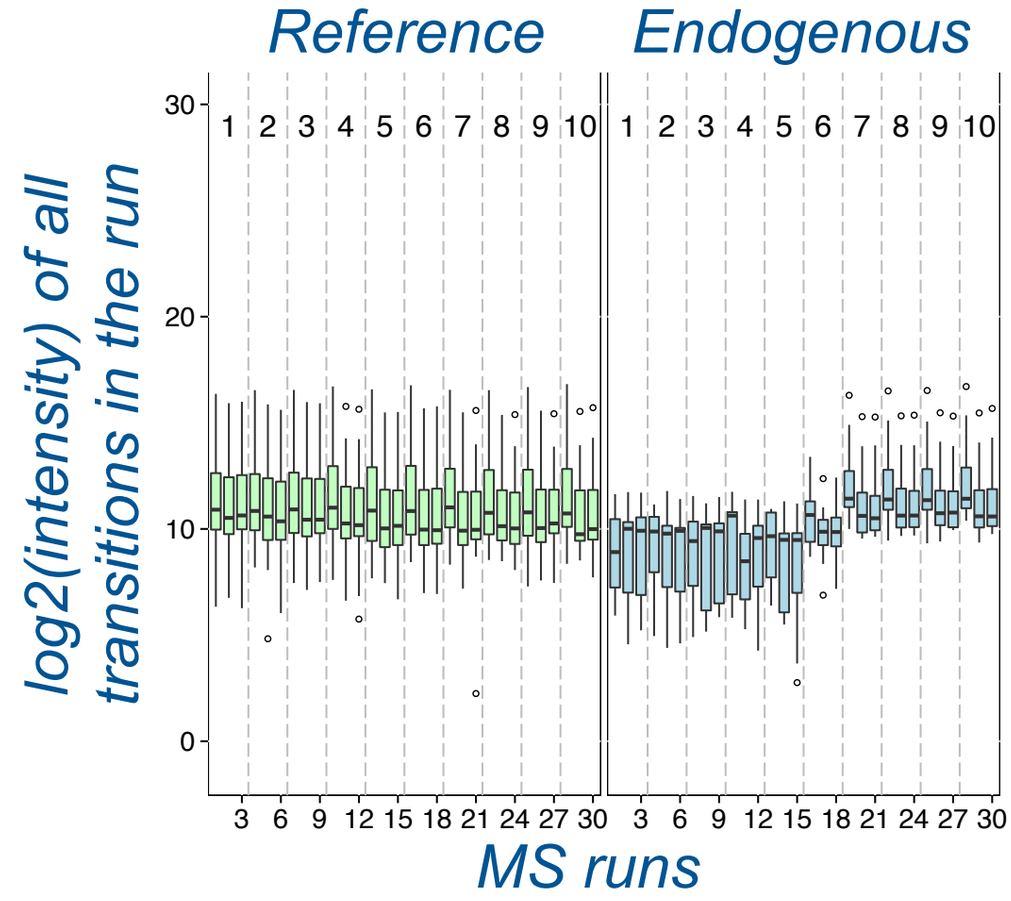

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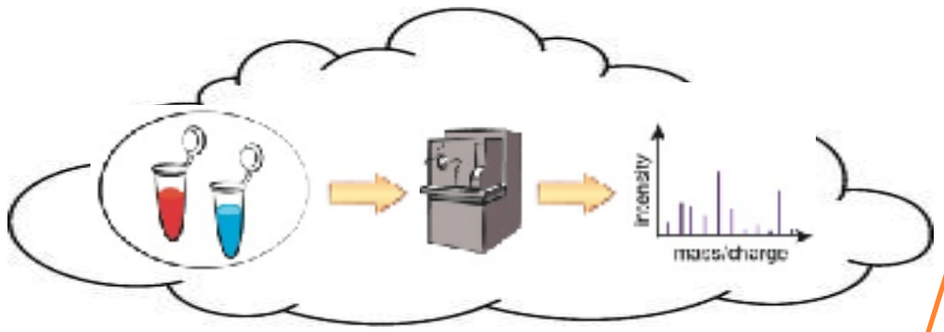

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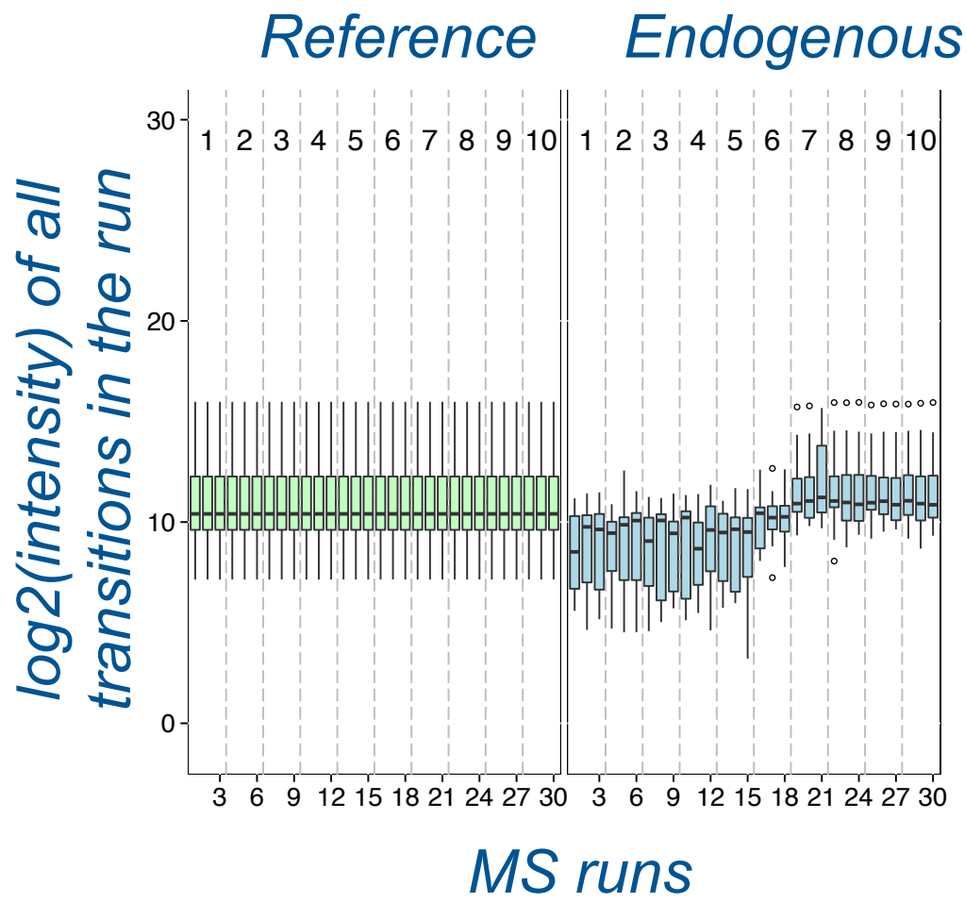

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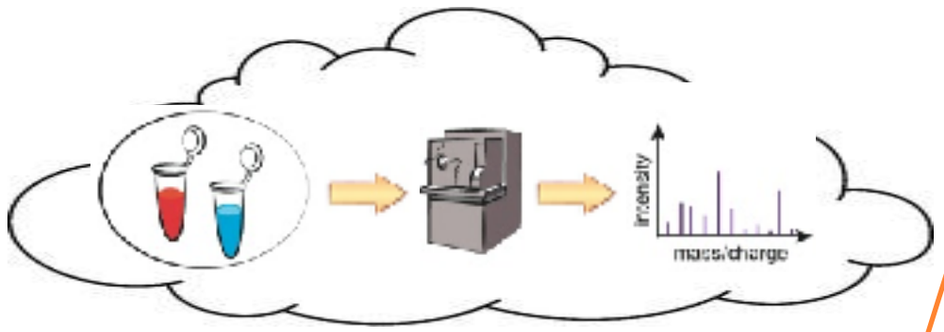
Experimental design

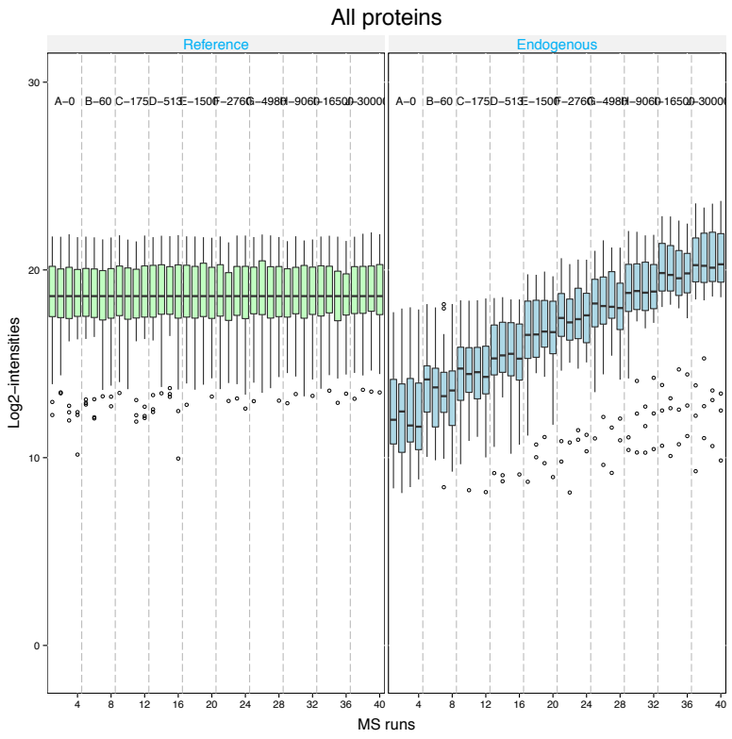


QC and normalization

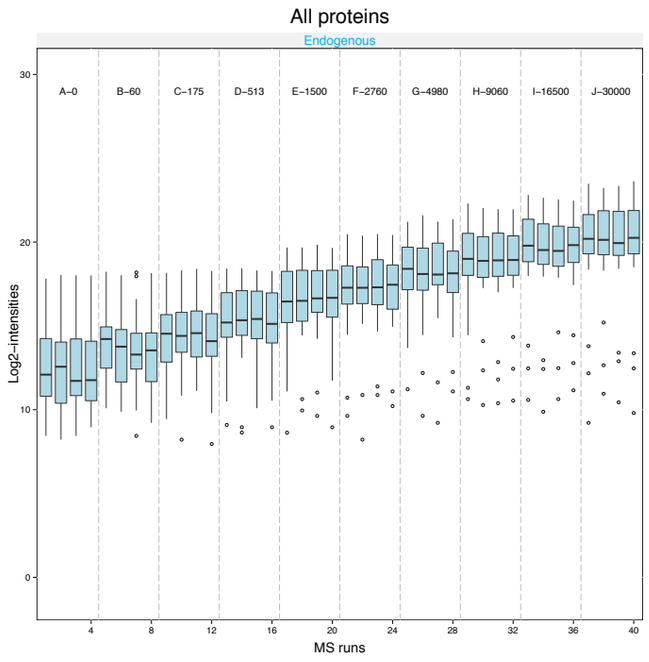
Data preparation

- Formatting
- Visualization
- Remove artifacts
- Normalization

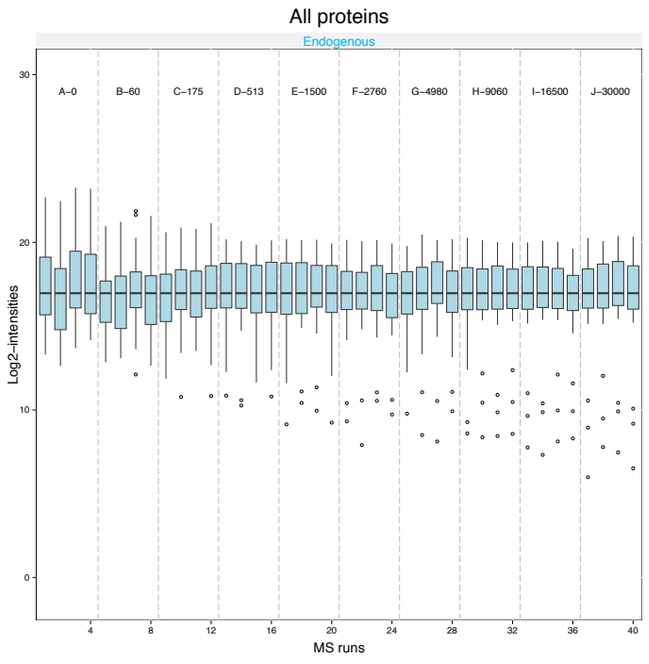
Equalize medians normalization



No normalization

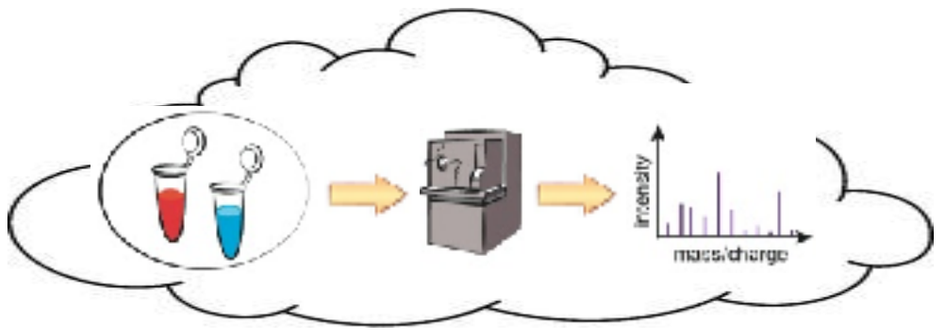


Equalize medians normalization



A TYPICAL ANALYSIS WORKFLOW

Experimental design



QC and normalization

Statistical modeling

Model-based conclusions

Experimental design

Summarize all protein features in a statistical model

- Systematic variation
- Random variation

Verify the assumptions!

