

# P162: PASEF-PRM LIVE on the timsTOF Pro

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# Overview

## ❑ **Overall objective**

- *Develop PASEF-PRM LIVE, a framework that implements on-the-fly adjustment of the detection window to maximize target peptide coverage while responding to changes in target elution times.*

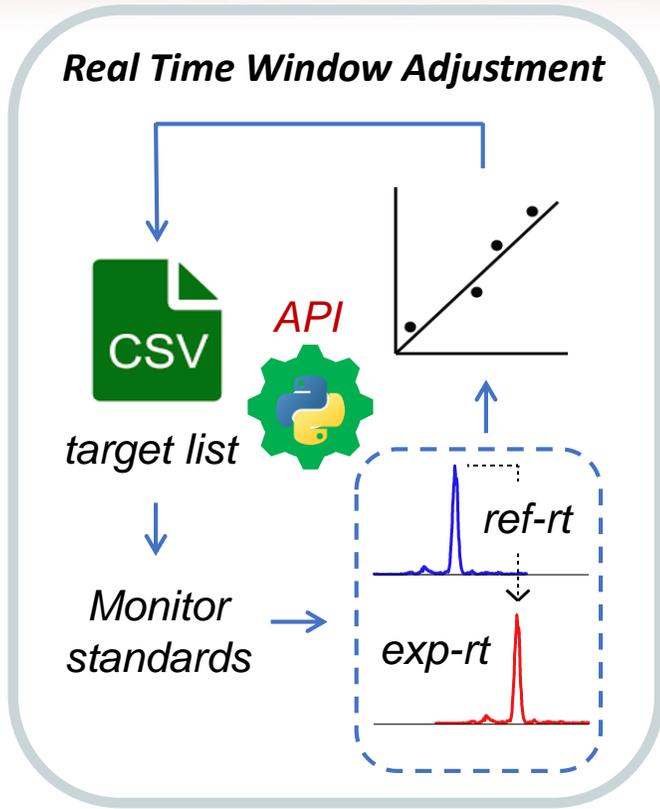
## ❑ **General development workflow**

- *Initial development based on iRT spiked CSF biofluids*
- *Evaluate impact of real time detection window adjustment algorithms*
- *Evaluate reproducibility*
- *Explore current limits for total # peptides in 1-hr gradient*

## ❑ **Application of PRM-LIVE in drug discovery**

- *Activity-based protein profiling (ABPP) for rapid selectivity analysis of inhibitors targeting deubiquitinating enzymes*

# Real-time RT correction algorithm



## Creating target files:

- PASEF run for CSF peptides spiked with iRTs
- Target peptides: m/z; charge; RT; 1/k0 range
- iRT peptides: m/z; RT; top6 frag ions with relative intensity

