



PulseDIA: Data-Independent Acquisition Mass Spectrometry Using Multi-Injection Pulsed Gas-Phase Fractionation

Cai Xue

Background

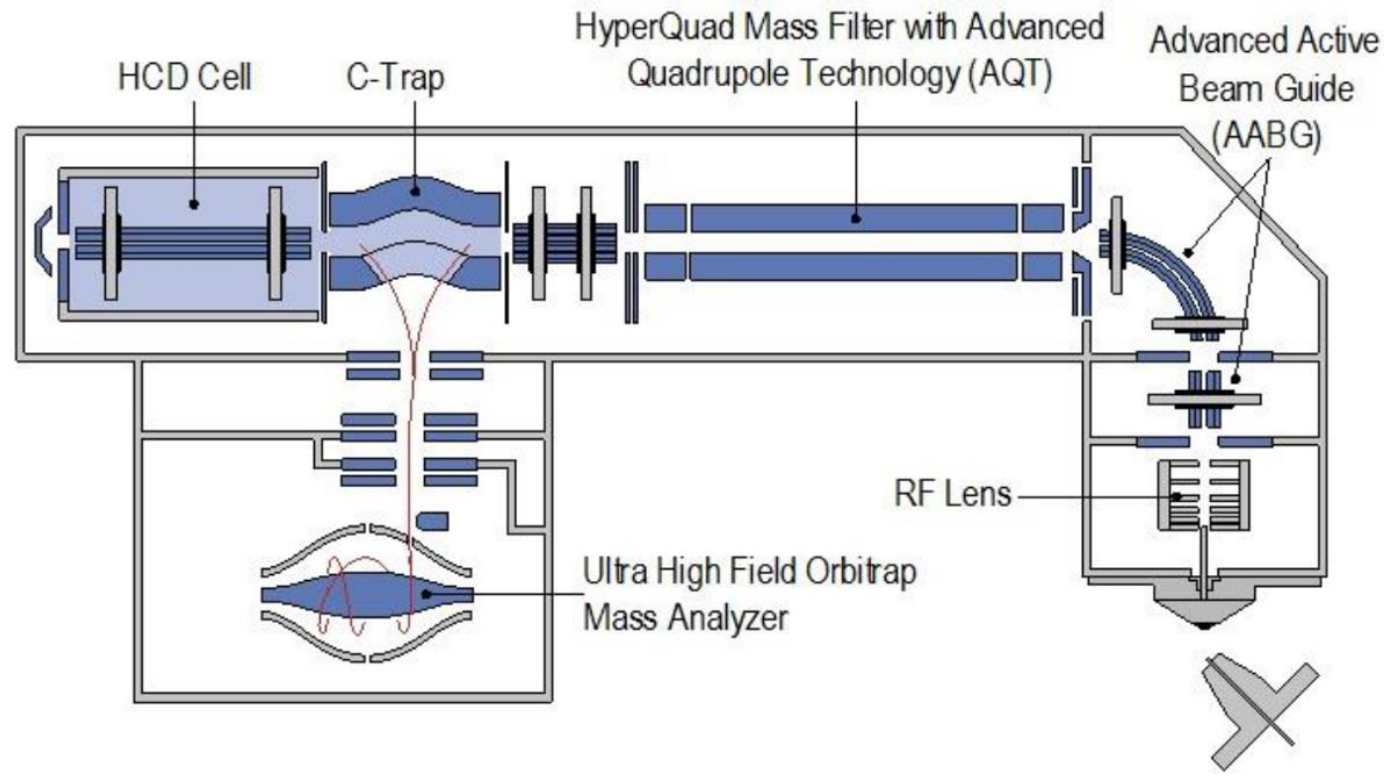
Gas Phase Fractionation



Analysis of
Accuracy, I
High throug
Consumpti
Depth of pr

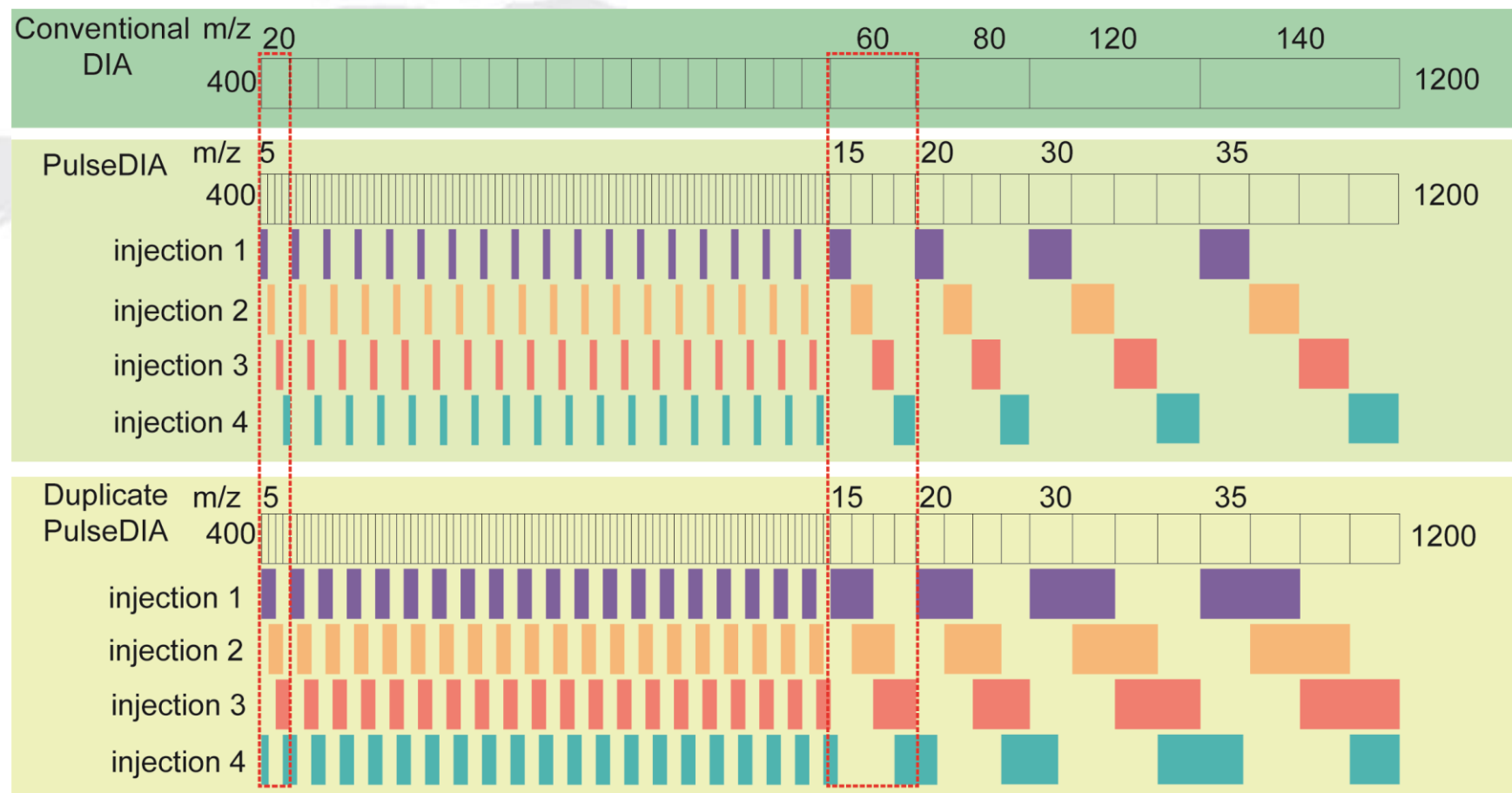
Depth opti
SCX, High
LC gradier

Gas Phase Fractionation



Low consumption of peptides,
Time-saving