- Describe statistical properties of
  - experimental design
  - biological variation
  - measurement technology

LINEAR MIXED MODELS

A split plot approach

Whole plot

Subplot	$Condition_1$						...				...	$Condition_I$									
	$Subject_1$			$Subject_2$			...	$Subject_J$			...	$Subject_{(I-1)J+1}$			$Subject_{(I-1)J+2}$			...	$Subject_{IJ}$		
	Run_1	Run_2	Run_3	Run_4	Run_5	Run_6	...	Run_{JK-2}	Run_{JK-1}	Run_{JK}	...	$Run_{(I-1)JK+1}$	$Run_{(I-1)JK+2}$	$Run_{(I-1)JK+3}$	$Run_{(I-1)JK+4}$	$Run_{(I-1)JK+5}$	$Run_{(I-1)JK+6}$	...	Run_{IJK-2}	Run_{IJK-1}	Run_{IJK}
$Feature_1$	y	y	y	y	y	y	...	y	y	y	...	y	y	y	y	y	y	...	y	y	y
$Feature_2$	y	y	y	y	y	**NA**	...	y	y	y	...	y	y	y	y	y	y	...	y	y	y
...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
$Feature_L$	y	**NA**	y	**NA**	**NA**	y	...	**NA**	y	y	...	**NA**	y	y	y	y	y	...	y	**NA**	y

Whole plot | Subplot

$$y_{ijkl} = \mu + \underbrace{\mathbf{Condition}_i + \underbrace{\mathbf{Subject(Condition)_{j(i)}}}_{\text{Whole-plot biological variation}}}_{\text{Whole plot}} + \underbrace{\underbrace{\mathbf{Run_{ijk}}}_{\text{Whole-plot technical variation}} + \mathbf{Feature}_l + \underbrace{\mathbf{Run \times Feature_{ijkl}}}_{\text{Subplot error}}}_{\text{Subplot}}$$

where $\sum_{i=1}^{I} \mathbf{Condition}_i = 0$, $\sum_{j=1}^{L} \mathbf{Feature}_l = 0$

$$\mathbf{Subject(Condition)_{j(i)}} \overset{\mathbf{iid}}{\sim} \mathcal{N}(\mathbf{0}, \sigma^2_{\mathbf{Subject}})$$

$$\mathbf{Run_{ijk}} = \psi_{\mathbf{ijk}} \overset{\mathbf{iid}}{\sim} \mathcal{N}(\mathbf{0}, \sigma^2_{\psi})$$

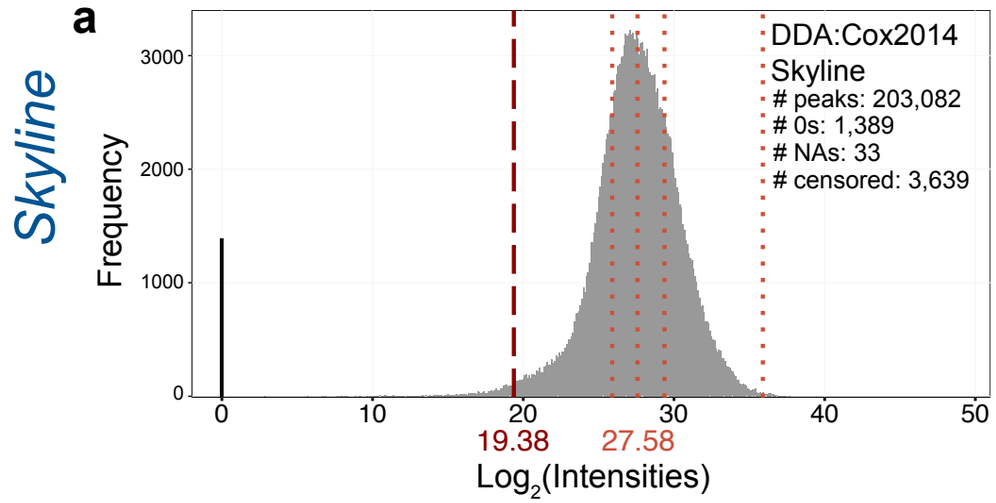
$$\mathbf{Run \times Feature_{ijkl}} = \epsilon_{\mathbf{ijkl}} \overset{\mathbf{iid}}{\sim} \mathcal{N}(\mathbf{0}, \sigma^2_{\epsilon})$$