## PRM LIVE:

- 112 target peptides @ 45min PASEF
- 30, 45, 60min gradients

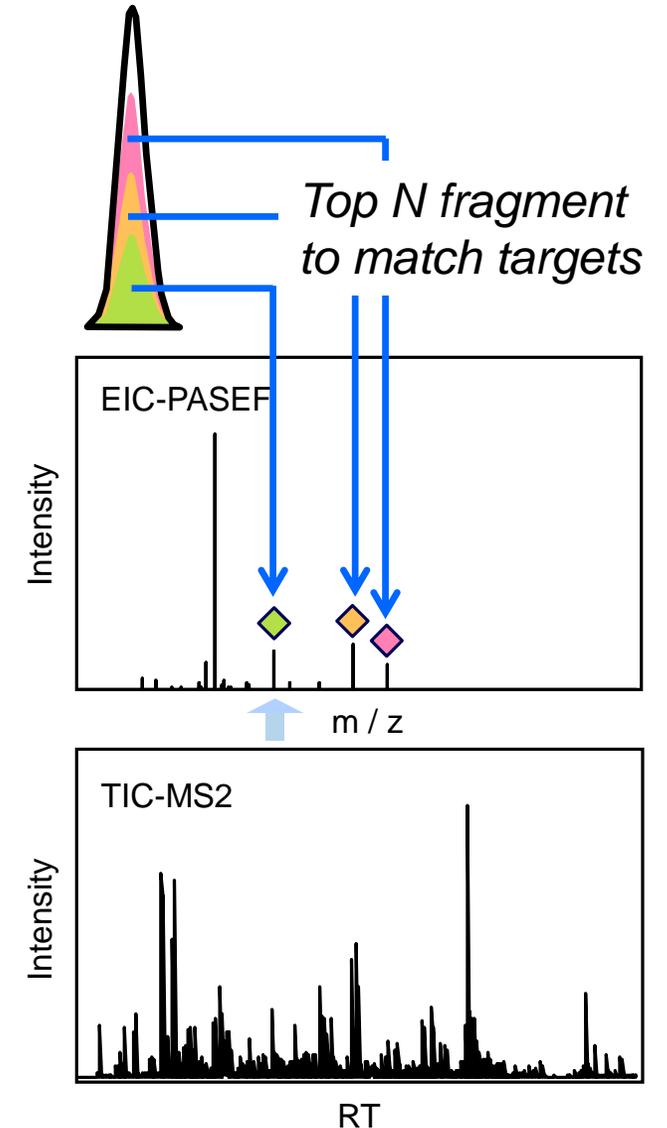
## Number of quantified peptides

gradient	#peps quantified
30min	104
45min	100
60min	103

## Algorithm:

- Time warping: **TW** (when  $\leq 2$  std peps detected)
- Time warping linear regression: **TWLR** (when  $\geq 3$  std peps detected)