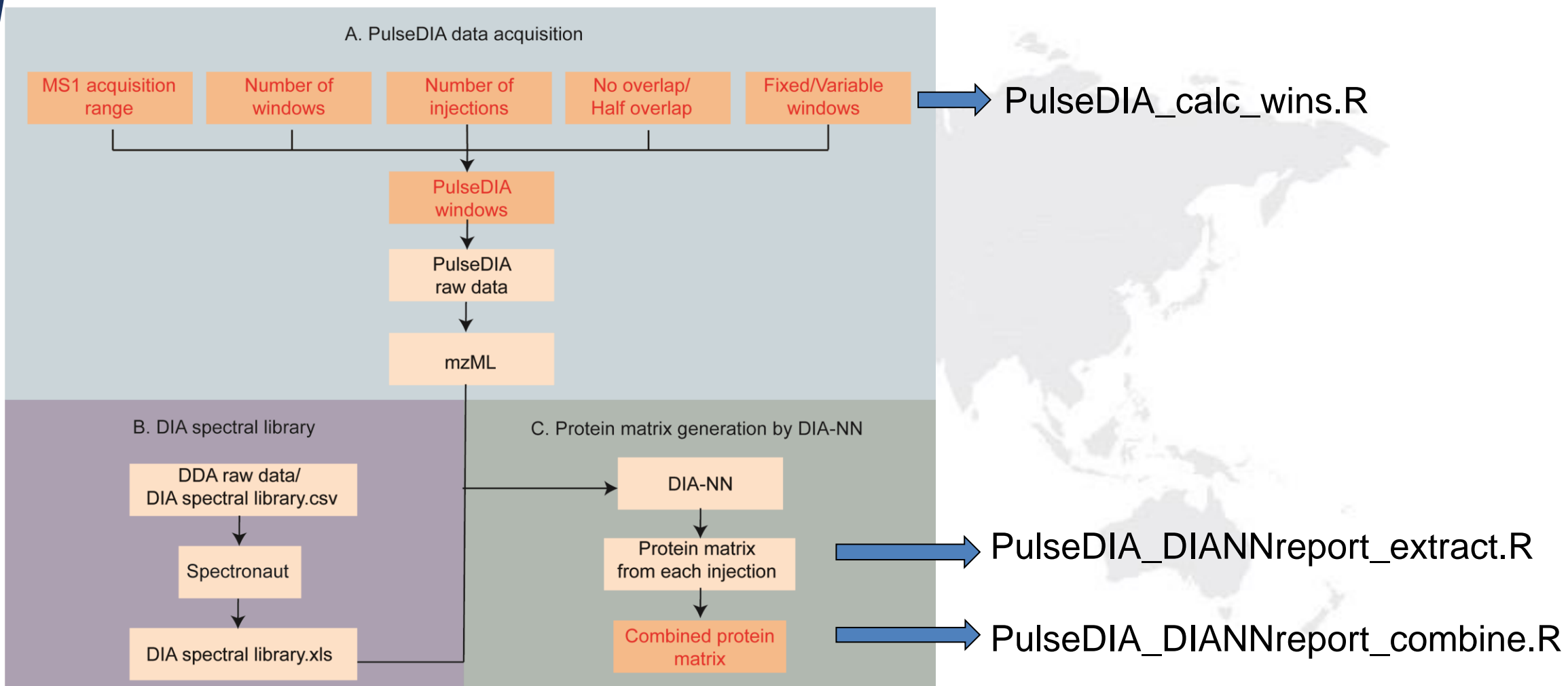
Schematic diagram of PulseDIA



Cai, X., Ge, W., Yi, X., Sun, R., Zhu, J., Lu, C., Sun, P., Zhu, T., Ruan, G., Yuan, C., et al. (2020a). PulseDIA: Data-Independent Acquisition Mass Spectrometry Using Multi-Injection Pulsed Gas-Phase Fractionation. *J Proteome Res.*

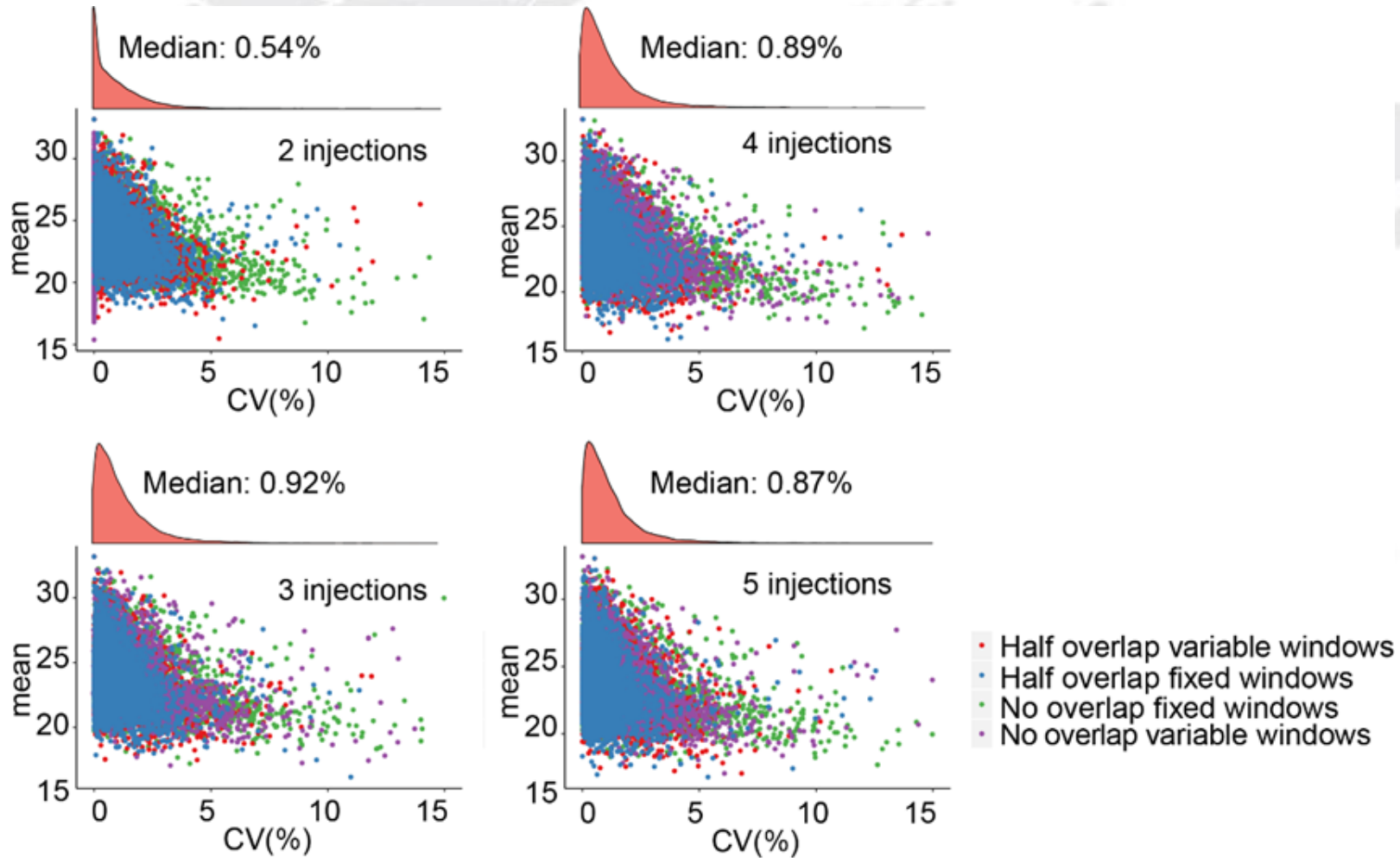
郭天南, 朱怡, 蔡雪, 葛伟刚。脉冲式数据非依赖性采集质谱检测蛋白质组的方法。发明专利, 授权公布日: 2019年11月15日, 专利号: 201910231539.0。