PROPERTIES OF PEAK INTENSITIES VARY BETWEEN DATA PROCESSING TOOLS

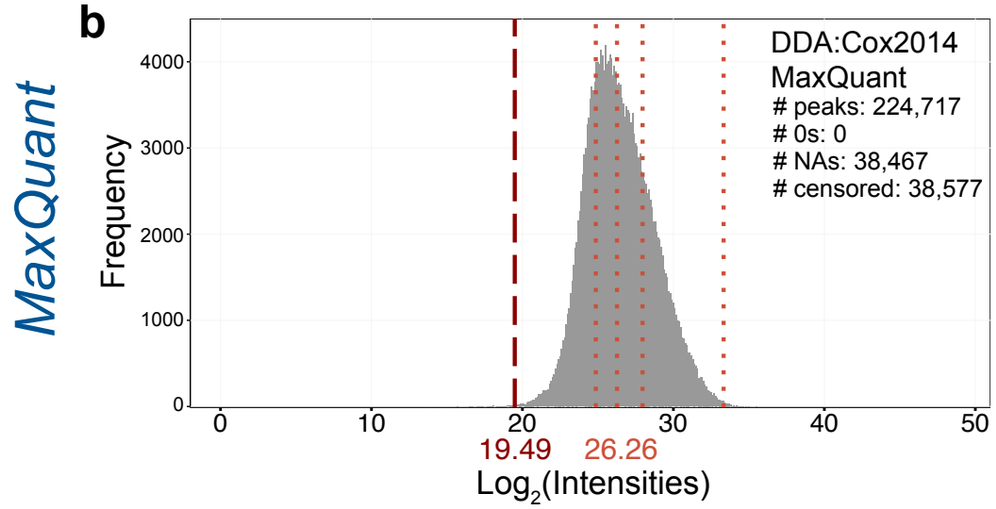
DDA: Cox 2014

DIA: Bruderer 2015

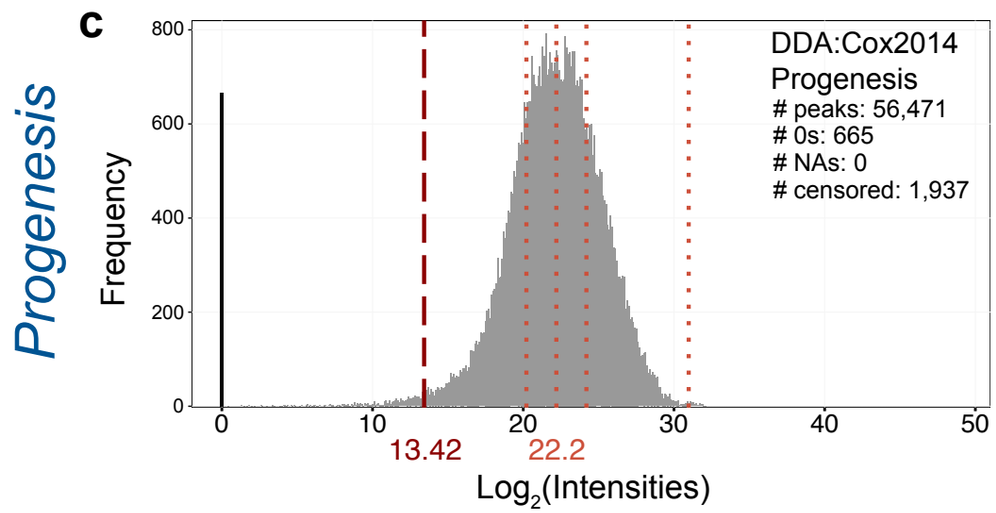
Skyline



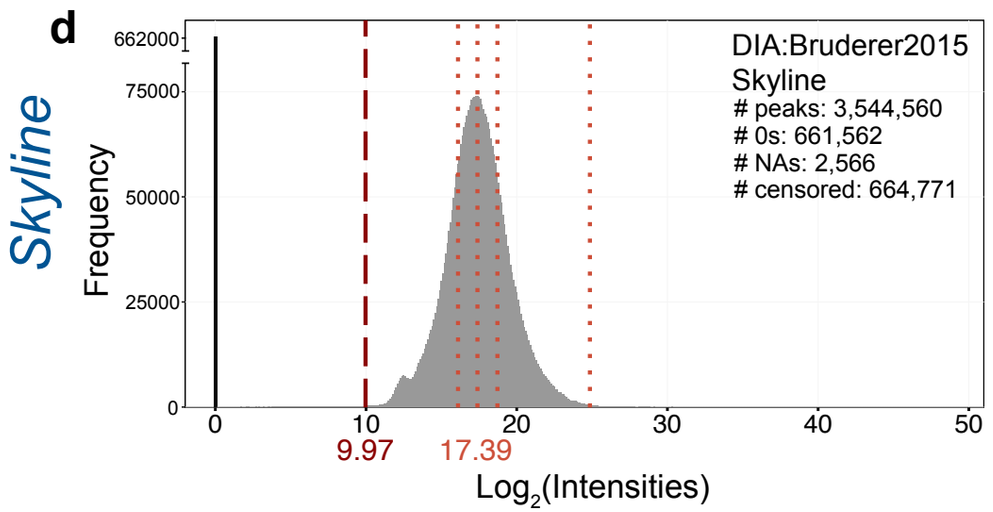
MaxQuant



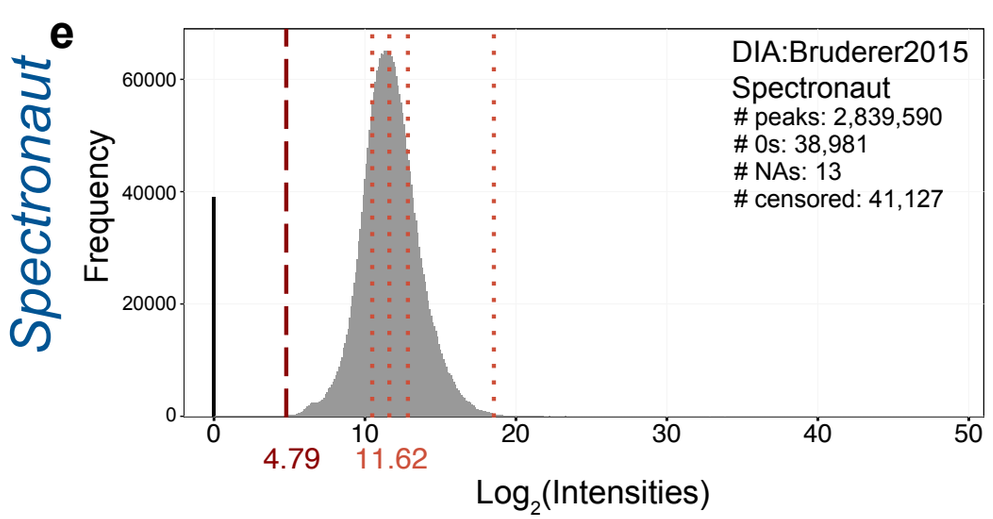
Progenesis

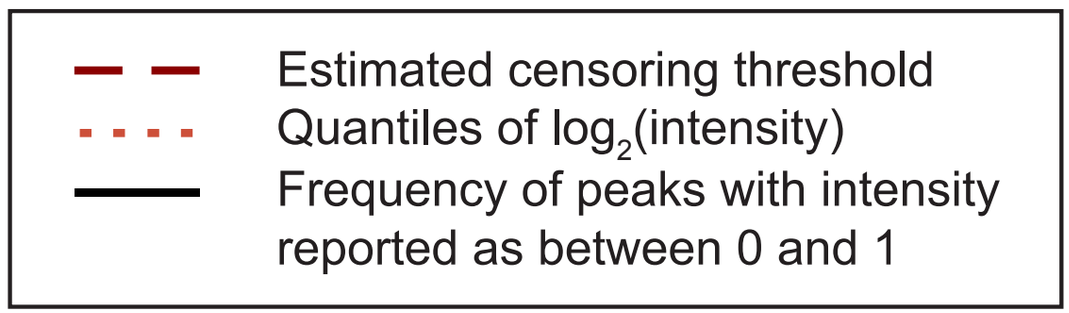


Skyline



Spectronaut





INTERPRETING CENSORED VALUES

A

	Run_1	Run_2	Run_3	Run_4	Run_5	Run_6	. . .	Run_{JK-2}	Run_{JK-1}	Run_{JK}	. . .	$Run_{(l-1)JK+1}$	$Run_{(l-1)JK+2}$	$Run_{(l-1)JK+3}$	$Run_{(l-1)JK+4}$	$Run_{(l-1)JK+5}$	$Run_{(l-1)JK+6}$	. . .	Run_{IJK-2}	Run_{IJK-1}	Run_{IJK}
$Feature_1$	y	y	y	y	y	y	. . .	y	y	y	. . .	y	y	y	y	y	y	. . .	y	y	y
$Feature_2$	y	y	y	y	y	$\mathbf{NA}_{rand}$	. . .	y	y	y	. . .	y	y	y	y	y	y	. . .	y	y	y
. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .
$Feature_L$	y	$\mathbf{NA}_{cen}$	y	$\mathbf{NA}_{cen}$	$\mathbf{NA}_{cen}$	y	. . .	$\mathbf{NA}_{cen}$	y	y	. . .	$\mathbf{NA}_{cen}$	y	y	y	y	y	. . .	y	$\mathbf{NA}_{cen}$	y

IMPUTING CENSORED VALUES

A

	Run_1	Run_2	Run_3	Run_4	Run_5	Run_6	· · ·	Run_{JK-2}	Run_{JK-1}	Run_{JK}	· · ·	$Run_{(I-1)JK+1}$	$Run_{(I-1)JK+2}$	$Run_{(I-1)JK+3}$	$Run_{(I-1)JK+4}$	$Run_{(I-1)JK+5}$	$Run_{(I-1)JK+6}$	· · ·	Run_{IJK-2}	Run_{IJK-1}	Run_{IJK}
$Feature_1$	y	y	y	y	y	y	· · ·	y	y	y	· · ·	y	y	y	y	y	y	· · ·	y	y	y
$Feature_2$	y	y	y	y	y	$\mathbf{NA}_{rand}$	· · ·	y	y	y	· · ·	y	y	y	y	y	y	· · ·	y	y	y
· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·
$Feature_L$	y	$\mathbf{NA}_{cen}$	y	$\mathbf{NA}_{cen}$	$\mathbf{NA}_{cen}$	y	· · ·	$\mathbf{NA}_{cen}$	y	y	· · ·	$\mathbf{NA}_{cen}$	y	y	y	y	y	· · ·	y	$\mathbf{NA}_{cen}$	y

Step 1 : Run-level subplot summarization

B

AFT model : Impute censored missing values by accelated failure model

$$y_{ijkl} = \mu + Run_{ijk} + Feature_l + \epsilon_{ijkl} \text{ , where } \sum_{ijk} Run_{ijk} = 0,\ \sum_{l} Feature_l = 0, \epsilon_{ijkl} \overset{iid}{\sim} \mathcal{N}(0, \sigma^2_\epsilon)$$

	Run_1	Run_2	Run_3	Run_4	Run_5	Run_6	· · ·	Run_{JK-2}	Run_{JK-1}	Run_{JK}	· · ·	$Run_{(I-1)JK+1}$	$Run_{(I-1)JK+2}$	$Run_{(I-1)JK+3}$	$Run_{(I-1)JK+4}$	$Run_{(I-1)JK+5}$	$Run_{(I-1)JK+6}$	· · ·	Run_{IJK-2}	Run_{IJK-1}	Run_{IJK}
$Feature_1$	y	y	y	y	y	y	· · ·	y	y	y	· · ·	y	y	y	y	y	y	· · ·	y	y	y
$Feature_2$	y	y	y	y	y		· · ·	y	y	y	· · ·	y	y	y	y	y	y	· · ·	y	y	y
· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·
$Feature_L$	y	$\mathbf{y}_{imp}$	y	$\mathbf{y}_{imp}$	$\mathbf{y}_{imp}$	y	· · ·	$\mathbf{y}_{imp}$	y	y	· · ·	$\mathbf{y}_{imp}$	y	y	y	y	y	· · ·	y	$\mathbf{y}_{imp}$	y

ROBUST RUN SUMMARIZATION

A

	Run_1	Run_2	Run_3	Run_4	Run_5	Run_6	· · ·	Run_{JK-2}	Run_{JK-1}	Run_{JK}	· · ·	$Run_{(l-1)JK+1}$	$Run_{(l-1)JK+2}$	$Run_{(l-1)JK+3}$	$Run_{(l-1)JK+4}$	$Run_{(l-1)JK+5}$	$Run_{(l-1)JK+6}$	· · ·	Run_{IJK-2}	Run_{IJK-1}	Run_{IJK}
$Feature_1$	y	y	y	y	y	y	· · ·	y	y	y	· · ·	y	y	y	y	y	y	· · ·	y	y	y
$Feature_2$	y	y	y	y	y	NA_{rand}	· · ·	y	y	y	· · ·	y	y	y	y	y	y	· · ·	y	y	y
· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·
$Feature_L$	y	NA_{cen}	y	NA_{cen}	NA_{cen}	y	· · ·	NA_{cen}	y	y	· · ·	NA_{cen}	y	y	y	y	y	· · ·	y	NA_{cen}	y

Step 1 : Run-level subplot summarization

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$Feature_1$	y	y	y	y	y	y	· · ·	y	y	y	· · ·	y	y	y	y	y	y	· · ·	y	y	y
$Feature_2$	y	y	y	y	y		· · ·	y	y	y	· · ·	y	y	y	y	y	y	· · ·	y	y	y
· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·
$Feature_L$	y	y_{imp}	y	y_{imp}	y_{imp}	y	· · ·	y_{imp}	y	y	· · ·	y_{imp}	y	y	y	y	y	· · ·	y	y_{imp}	y

TMP : Parameter estimation by robust method

$$y_{ijkl} = \mu + Run_{ijk} + Feature_l + \epsilon_{ijkl} \text{ , where}$$
$$median_{ijk}(Run_{ijk}) = 0,\ median_l(Feature_l) = 0,\ and\ median_{ijk}(\epsilon_{ijkl}) = median_l(\epsilon_{ijkl}) = 0$$

	Run_1	Run_2	Run_3	Run_4	Run_5	Run_6	· · ·	Run_{JK-2}	Run_{JK-1}	Run_{JK}	· · ·	$Run_{(l-1)JK+1}$	$Run_{(l-1)JK+2}$	$Run_{(l-1)JK+3}$	$Run_{(l-1)JK+4}$	$Run_{(l-1)JK+5}$	$Run_{(l-1)JK+6}$	· · ·	Run_{IJK-2}	Run_{IJK-1}	Run_{IJK}
Summarized	$\hat{y}$	$\hat{y}$	$\hat{y}$	$\hat{y}$	$\hat{y}$	$\hat{y}$	· · ·	$\hat{y}$	$\hat{y}$	$\hat{y}$	· · ·	$\hat{y}$	$\hat{y}$	$\hat{y}$	$\hat{y}$	$\hat{y}$	$\hat{y}$	· · ·	$\hat{y}$	$\hat{y}$	$\hat{y}$

SUB-PLOT

Summarization over all features in a run

log(Feature int)	Run 1	2	...	12
1	x_{11}	x_{12}	...	$x_{1\,12}$
2	x_{21}	x_{22}	...	$x_{2\,12}$
...			...	
n	x_{n1}	x_{n2}	...	$x_{n\,12}$

Tukey median polish

Represent features and runs in a sub-plot as 2-way Analysis of Variance

$$x_{ij} = feature_i + run_j + error_{ij}$$

Addition: censored data

Impute missing values by assuming that they have intensities below detection threshold

- Robust parameter estimation
  - subtract column median from each value
  - subtract row median from each value
  - continue until no change
  - obtain fitted values
    - subtract the resulting residuals from the original values
  - obtain array-based summary
    - average fitted values over the column

LINEAR MODELS FOR THE DESIGN

	Run_1	Run_2	Run_3	Run_4	Run_5	Run_6	· · ·	Run_{JK-2}	Run_{JK-1}	Run_{JK}	· · ·	$Run_{(I-1)JK+1}$	$Run_{(I-1)JK+2}$	$Run_{(I-1)JK+3}$	$Run_{(I-1)JK+4}$	$Run_{(I-1)JK+5}$	$Run_{(I-1)JK+6}$	· · ·	Run_{IJK-2}	Run_{IJK-1}	Run_{IJK}
Summarized	$\hat{y}$	$\hat{y}$	$\hat{y}$	$\hat{y}$	$\hat{y}$	$\hat{y}$	· · ·	$\hat{y}$	$\hat{y}$	$\hat{y}$	· · ·	$\hat{y}$	$\hat{y}$	$\hat{y}$	$\hat{y}$	$\hat{y}$	$\hat{y}$	· · ·	$\hat{y}$	$\hat{y}$	$\hat{y}$