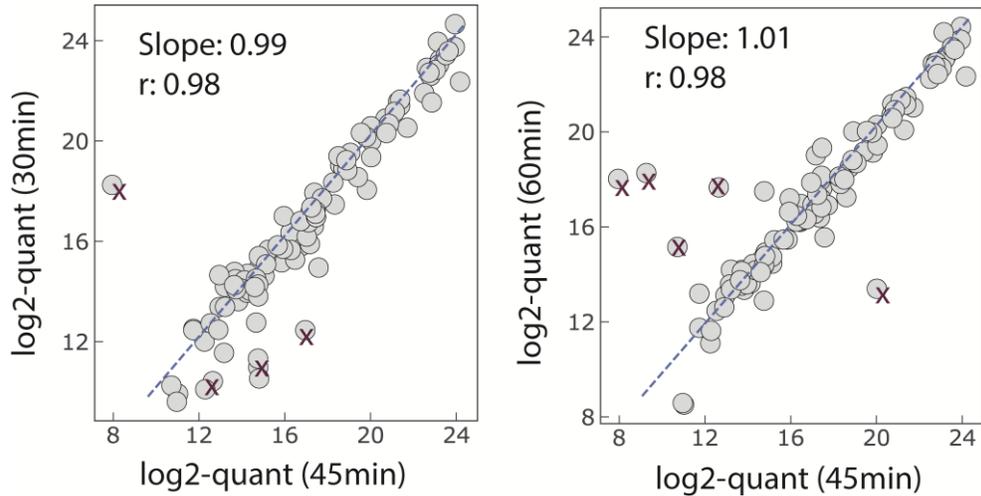
## Quantification



# Real-time RT correction algorithm

## Quant Correlation

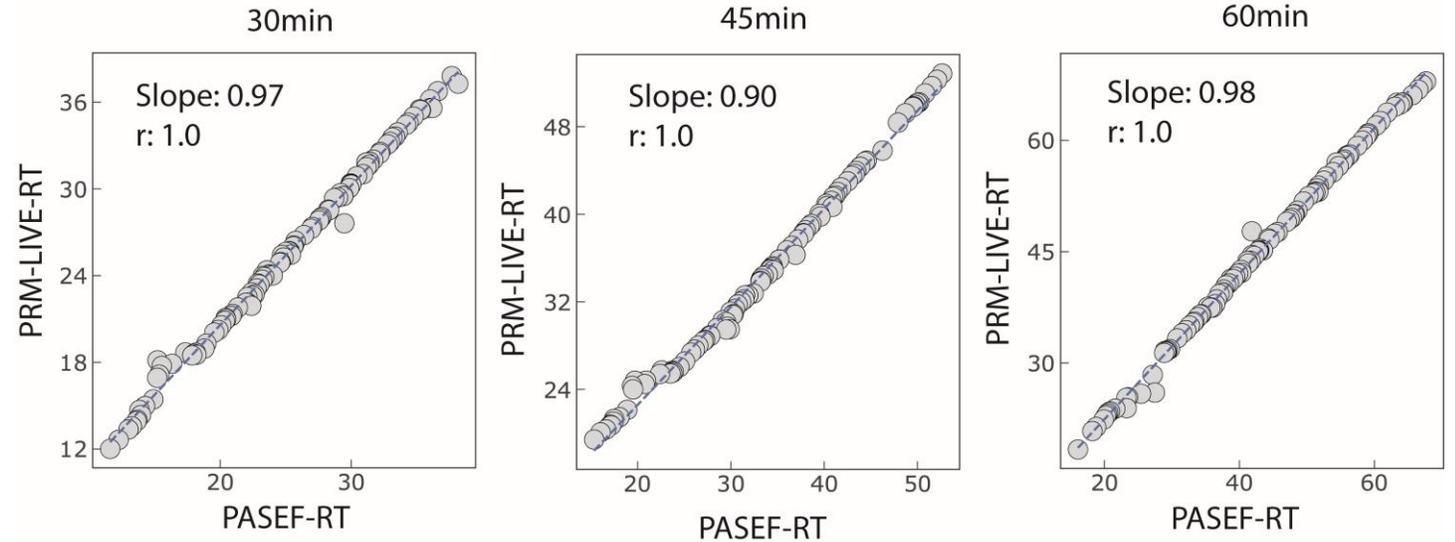
Gradients: 30min, 45min and 60 min



- Good quant correlation of targeted peptides between 30min, 45min and 60min gradients.

## RT Correlation

Accuracy of algorithm TW/TWLR: RT-PRM-LIVE vs. RT-PASEF