Workflow of PulseDIA



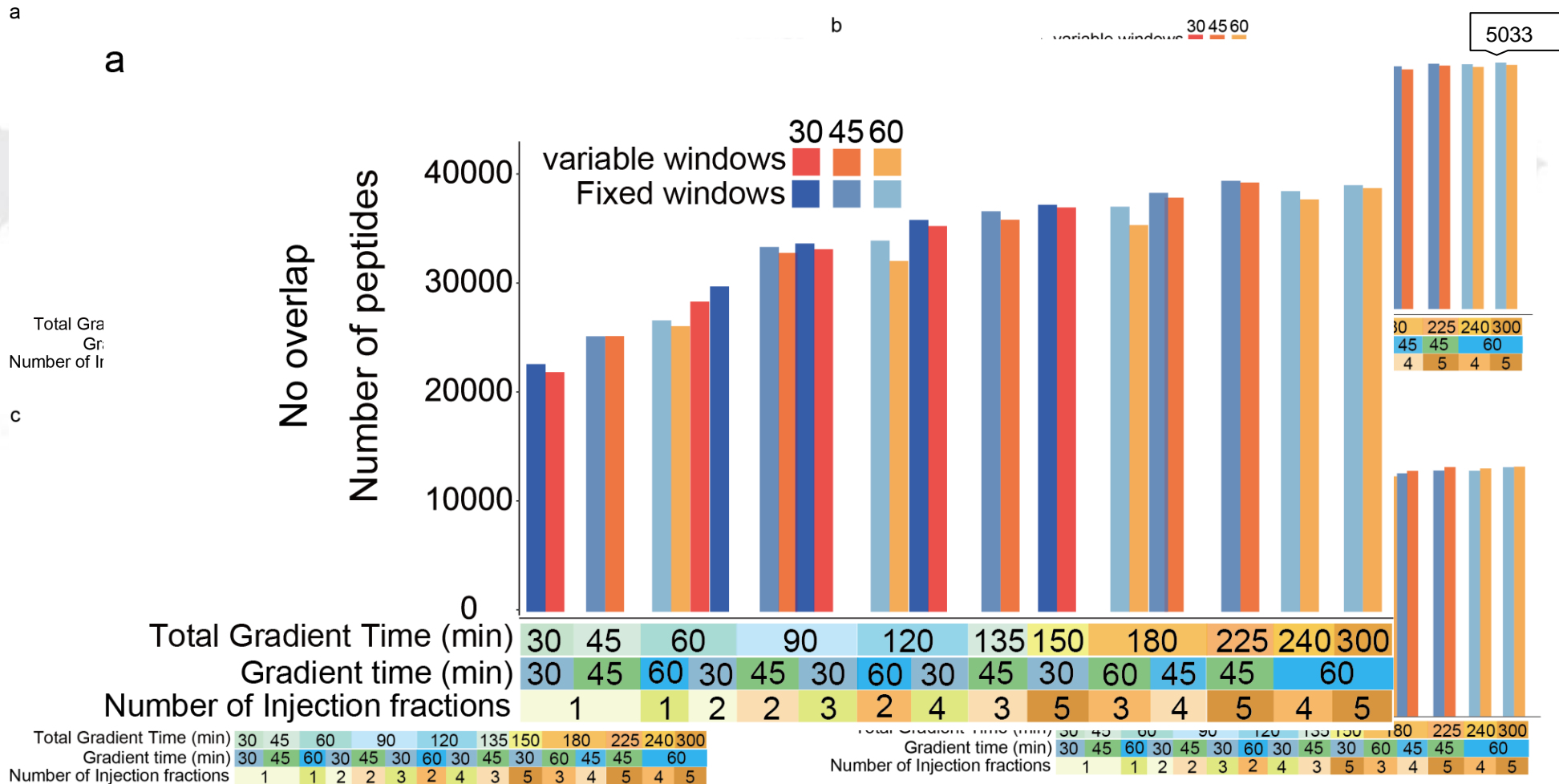
Code地址: <https://github.com/guomics/PulseDIA>

Technical repeatability of PulseDIA



Optimization of PulseDIA

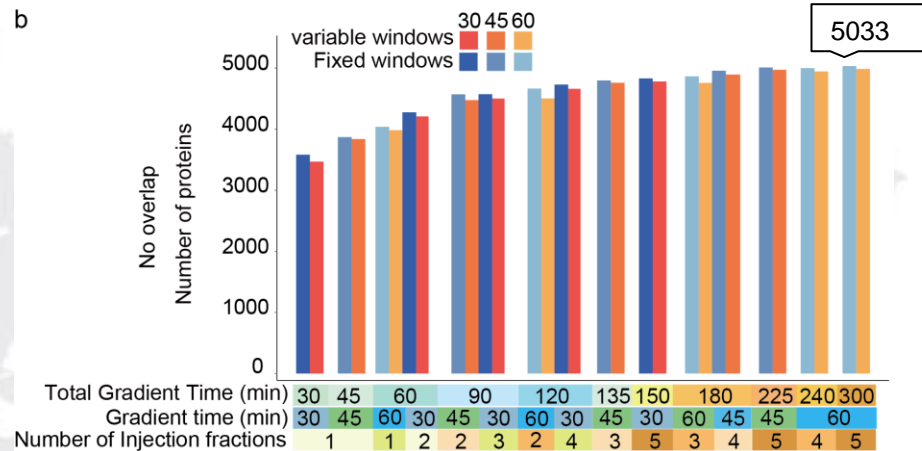
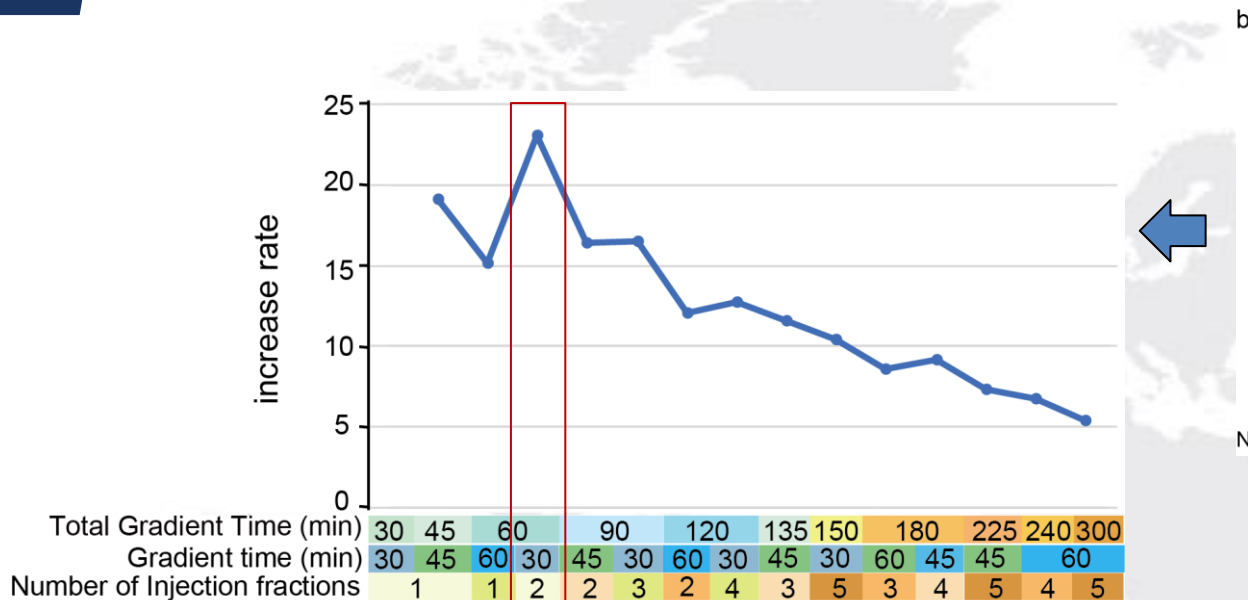
Breast cell line samples DIA library: 58,015 peptides
6,331 proteins