C

Step 2 : Model-based inference by whole plot

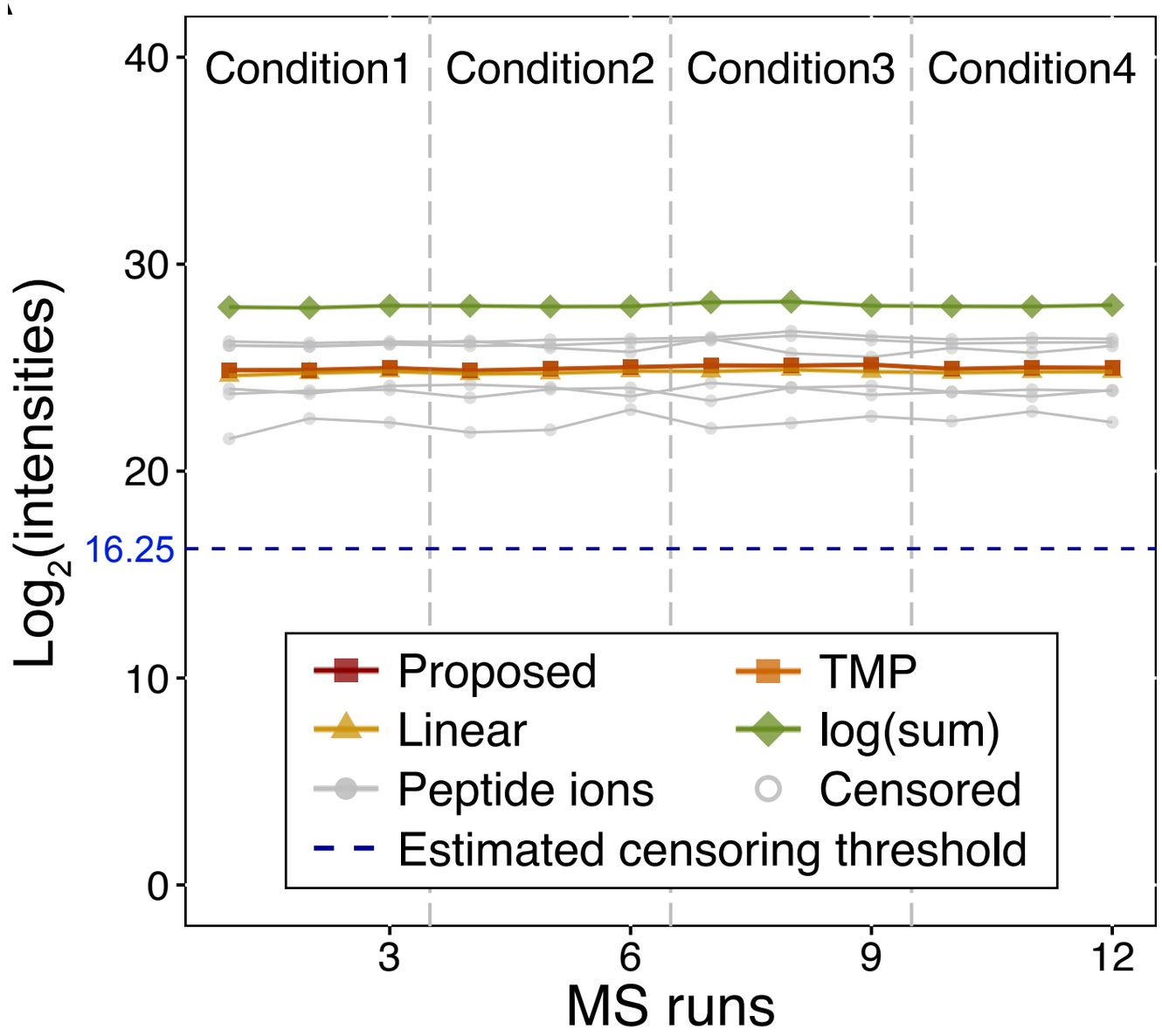
$$\hat{y}_{ijk} = \mu + Condition_i + Subject(Condition)_{j(i)} + \psi_{ijk}, \text{where}$$

$$\sum_i Condition_i = 0,\ Subject(Condition)_{j(i)} \overset{iid}{\sim} \mathcal{N}(0, \sigma^2_{Subject}),\ \psi_{ijk} \overset{iid}{\sim} \mathcal{N}(0, \sigma^2_{\psi})$$

	$Condition_1$						· · ·				· · ·	$Condition_I$						· · ·			
	$Subject_1$			$Subject_2$			· · ·	$Subject_J$			· · ·	$Subject_{(I-1)J+1}$			$Subject_{(I-1)+2}$			· · ·	$Subject_{IJ}$		
	Run_1	Run_2	Run_3	Run_4	Run_5	Run_6	· · ·	Run_{JK-2}	Run_{JK-1}	Run_{JK}	· · ·	$Run_{(I-1)JK+1}$	$Run_{(I-1)JK+2}$	$Run_{(I-1)JK+3}$	$Run_{(I-1)JK+4}$	$Run_{(I-1)JK+5}$	$Run_{(I-1)JK+6}$	· · ·	Run_{IJK-2}	Run_{IJK-1}	Run_{IJK}
Summarized	$\hat{y}$	$\hat{y}$	$\hat{y}$	$\hat{y}$	$\hat{y}$	$\hat{y}$	· · ·	$\hat{y}$	$\hat{y}$	$\hat{y}$	· · ·	$\hat{y}$	$\hat{y}$	$\hat{y}$	$\hat{y}$	$\hat{y}$	$\hat{y}$	· · ·	$\hat{y}$	$\hat{y}$	$\hat{y}$

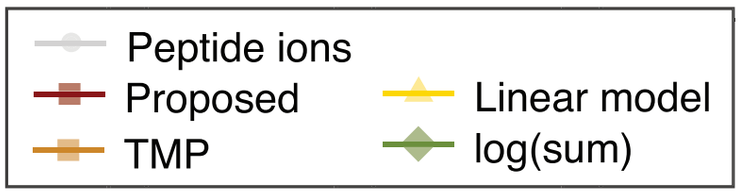
ROBUSTNESS TO OUTLIERS

Methods perform similarly with high quality data



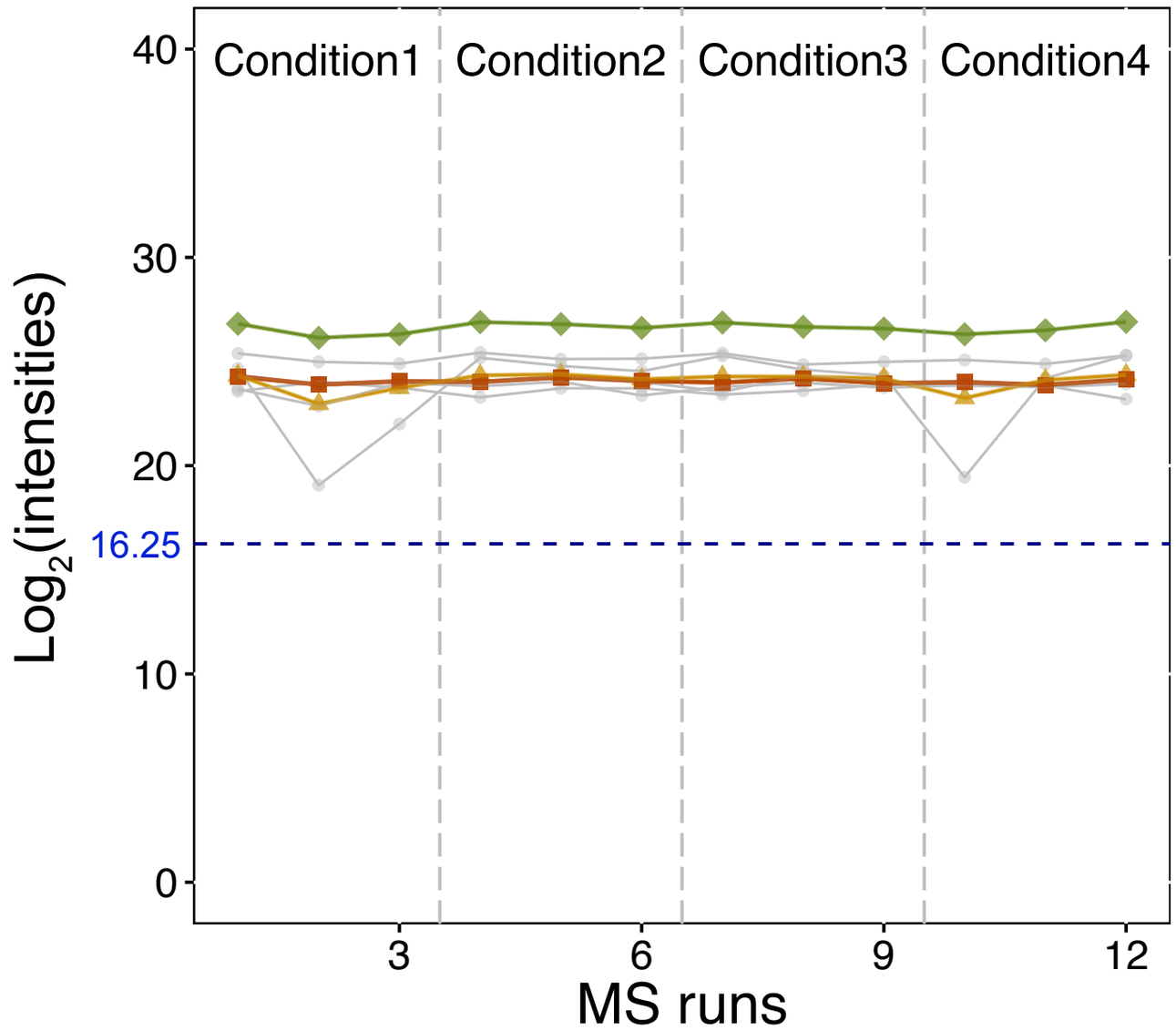
Condition2-Condition1 : True fold change=1

	EstimatedFC	Adj.pvalue
Proposed	1.016	0.999
TMP	1.016	0.999
Linear model	1.020	0.999
log(sum)	1.019	0.999



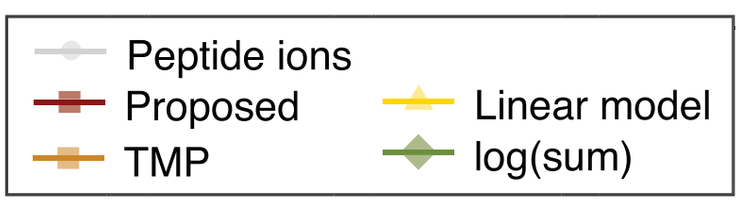
ROBUSTNESS TO OUTLIERS

Outliers in low intensities: robust summarization with TMP improves upon linear model



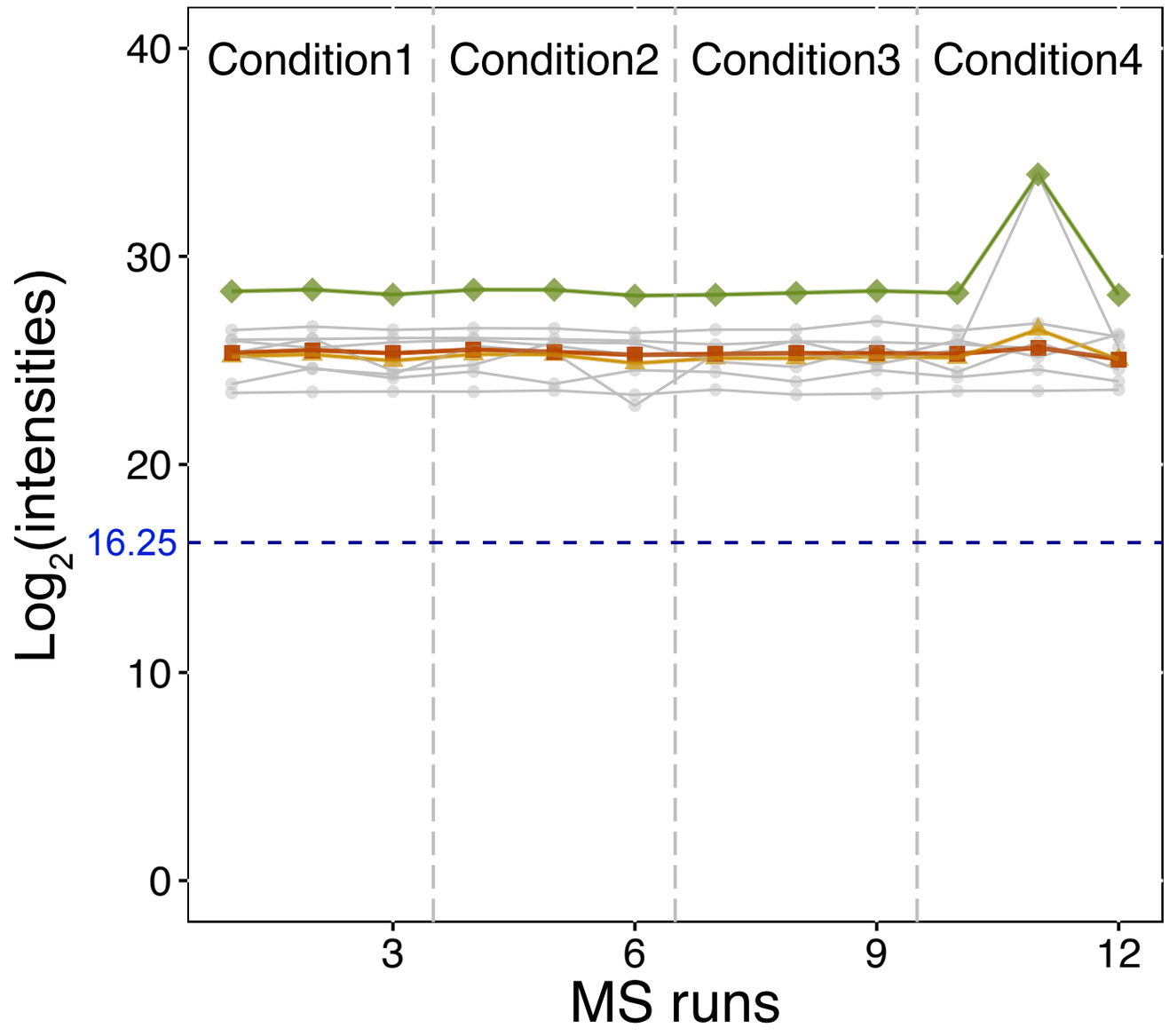
Condition3-Condition1 : True fold change=1