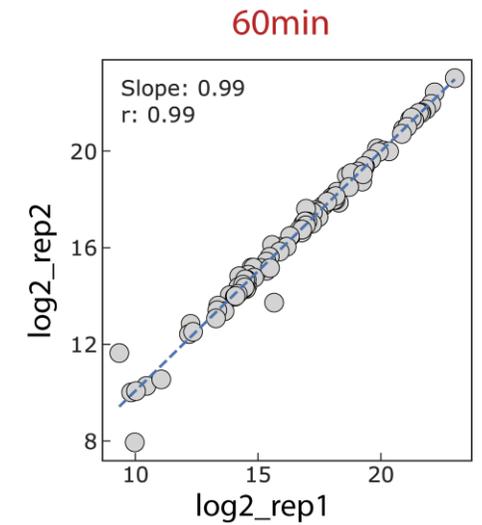
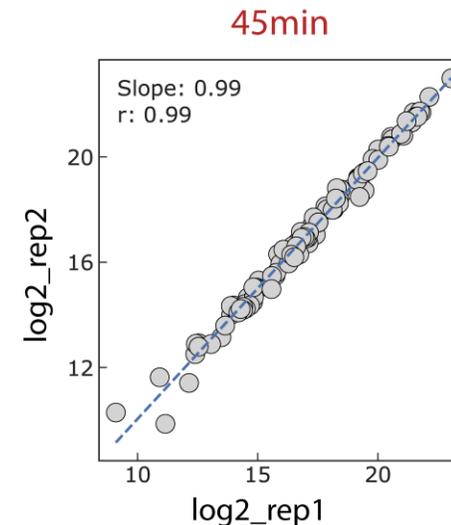
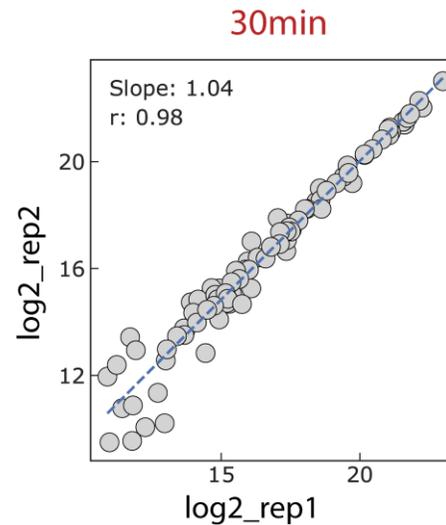
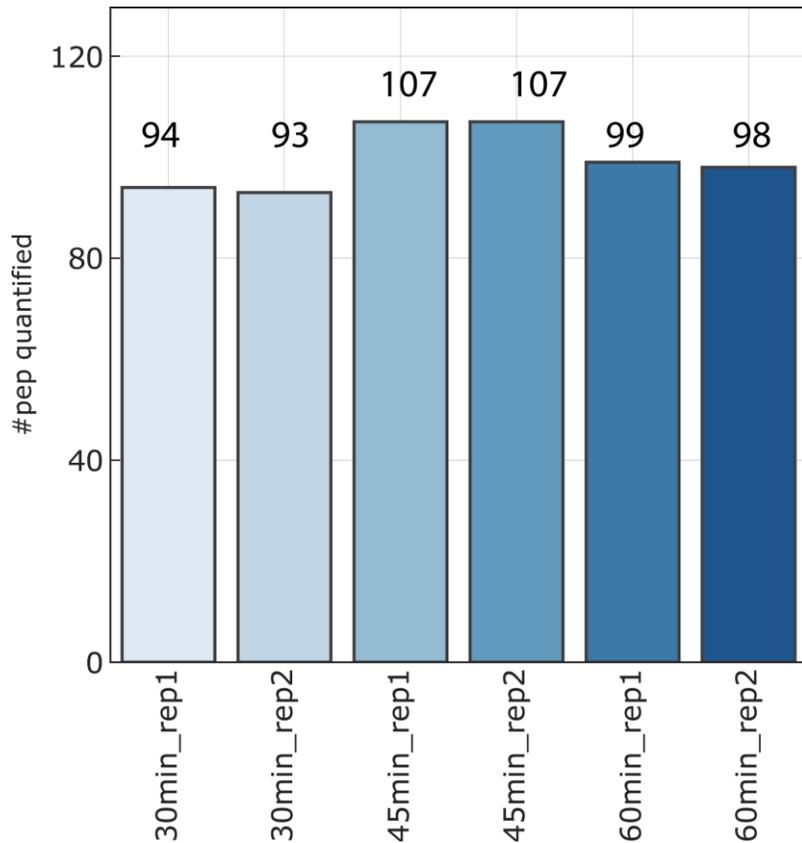
- RT of targets in PRM-LIVE has a good alignment with RT in the corresponding PASEF runs, indicating that the current real-time RT correction algorithm can accurately predict RT of targeted peptides.