Optimization of PulseDIA using breast cell line samples. Four parameters were tested and optimized to maximize the performance of PulseDIA: i) number of injection fractions (n=1, 2, 3, 4, 5); ii) length of LC gradient (30, 45, 60 min); iii) fixed or variable window; iv) PulseDIA (a, b) or duplicate PulseDIA (c, d).

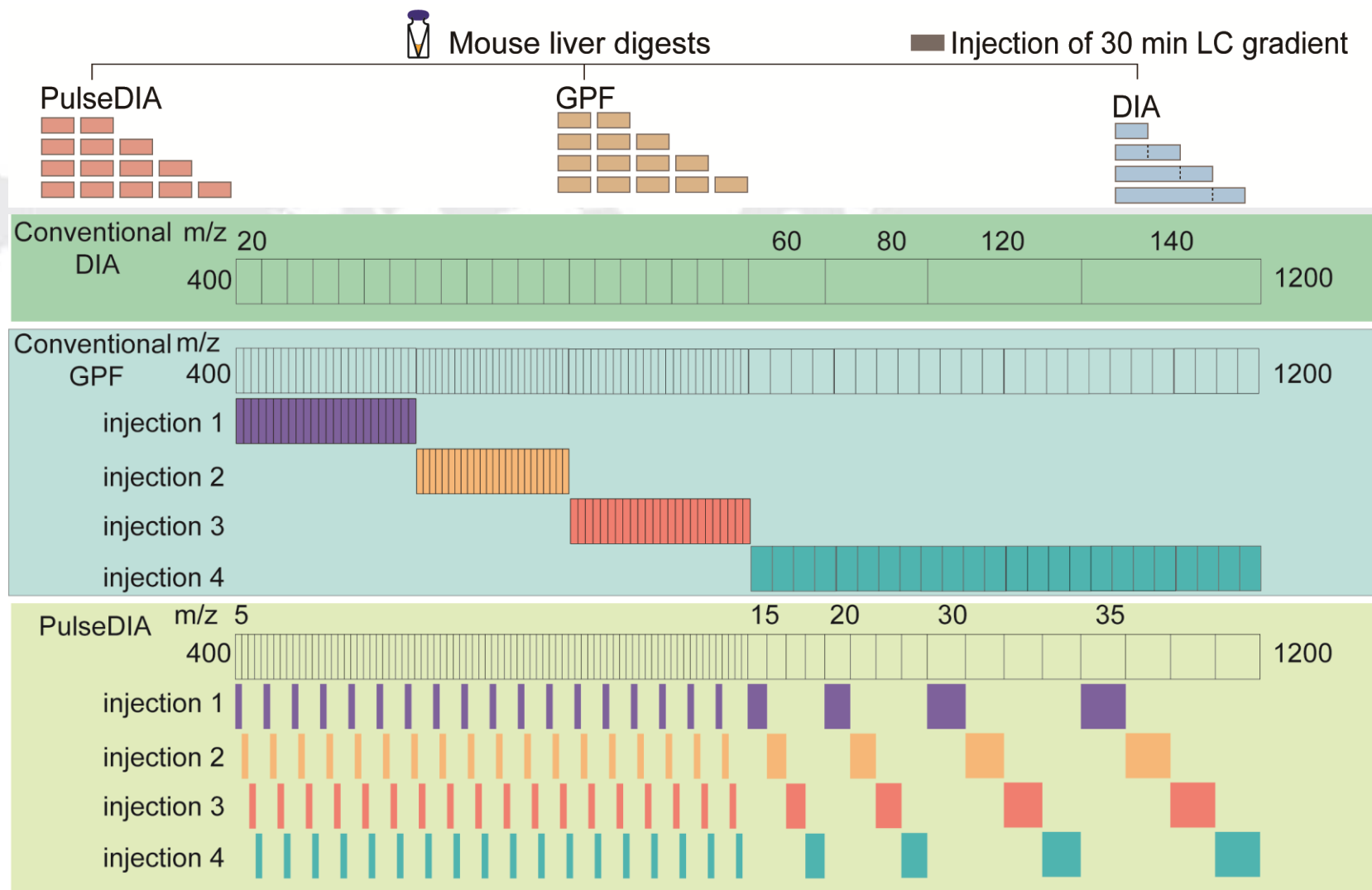
Optimization of PulseDIA

Breast cell line samples DIA library: 58,015 peptides
6,331 proteins



Under the condition of fixed windows without overlap, the number of increased proteins identified per increased time unit (min) compare to the conventional DIA of 30 minutes LC gradient in breast cancer line sample.