	EstimatedFC	Adj.pvalue
Proposed	0.979	0.952
TMP	0.979	0.956
Linear model	1.488	0.815
log(sum)	1.218	0.734



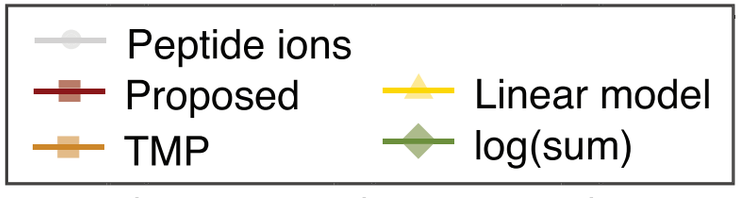
ROBUSTNESS TO OUTLIERS

Outliers in high intensities: robust summarization with TMP improves upon log(sum)



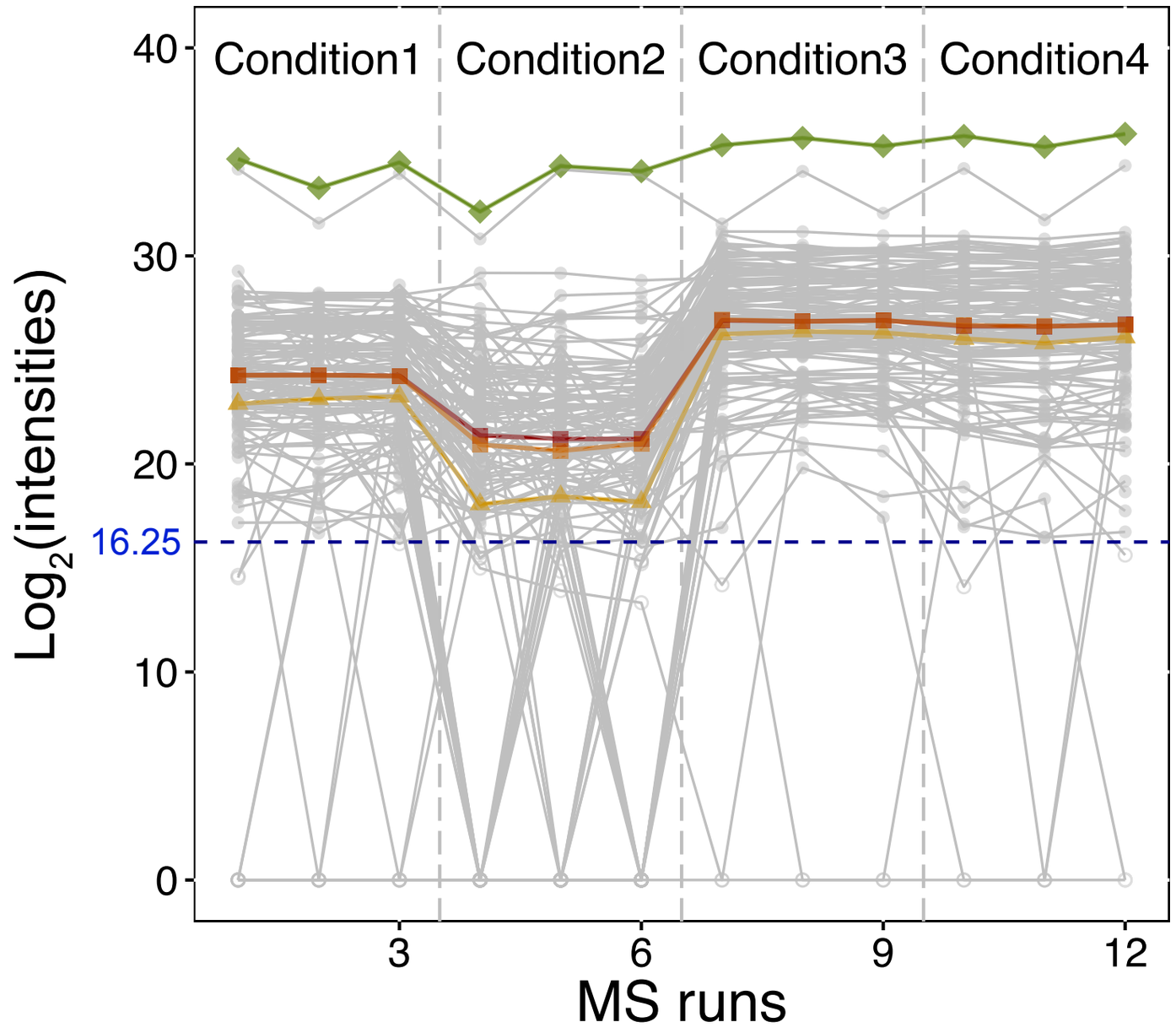
Condition4-Condition1 : True fold change=1

	EstimatedFC	Adj.pvalue
Proposed	0.951	0.948
TMP	0.951	0.948
Linear model	1.317	0.881
log(sum)	3.514	0.741



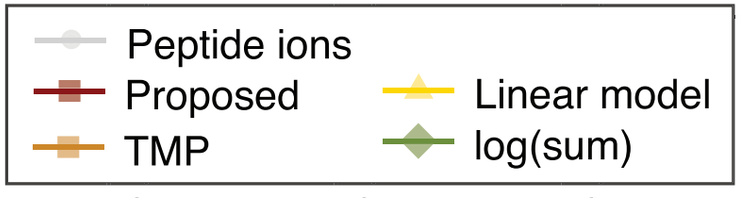
ROBUSTNESS TO OUTLIERS

Outliers in both high and low intensities: TMP improves upon linear model and log(sum)



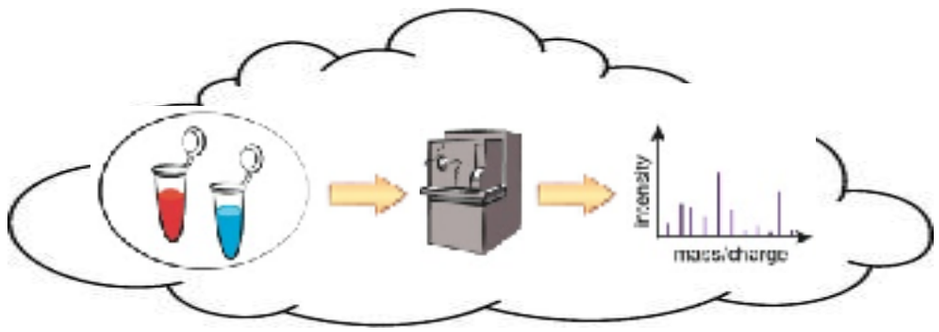
Condition1-Condition2 : True fold change=7.5

	EstimatedFC	Adj.pvalue
Proposed	8.015	< 0.001
TMP	10.605	< 0.001
Linear model	29.106	< 0.001
log(sum)	1.552	0.999