Note: X annotates outliers suffering from peptides stability issue and variability of targeting window (under investigation).

# Reproducibility

## Quant correlation: replicates with different gradients

- 108 target CSF peptides @ 45 min PASEF
- 30, 45, 60min grads with 2 replicates (every 5.5h/replicate)

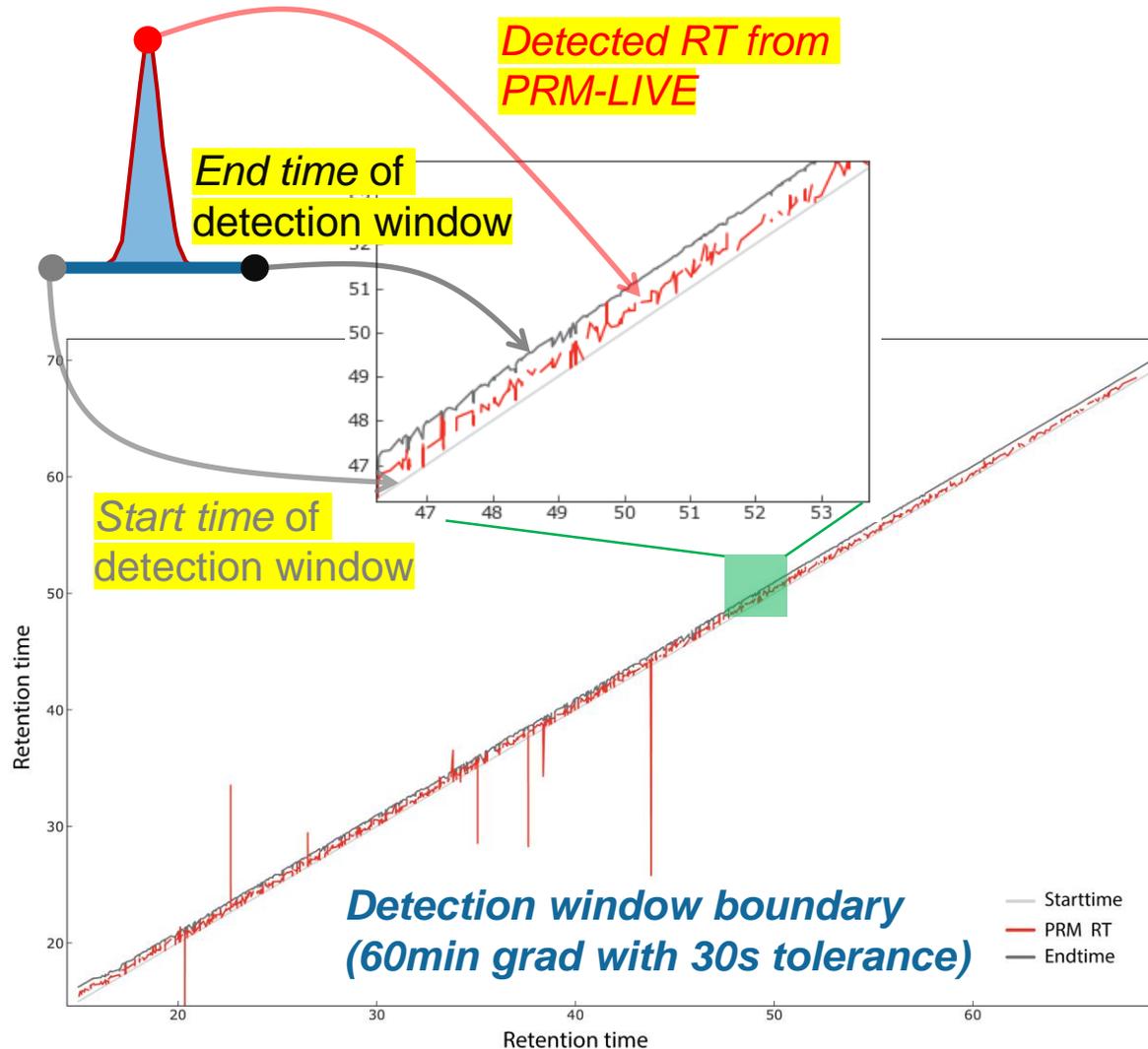


- *PRM-LIVE with 45min gradient has a largest number of quantified peptides.*
- *All the quantified peptides across different gradients shows good quant correlation.*

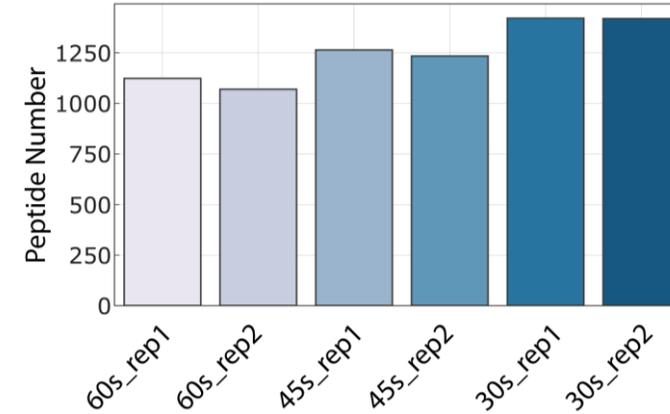
# Current maximum targeted peptide# from CSF biofluids

Targets: 1731 CSF peps @ 60 min PASEF

- RT variance  $\leq$  1min from 3 replicates

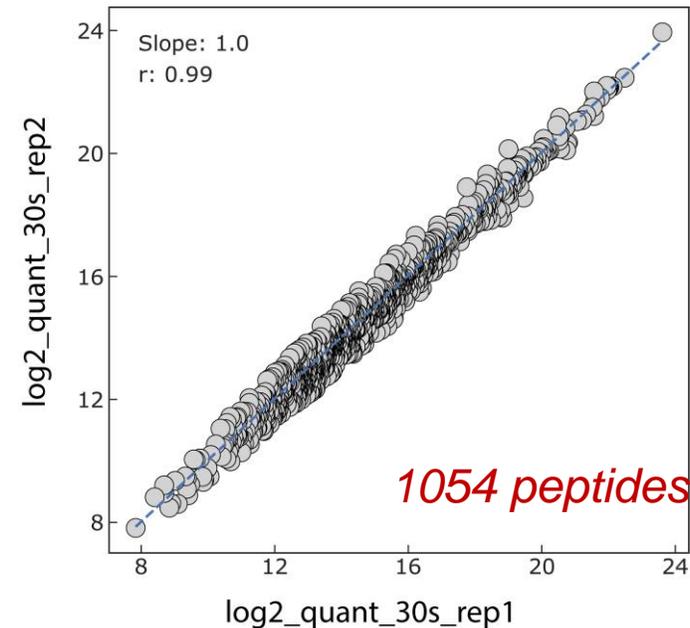


## Quantified peptides # under different RT tolerances



With 30s RT tolerance, around 1400 peptides were quantified.