Performance of PulseDIA

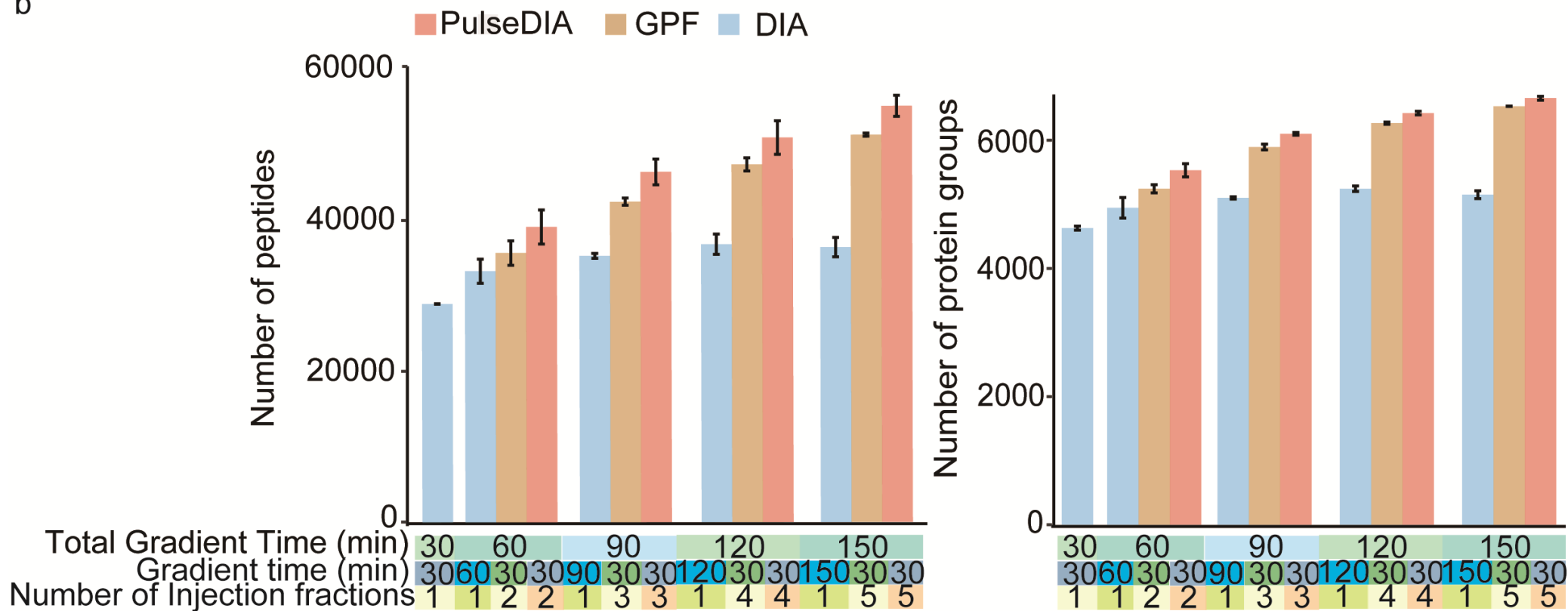


The experiment design for PulseDIA, GPF and DIA using mouse liver digests.

Performance of PulseDIA

Mouse liver DIA library: 196,728 peptides
12,298 proteins

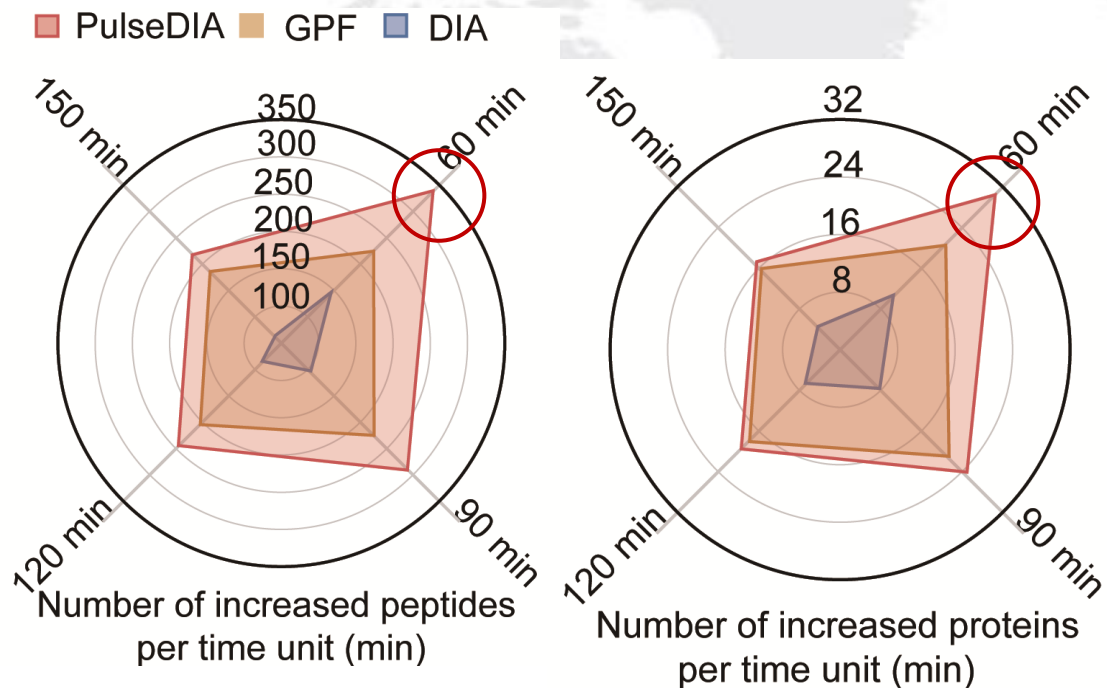
b



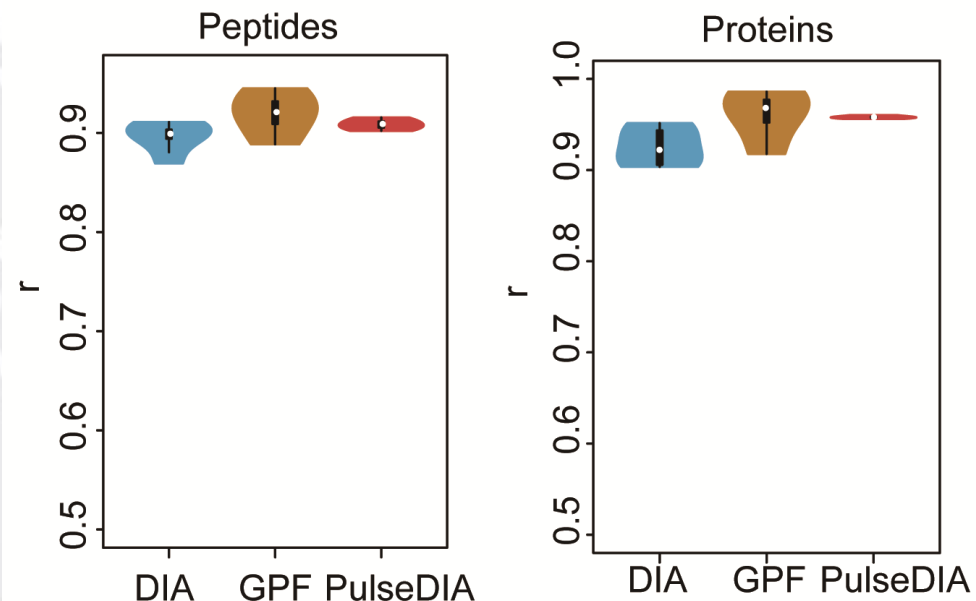
Number of identified peptides and protein groups using PulseDIA, GPF and DIA with different numbers of injections and different lengths of LC gradient.