A TYPICAL ANALYSIS WORKFLOW

Experimental design



QC and normalization

Statistical modeling

Model-based conclusions

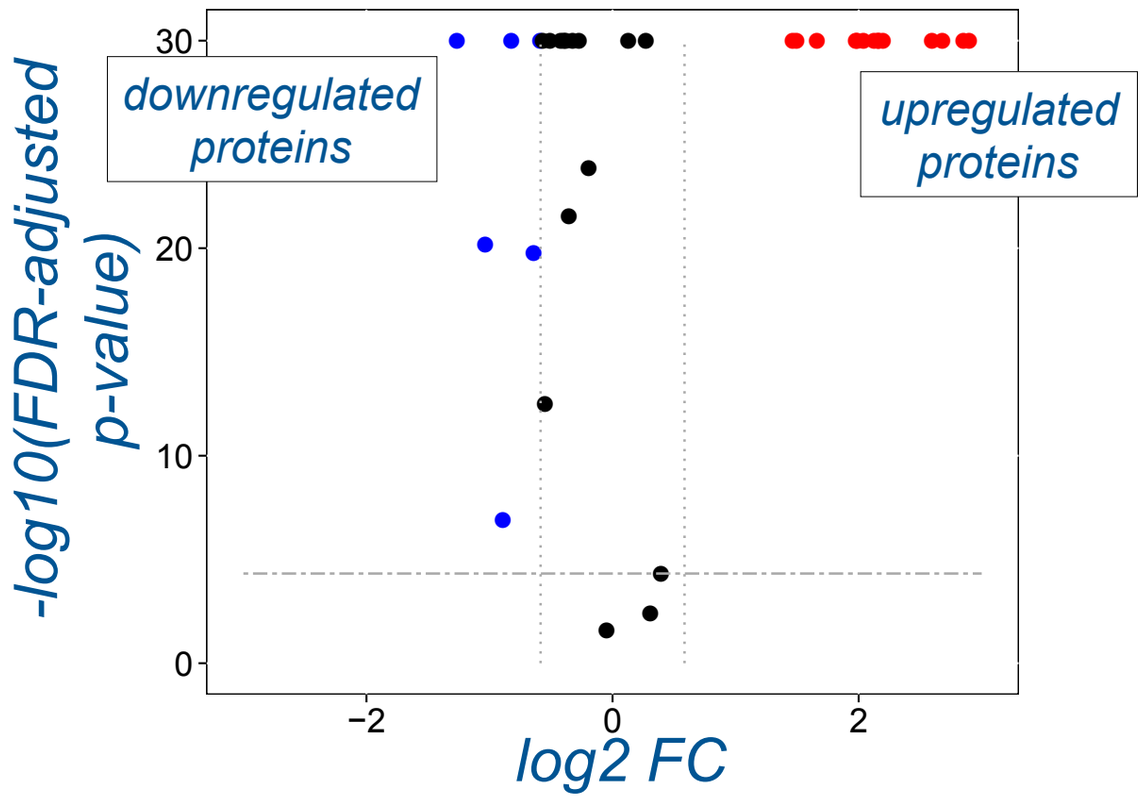
Experimental design

Model-based group comparisons

- Quantify the uncertainty
- Adjust p-values to control FDR

Relative protein quantification

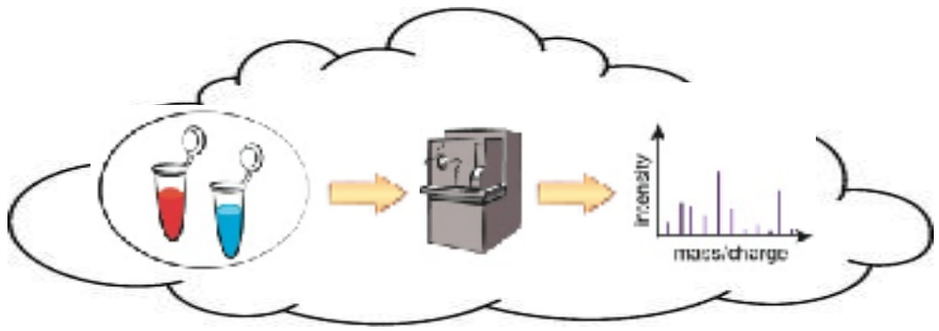
- In one sample
- In one condition



C.-Y. Chang et al. *MCP*, 2012

A TYPICAL ANALYSIS WORKFLOW

Experimental design



QC and normalization

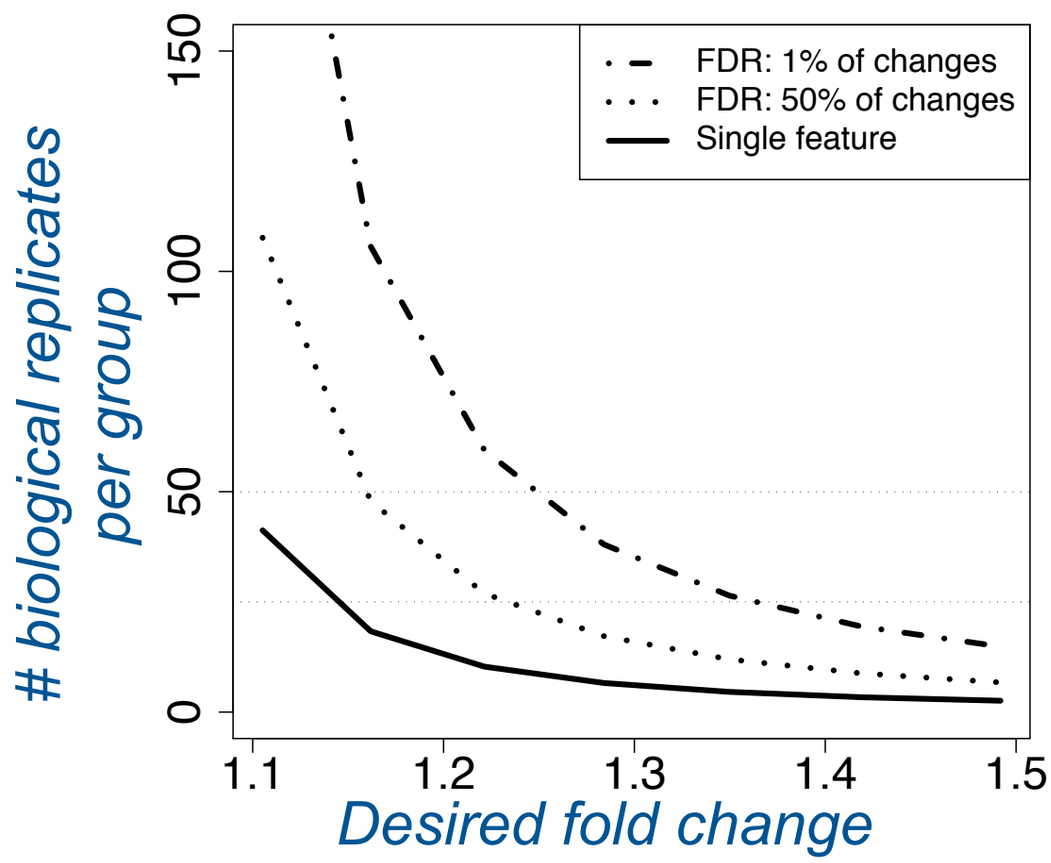
Statistical modeling

Model-based conclusions

Experimental design

Use the dataset to improve:

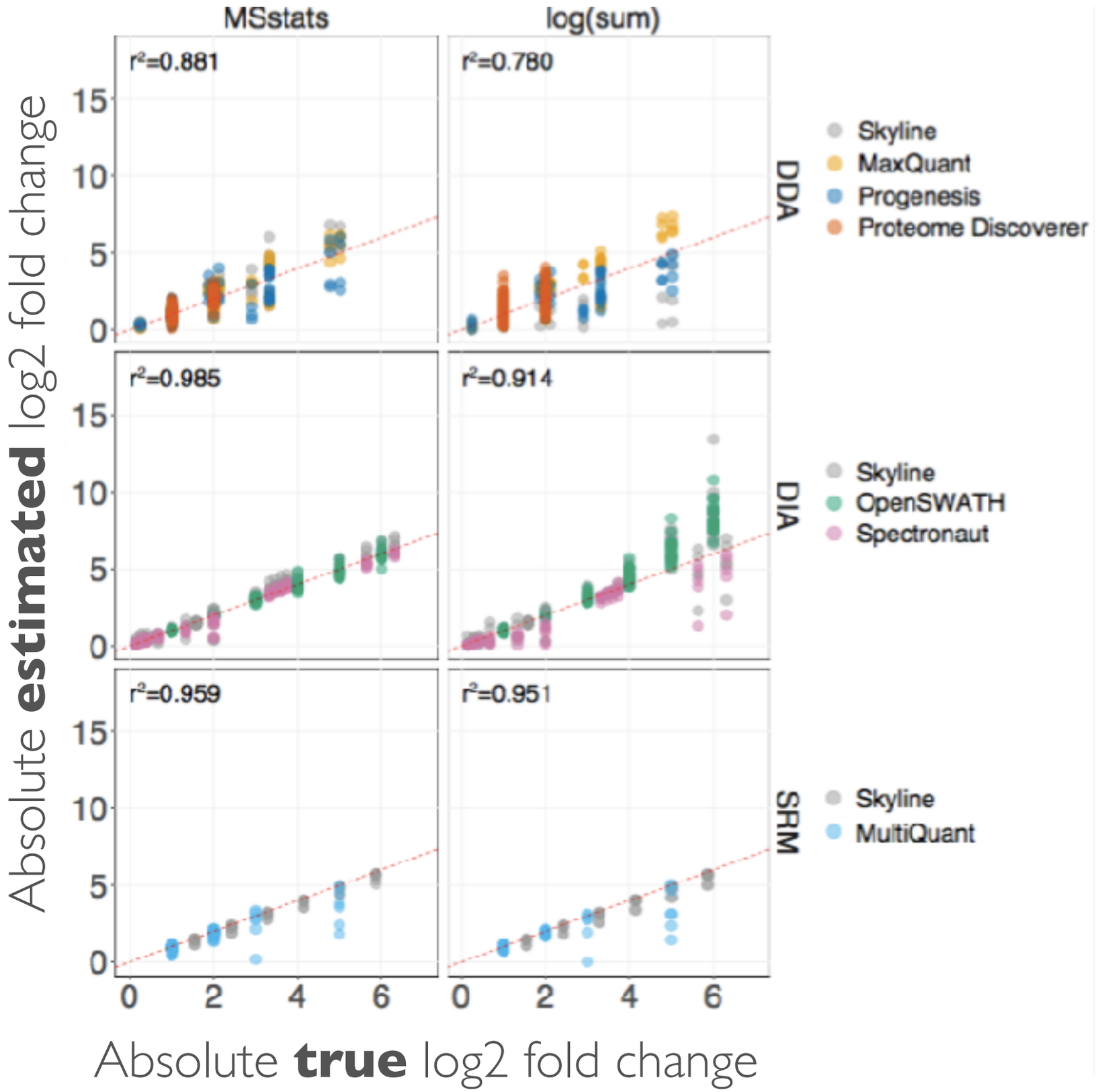
- Subject selection: matching
- Resource allocation: blocking
- Calculation of sample size



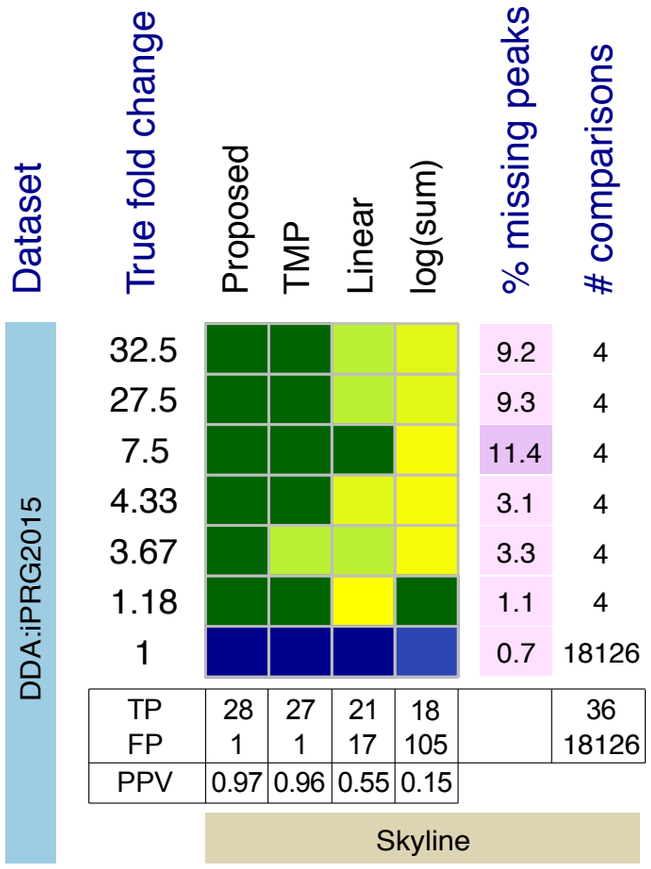
OUTLINE

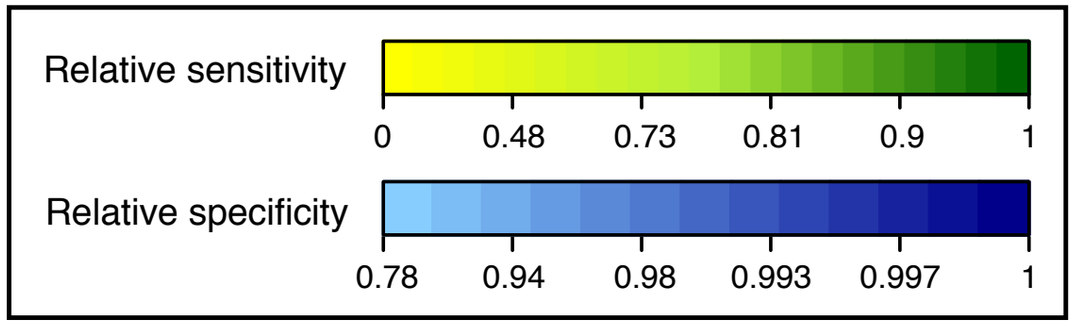
- Methods: background
  - Linear mixed effects models
- Evaluation
  - Spike-in and experimental datasets

ESTIMATION OF LOG-FOLD CHANGE

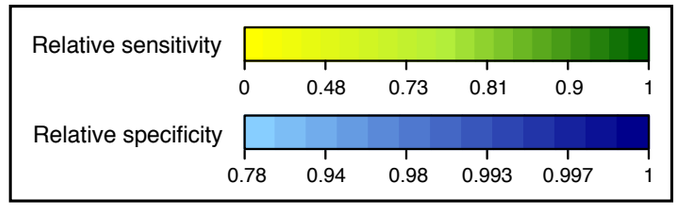


TESTING: DDA


Dataset
True fold change
Proposed
TMP
Linear
log(sum)
% missing peaks
# comparisons
DDA:iPRG2015
32.5
27.5
7.5
4.33
3.67
1.18
1
9.2
9.3
11.4
3.1
3.3
1.1
0.7
4
4
4
4
4
4
18126
TP 28 27 21 18 36
FP 1 1 17 105 18126
PPV 0.97 0.96 0.55 0.15
Skyline


Relative sensitivity
0
0.48
0.73
0.81
0.9
1
Relative specificity
0.78
0.94
0.98
0.993
0.997
1

TESTING: DDA



a

Dataset	True fold change	Proposed	TMP	Linear	log(sum)	% missing peaks	# comparisons
DDA:iPRG2015	32.5					9.2	4
	27.5					9.3	4
	7.5					11.4	4
	4.33					3.1	4
	3.67					3.3	4
	1.18					1.1	4
	1					0.7	18126
	TP	28	27	21	18		36
	FP	1	1	17	105		18126
	PPV	0.97	0.96	0.55	0.15		
		Skyline					

		Proposed	TMP	Linear	log(sum)	% missing peaks	# comparisons
DDA:iPRG2015	32.5					40.5	4
	27.5					41.0	4
	7.5					55.8	4
	4.33					20.4	4
	3.67					20.9	4
	1.18					5.5	4
	1					10.7	17205
	TP	26	23	19	25		30
	FP	7	1	5	20		17205
	PPV	0.79	0.96	0.79	0.56		
		MaxQuant					

		Proposed	TMP	Linear	log(sum)	% missing peaks	# comparisons
DDA:iPRG2015	32.5					4.2	4
	27.5					4.2	4
	7.5					6.2	4
	4.33					2.1	4
	3.67					2.1	4
	1.18					0	4
	1					0.4	18054
	TP	26	23	24	30		36
	FP	106	96	183	212		18054
	PPV	0.20	0.19	0.12	0.11		
		Progenesis					