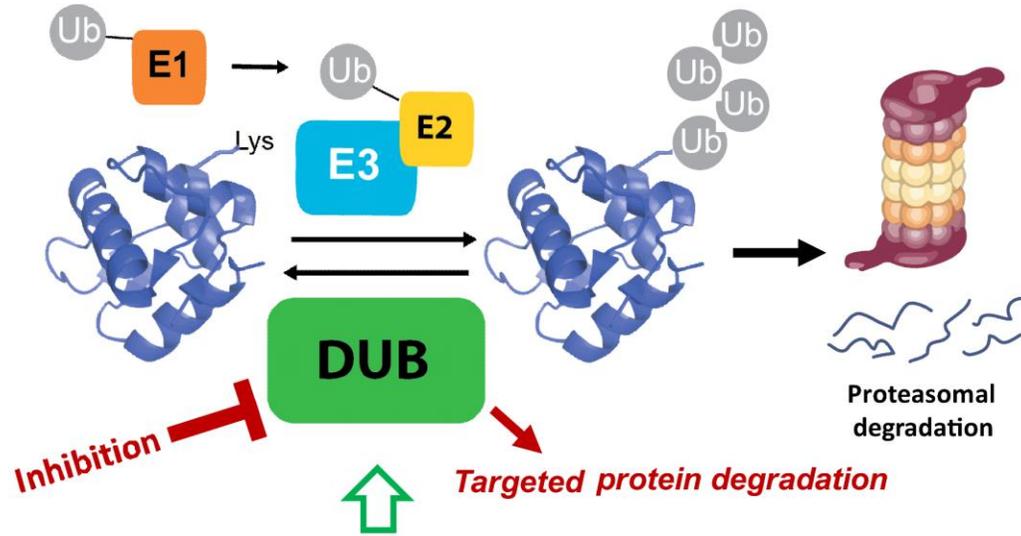
## Quant Correlation



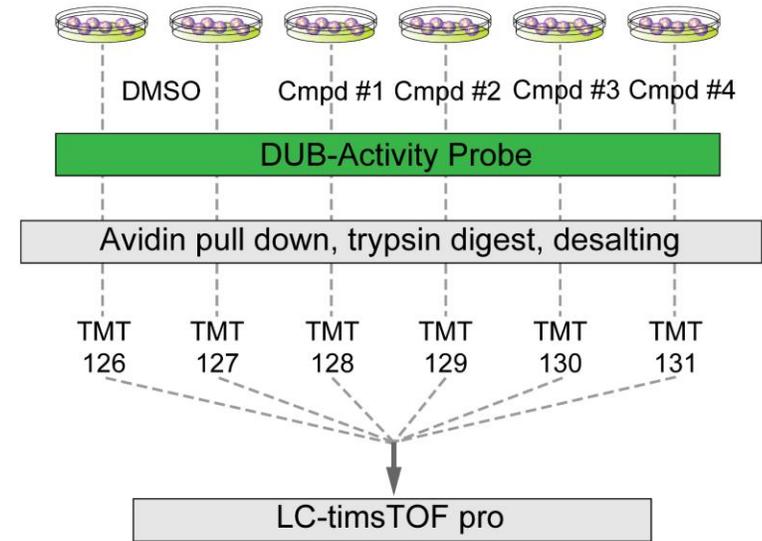
1352 quantified peptides were overlapped between two replicates, among which 1054 peptides ( $cv \leq 0.05$ ) showed good quant correlation.