Performance of PulseDIA

Mouse liver DIA library: 196,728 peptides
12,298 proteins

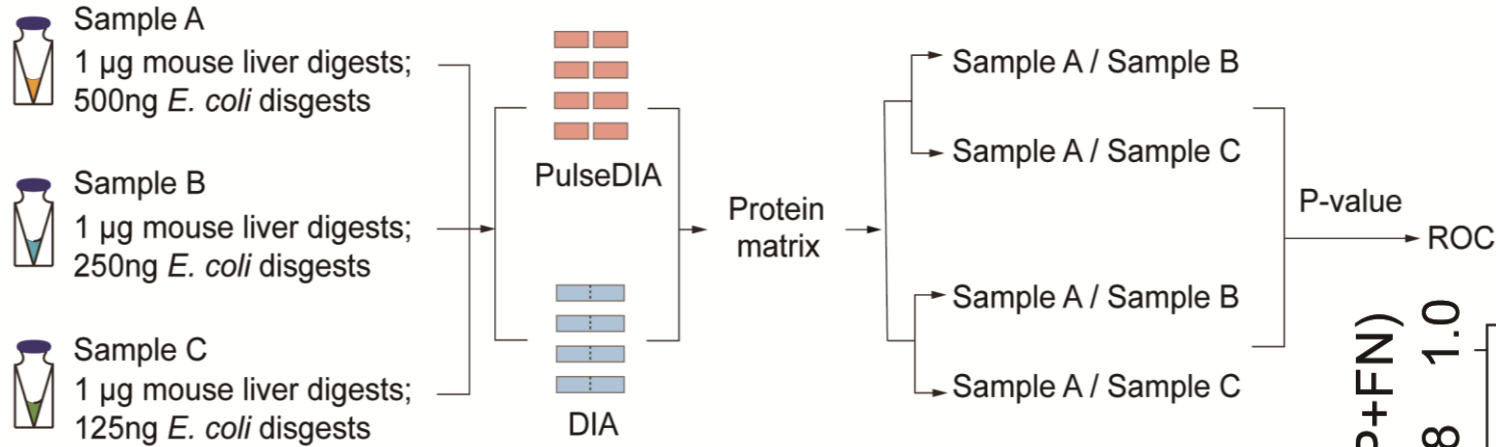


The number of increased peptides and protein groups identified per time unit (min) compared to the conventional DIA of 30 min LC gradient.

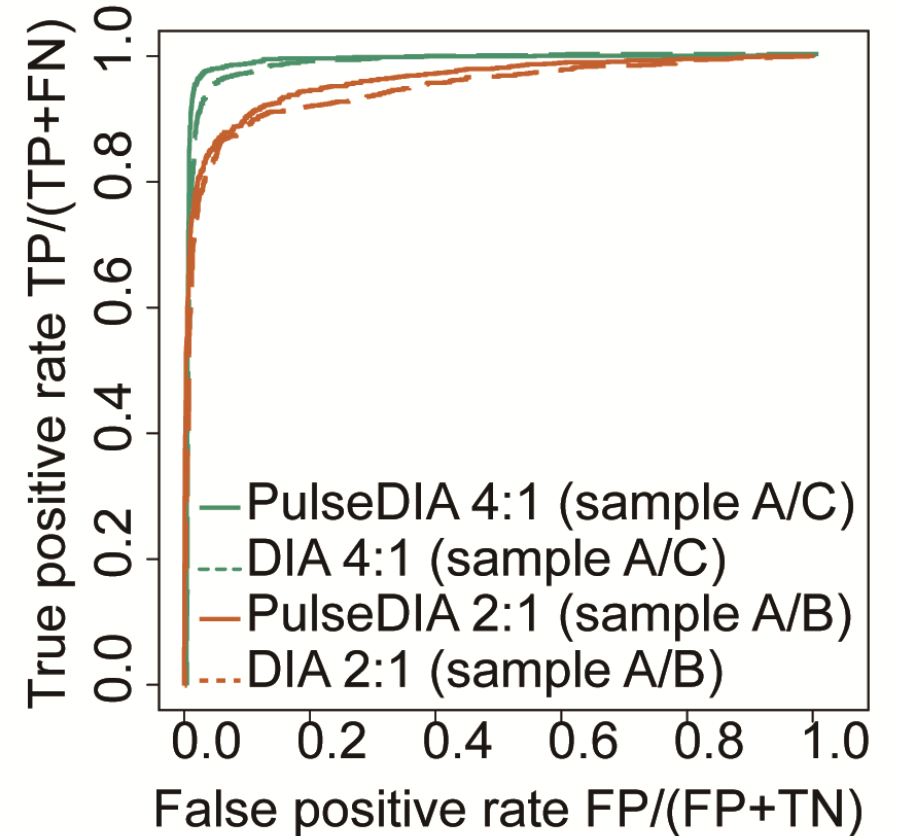
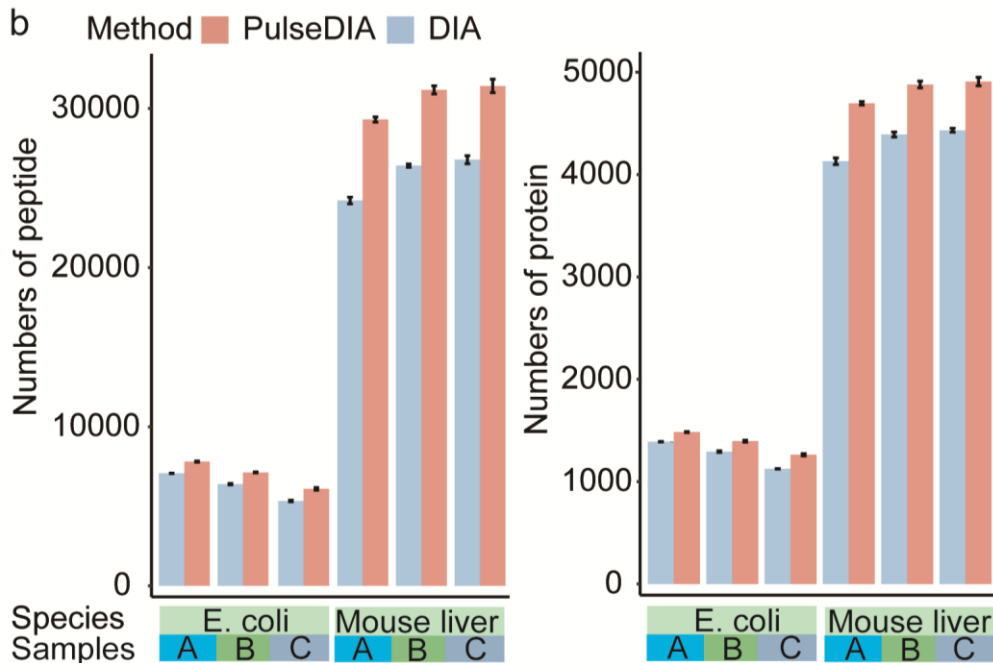


Technical reproducibility of PulseDIA, GPF and DIA using mouse liver digests.