		Proposed	TMP	Linear	log(sum)	% missing peaks	# comparisons
DDA:Cox2014	10					5.5	8
	0.1					1.9	8
	1					1.6	1931
	TP	38	38	34	35		39
	FP	13	13	2	14		1931
	PPV	0.75	0.75	0.94	0.71		
		Skyline					

		Proposed	TMP	Linear	log(sum)	% missing peaks	# comparisons
DDA:Cox2014	10					29.3	8
	0.1					10.2	7
	1					14.0	2046
	TP	34	29	26	34		35
	FP	5	6	5	10		2046
	PPV	0.87	0.83	0.84	0.77		
		MaxQuant					

		Proposed	TMP	Linear	log(sum)	% missing peaks	# comparisons
DDA:Cox2014	10					2.6	8
	0.1					0	7
	1					2.2	1877
	TP	38	37	36	38		38
	FP	17	17	25	31		1877
	PPV	0.69	0.69	0.59	0.55		
		Progenesis					

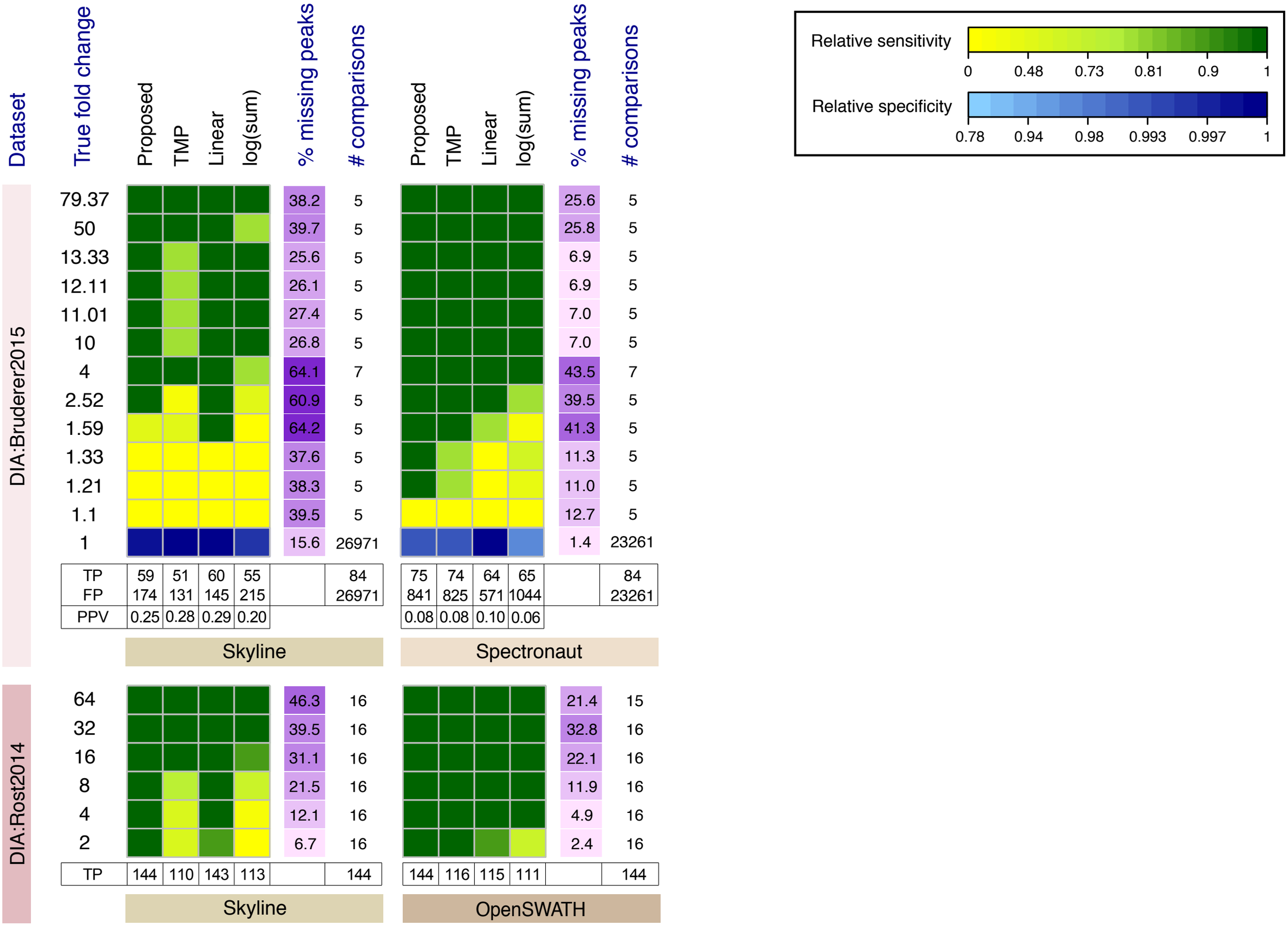
		Proposed	TMP	Linear	log(sum)	% missing peaks	# comparisons
DDA:SpikeIn	4					0.9	48
	2					1.1	144
	1					1.4	12258
	TP	169	168	129	165		192
	FP	101	94	69	178		12258
	PPV	0.63	0.64	0.65	0.48		
		Skyline					

		Proposed	TMP	Linear	log(sum)	% missing peaks	# comparisons
DDA:SpikeIn	4					12.4	56
	2					12.9	168
	1					13.6	15262
	TP	188	192	110	190		224
	FP	76	84	62	109		15262
	PPV	0.71	0.70	0.64	0.64		
		MaxQuant					

		Proposed	TMP	Linear	log(sum)	% missing peaks	# comparisons
DDA:SpikeIn	4					3.1	54
	2					3.0	162
	1					1.0	13084
	TP	190	186	97	202		216
	FP	53	49	40	143		13084
	PPV	0.78	0.79	0.71	0.59		
		Progenesis					

		Proposed	TMP	Linear	log(sum)	% missing peaks	# comparisons
DDA:SpikeIn	4					28.2	54
	2					29.3	162
	1					30.1	12314
	TP	187	203	46	159		216
	FP	98	127	8	101		12314
	PPV	0.66	0.62	0.85	0.61		
		Proteome Discover					

TESTING: DIA


Dataset
True fold change
Proposed
TMP
Linear
log(sum)
% missing peaks
# comparisons
DIA:Bruderer2015
79.37 50 13.33 12.11 11.01 10 4 2.52 1.59 1.33 1.21 1.1 1
38.2 39.7 25.6 26.1 27.4 26.8 64.1 60.9 64.2 37.6 38.3 39.5 15.6
5 5 5 5 5 5 7 5 5 5 5 5 26971
25.6 25.8 6.9 6.9 7.0 7.0 43.5 39.5 41.3 11.3 11.0 12.7 1.4
5 5 5 5 5 5 7 5 5 5 5 5 23261
TP 59 51 60 55 84
FP 174 131 145 215 26971
PPV 0.25 0.28 0.29 0.20
TP 75 74 64 65 84
FP 841 825 571 1044 23261
PPV 0.08 0.08 0.10 0.06
Skyline
Spectronaut
DIA:Rost2014
64 32 16 8 4 2
46.3 39.5 31.1 21.5 12.1 6.7
16 16 16 16 16 16
21.4 32.8 22.1 11.9 4.9 2.4
15 16 16 16 16 16
TP 144 110 143 113 144
TP 144 116 115 111 144
Skyline
OpenSWATH

Relative sensitivity
0 0.48 0.73 0.81 0.9 1
Relative specificity
0.78 0.94 0.98 0.993 0.997 1

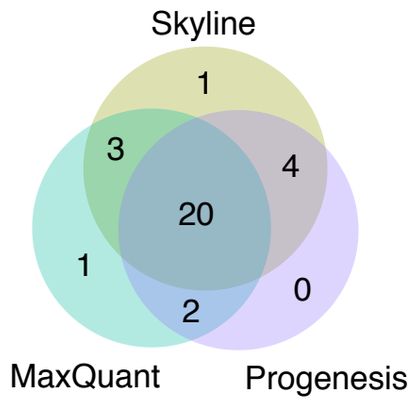
BETTER STATISTICAL METHODS ENHANCE REPRODUCIBLE RESEARCH

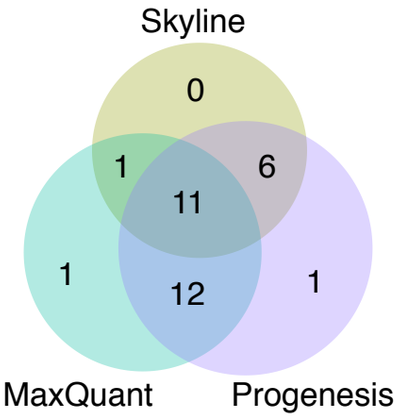
Better agreement in #differentially abundant proteins between tools

Proposed

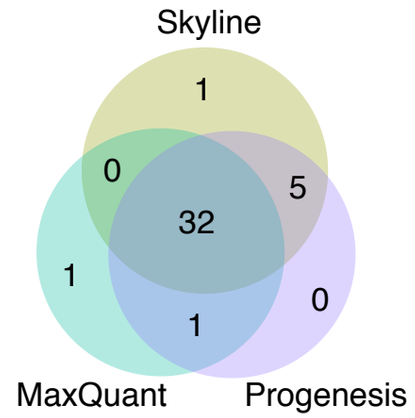
Log(sum)