# Targeted DUBome analysis by high throughput Zip-Chip-timsTOF

## Targeted protein degradation by inhibiting deubiquitination (DUB)



## Specific DUB inhibitor screening by multiplexed ABPP



## High throughput analysis

57 DUBs @ 2h on LC-timsTOF

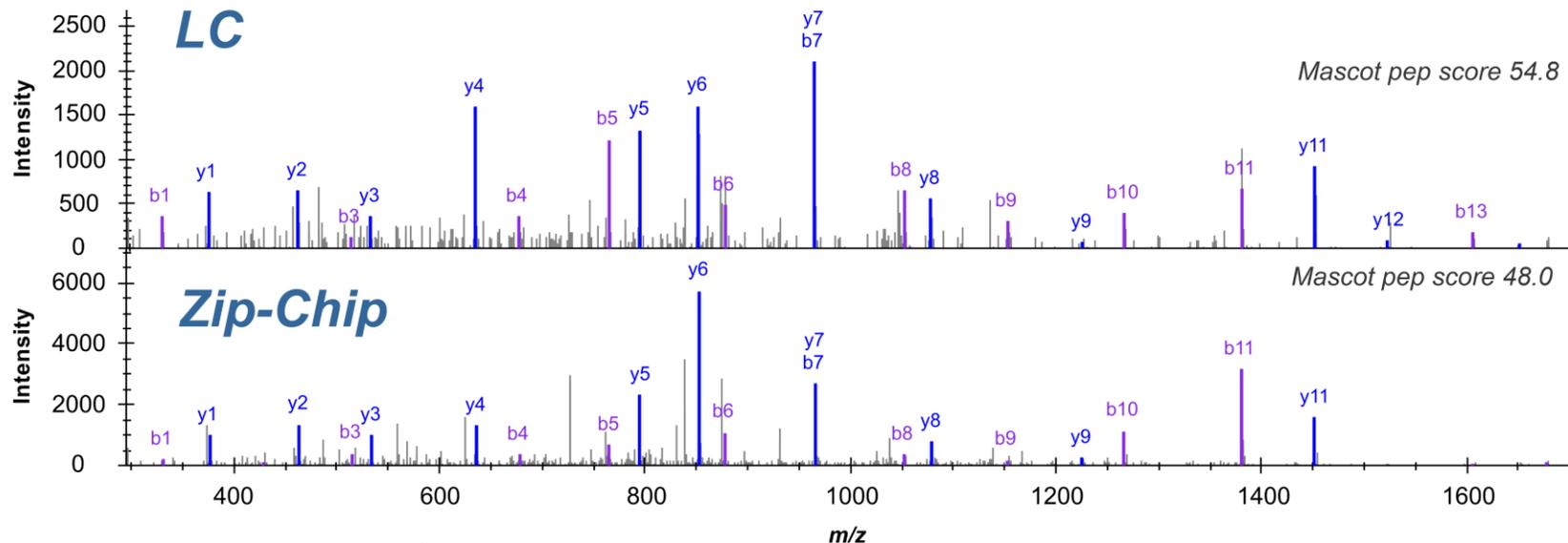
? #DUBs @ 15 min on Zip-Chip-timsTOF

Human DUBome: ~ 100 enzymes

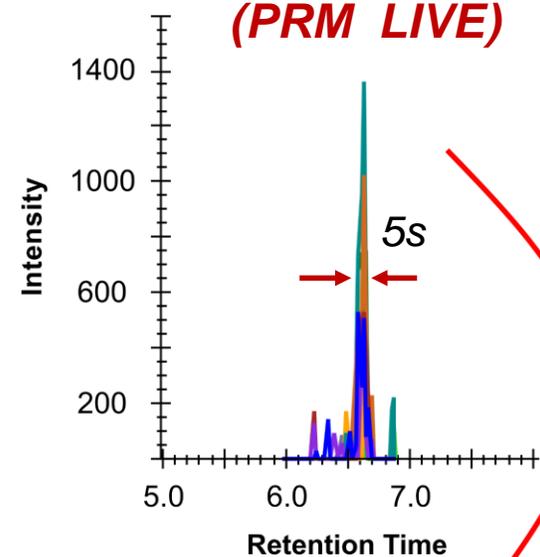
# Targeted DUBome analysis by high throughput Zip-Chip-timsTOF