Quantitative accuracy evaluation of PulseDIA



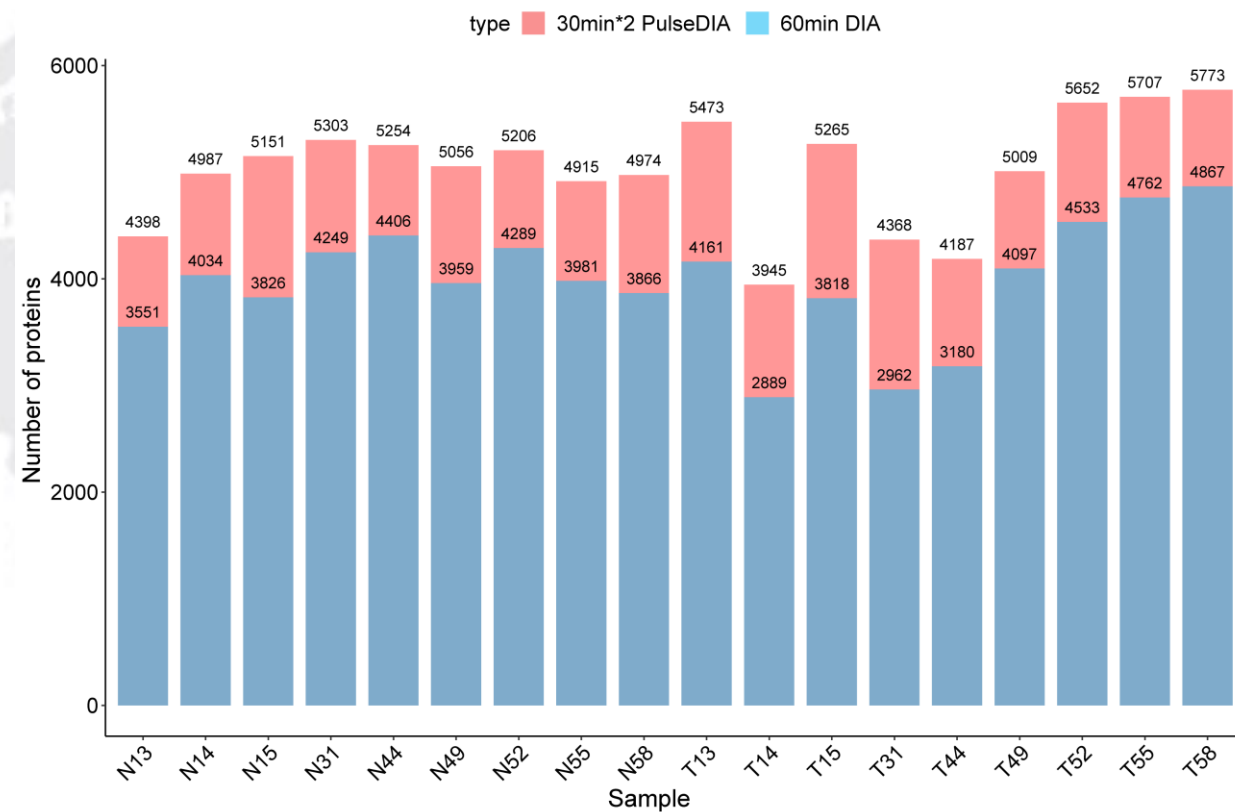
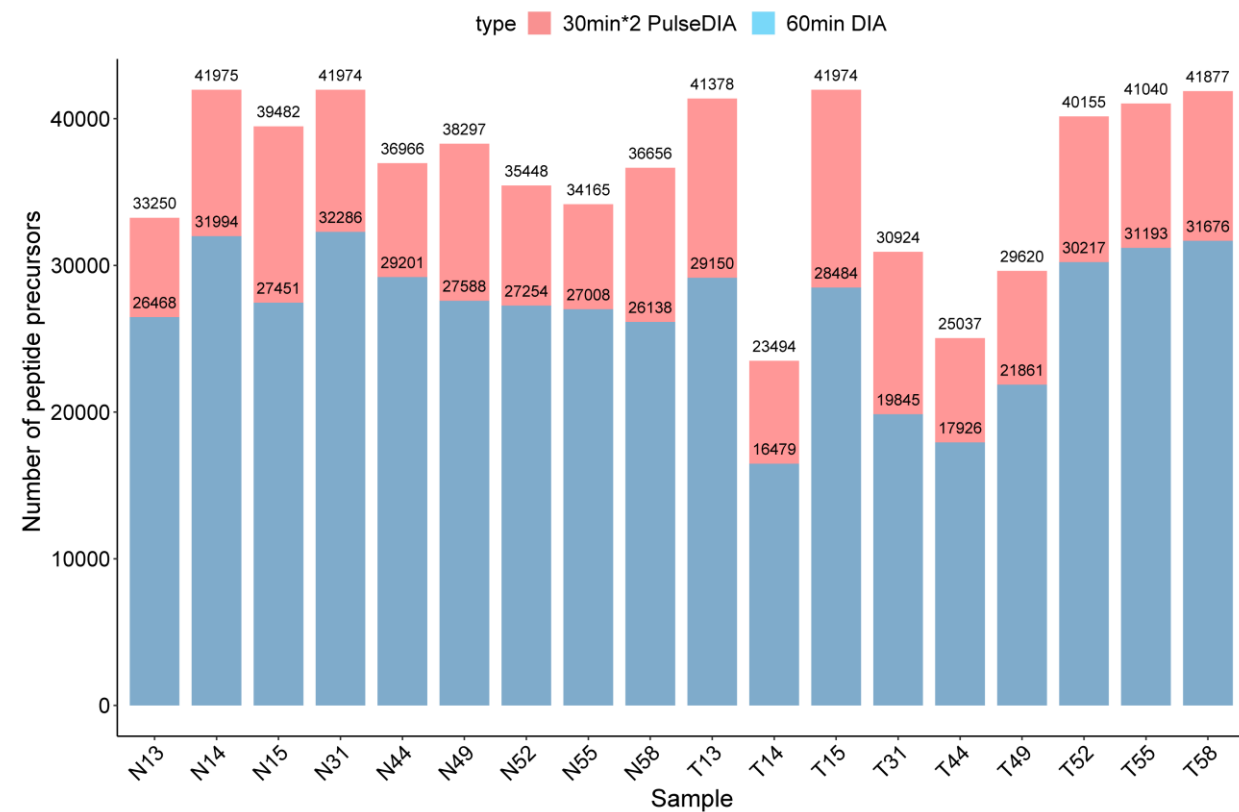
Mix DIA library: 92,303 mouse liver peptides
 7696 mouse liver proteins
 15,854 *E. coli* peptides
 1957 *E. coli* proteins