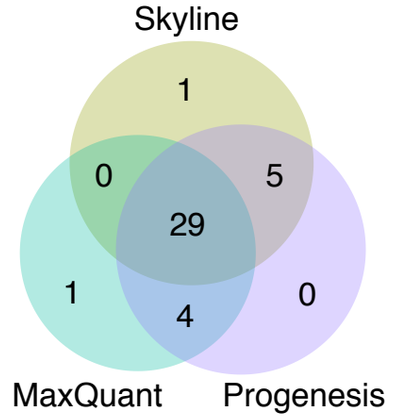
DDA: iPRG2015



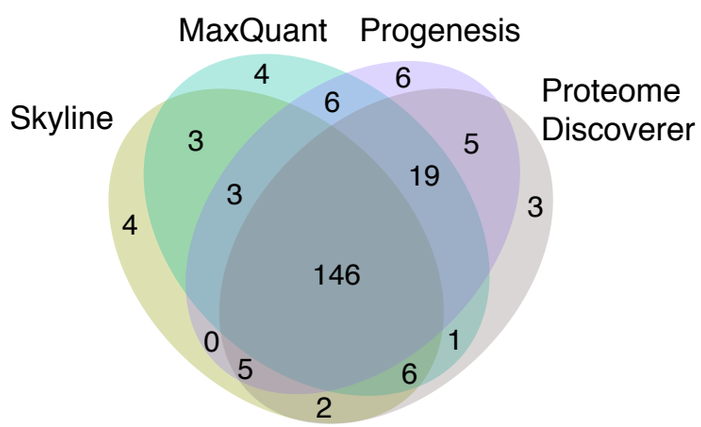


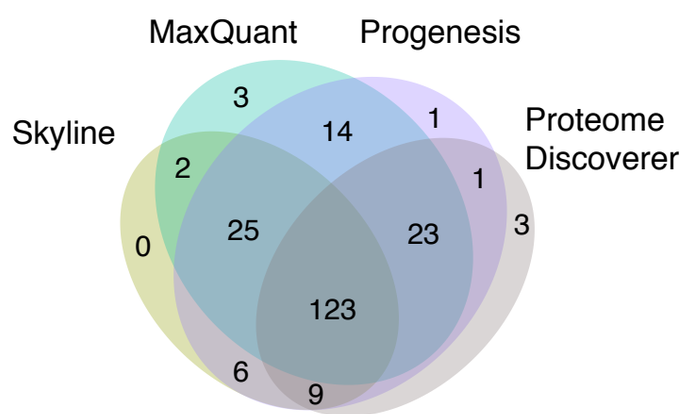
DDA: Cox 2014





DDA: Spike-in

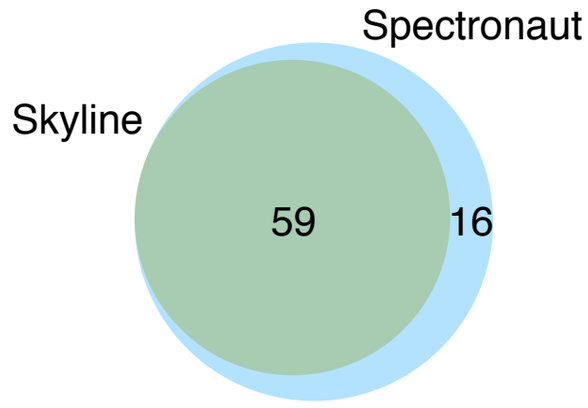




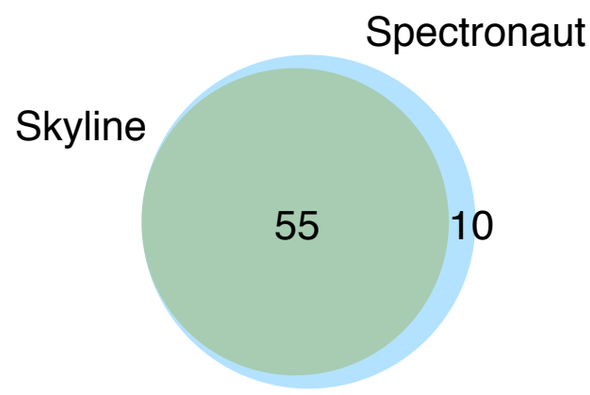
BETTER STATISTICAL METHODS ENHANCE REPRODUCIBLE RESEARCH

DIA: Bruderer2015

Proposed

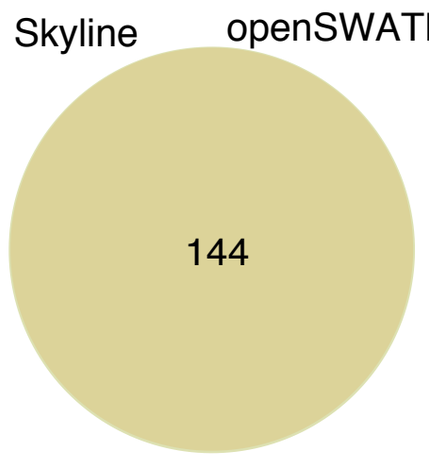


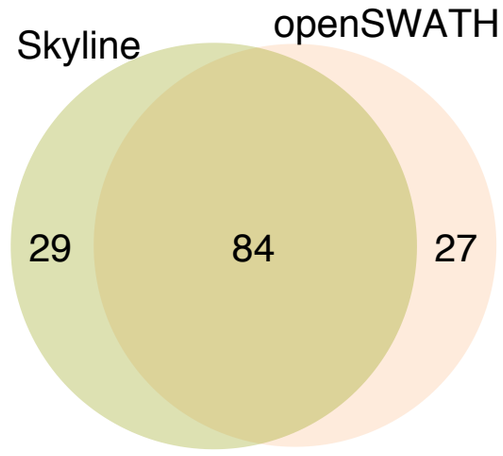
Log(sum)



Better agreement in #differentially abundant proteins between tools

DIA; Roest2014





MSSTATS IS OPEN-SOURCE, R-BASED AND PUBLICLY AVAILABLE

msstats.org

MSstats

Statistical Tool For Quantitative Mass Spectrometry-Based Proteomics

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Overview

MSstats is an open-source R package for statistical relative quantification of proteins and peptides in global, targeted and data-independent proteomics. It provides workflows for

- detecting differentially abundant proteins for MS experiments with chromatography-based quantification, with complex designs,
- characterizing MS assays in terms of limit of blank and limit of detection (LOB/LOD),
- longitudinal monitoring of quality control and system suitability testing (SST).

News

- The upcoming NEU course (Computation and Statistics for Mass Spectrometry) on May 1-12, 2017. The detailed information will follow shortly.
- MSstats v3.6 is available on Bioconductor 3.4.
- 11/20/2016 – **MSstats v3.7.1 is updated! This is the most recent development version.** Please see 'Installation' page for more details. W will continue to update the versioning of the main package several time year, to synchronize with the Bioconductor release. – Changes since th previous version

STATISTICAL METHODS FOR ASSAY CHARACTERIZATION

MSstats

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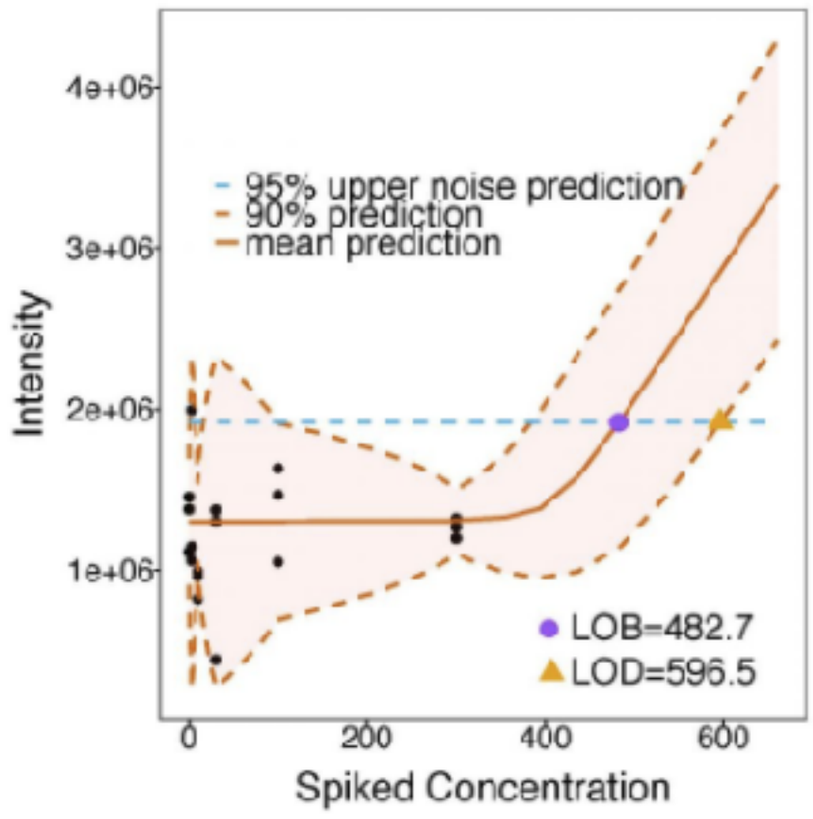
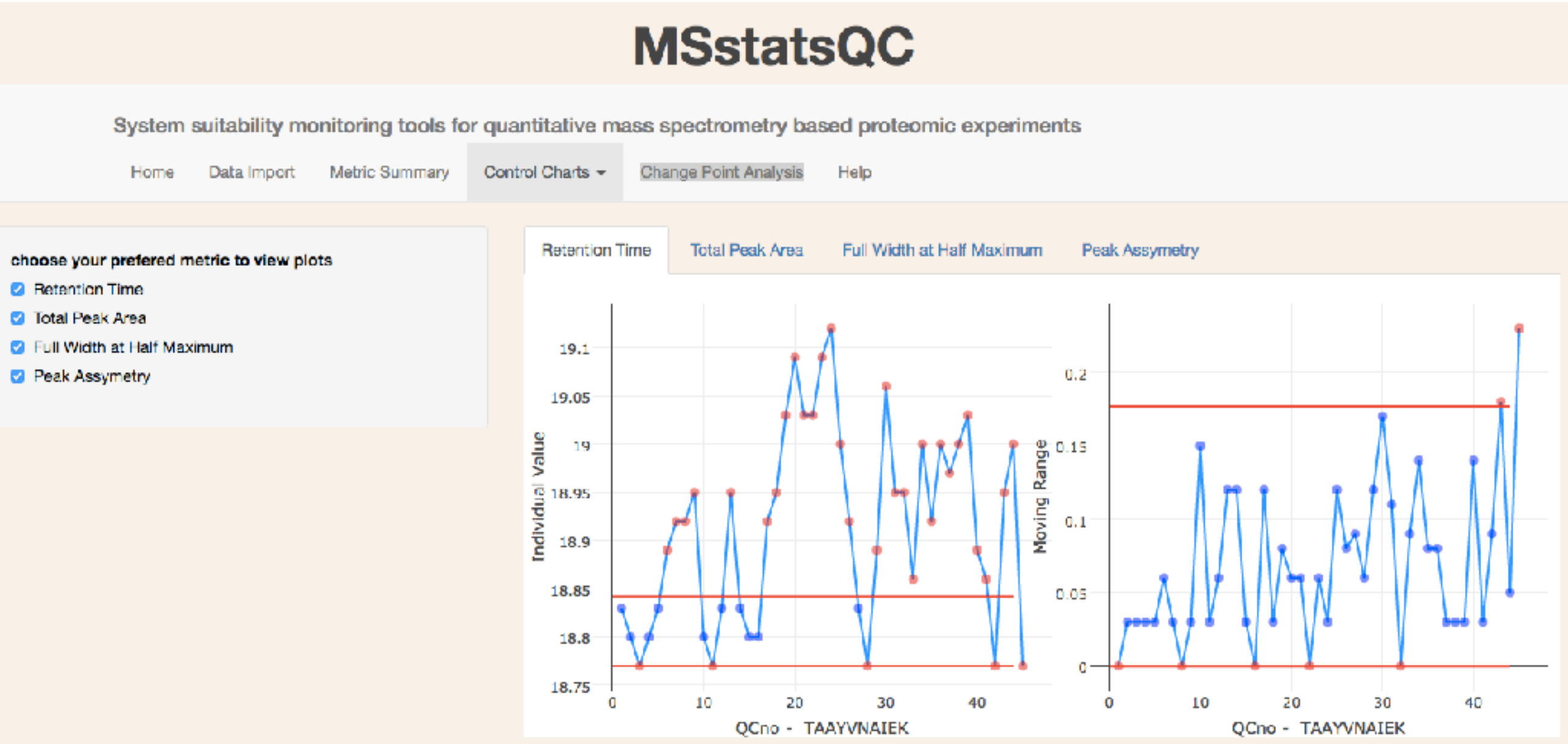
LOB/LOD ANALYSIS

Assay characterization : estimation of limit of blanc (LoB) and limit of detection (LoD)

The need for assay characterization is ubiquitous in quantitative mass spectrometry-based proteomics. Although many assay characteristics exist, the limit of blank (LOB) and limit of detection (LOD) are particularly useful figures of merit. LOB and LOD are determined by repeatedly measuring the peak intensities of peptides in samples with known peptide concentrations, and deriving an intensity versus concentration response curve. Most commonly, a weighted linear regression is fit to the intensity-concentration response, and LOB and LOD are estimated from the fit. Linear methods, however, inaccurately characterize assays containing a noise threshold at low concentrations, which is a very common situation. We propose a new approach based on non-linear regression that correctly captures the noise threshold. In absence of a noise threshold, the estimates of LOB/LOD obtained with non-linear statistical modeling are identical to those of weighted linear regression. However, in presence of a noise threshold the non-linear model changed the estimates of LOB/LOD by up to 20-40%. It improved the accuracy of the results, and avoided the unduly optimistic estimation of these figures of merit. We implemented the non-linear regression approach in the open-source R-based software MSstats, and advocate its general use for mass spectrometric protein assay characterization.



LONGITUDINAL PROFILING FOR SYSTEM SUITABILITY AND QUALITY CONTROL



ACKNOWLEDGEMENTS

Northeastern University

Kylie Bemis
Meena Choi
Eralp Dogu
Dan Guo
April Harry
Ting Huang
Cyril Galitzine
Robert Ness
Sara Taheri
Tsung-Heng Tsai

ETH Zurich

Ruedi Aebersold
Tiannan Guo
Ruth Huttenhain
Paola Picotti
Silvia Surinova
Bernd Wollscheid

University of Washington

Michael MacCoss
Brendan MacLean
Jarrett Egertson

Biognosis

Lukas Reiter

iPRG 2015

Zeynep Eren-Dogu
Chris Colangelo
John Cottrell
Michael Hopman
Eugene Kapp
Santa Kim
Henry Lam
Tom Neubert
Magnus Palmblad
Brett Phinney
Sue Weintraub,