**PASEF**

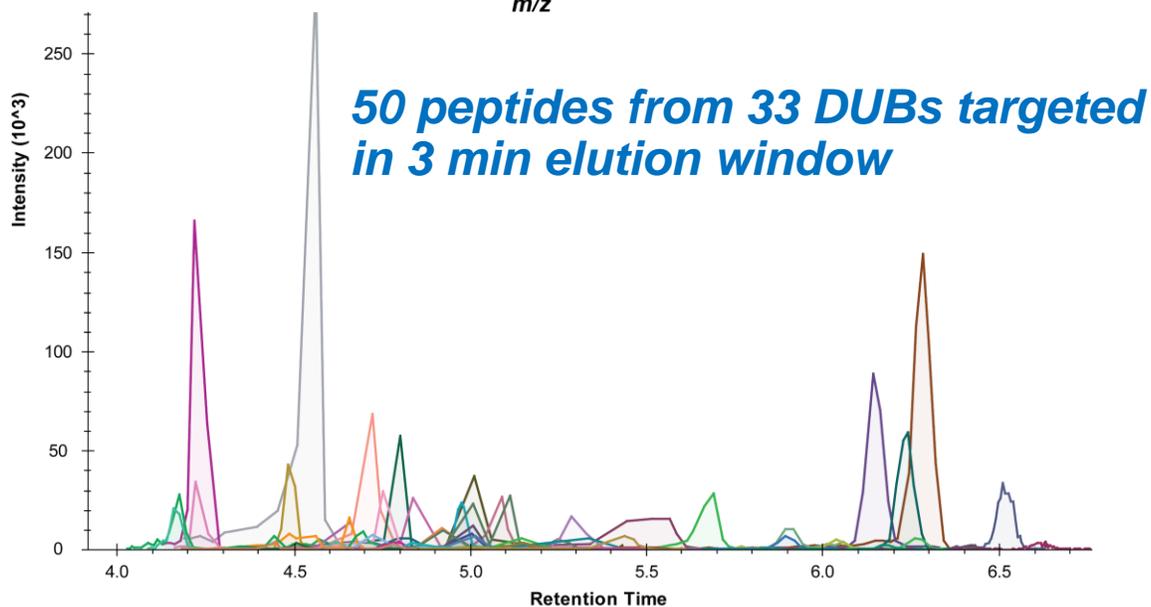
USP10 TPSYSISSTLNPQAPEFILGCTASK<sup>+3</sup>



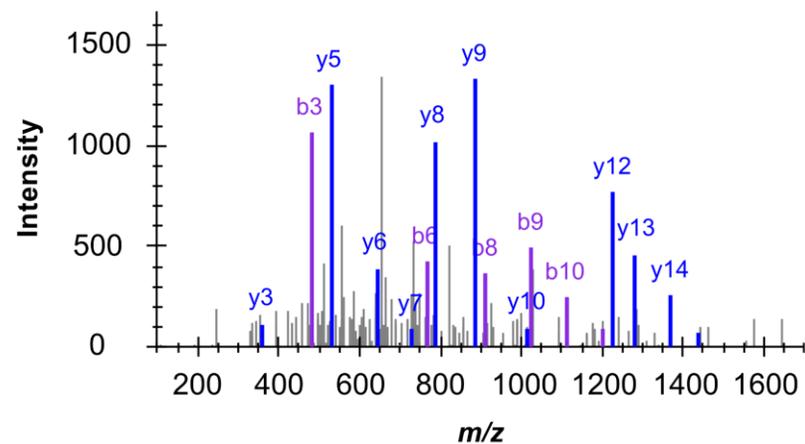
**Low abundance target on Zip-Chip (PRM LIVE)**



**Targeted DUBome at a total of 15 min run**



GPVPSTSGISSEMLASGPIEVGSLPAGER<sup>+3</sup>



# Summary

- ❖ *Real time RT adjusting algorithm of PRM-LIVE can predict the correct RT of targeted peptides across different gradients.*
- ❖ *Preliminary results shows that PRM-LIVE has a good quant reproducibility of peptide targets, and PRM-LIVE is promising to target around 1,000 peptides from CSF biofluids at a 60 min LC-gradient.*
- ❖ *A promising initial application to activity-based protein profiling for rapid selectivity analysis of small molecular targeting deubiquitinating enzymes (DUBs); PRM-LIVE integrated with Zip-Chip separation can efficiently target peptides from 33 DUBs in a 3 min elution window (a total of 15 min run).*

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