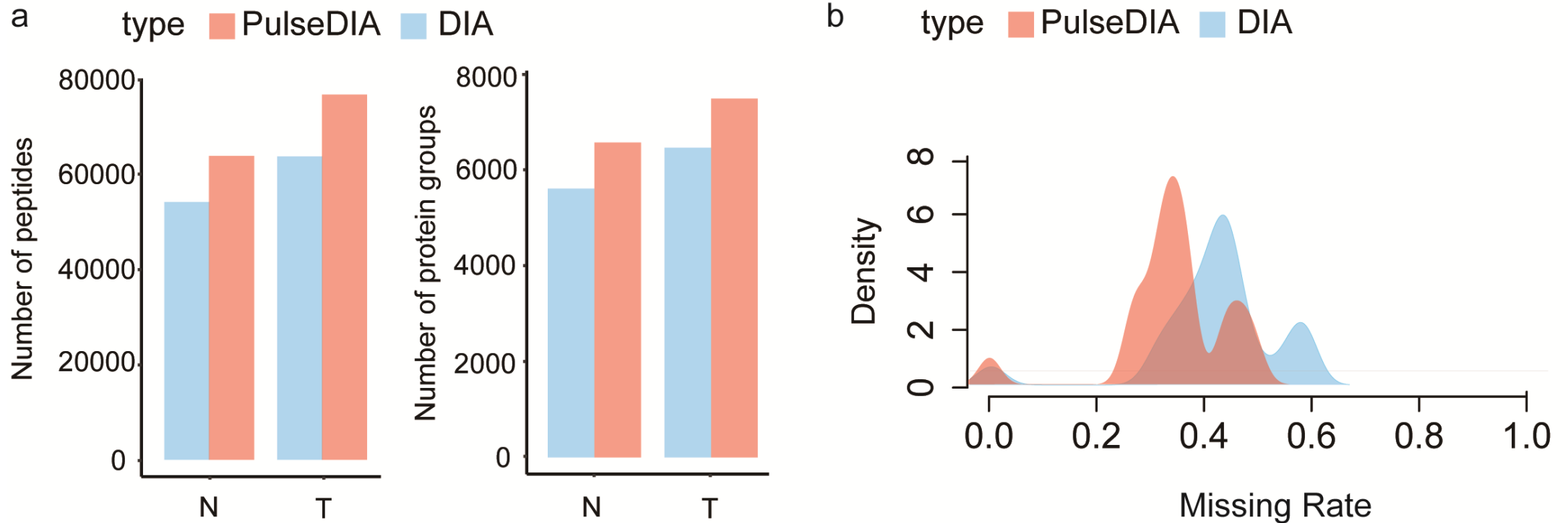
Application of PulseDIA to CCA

DIA Pan Human library: 396,245 peptides
14,786 proteins

Application of PulseDIA to 18 tissue samples from 9 CCA patients. N, benign; T, tumor.



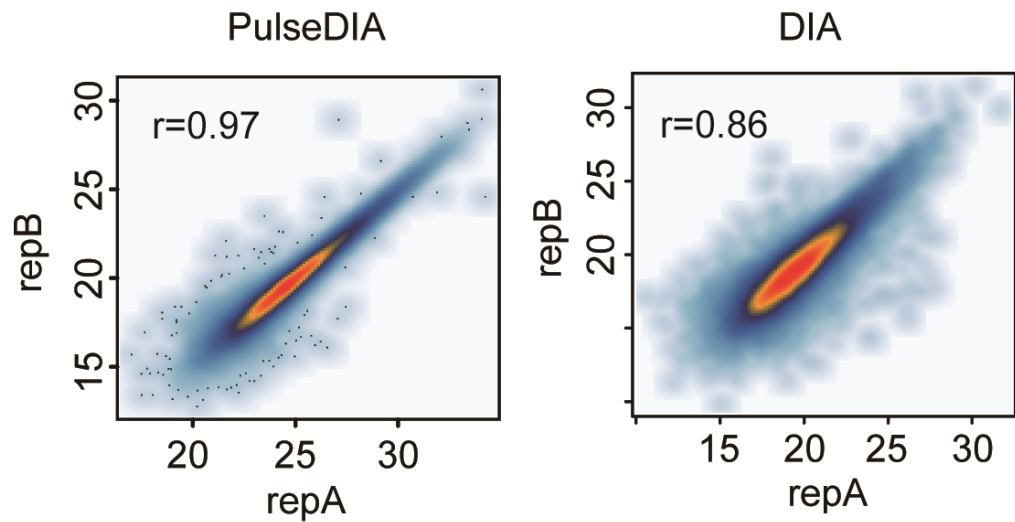
Application of PulseDIA to CCA



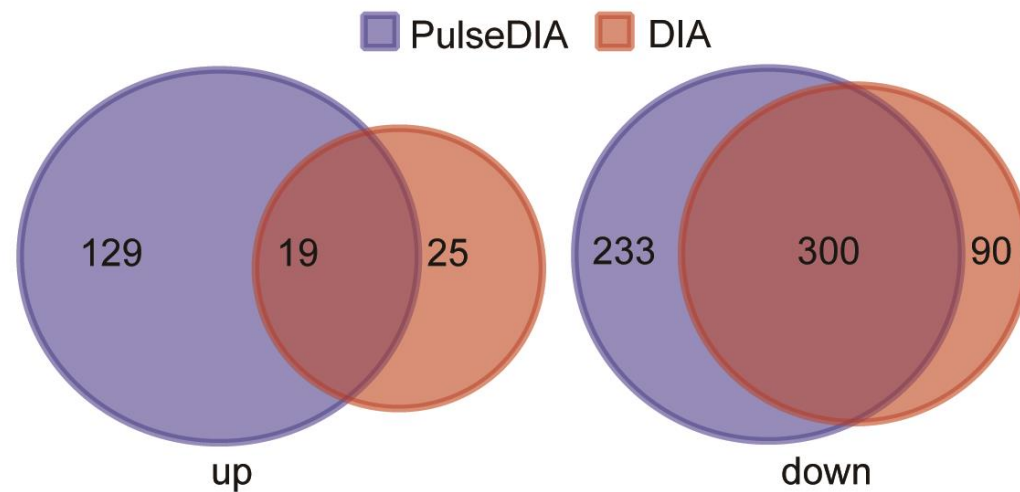
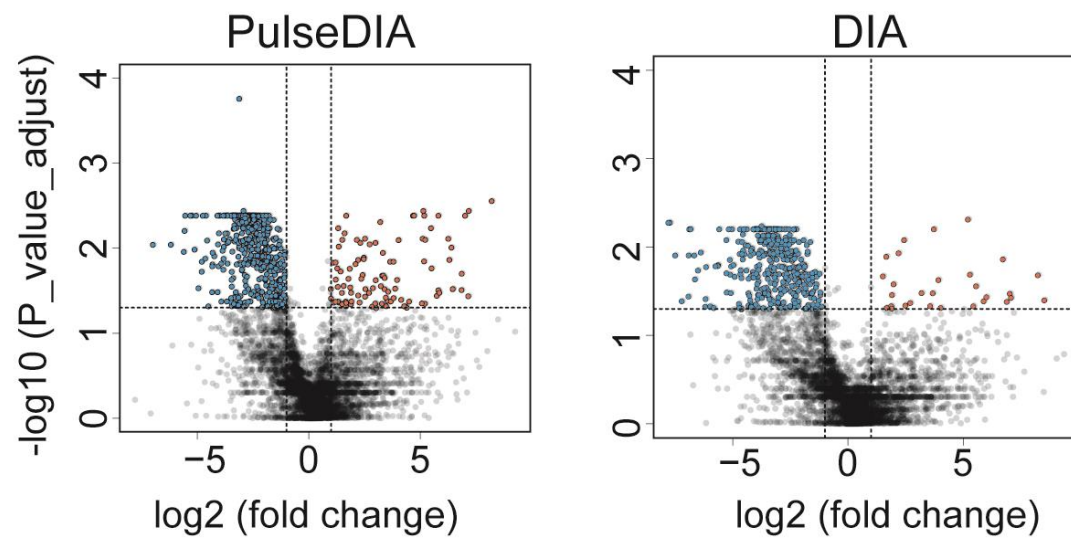
(a) The total number of identified peptides and protein groups for all non-tumorous tissues and tumorous tissues by PulseDIA and DIA.

(b) The missing rate distribution of quantified protein groups by two methods. The distribution of protein missing rate for the tissue samples is shown as a density curve.

Application of PulseDIA to CCA



Pearson correlation between technical replicates



Volcano plots of regulated proteins for PulseDIA and DIA data sets.



THANK YOU

西湖大學
WESTLAKE UNIVERSITY