THE OHIO STATE UNIVERSITY

Generating fit-for-purpose targeted PRM assays from DIA experiments

2023 Skyline User Meeting

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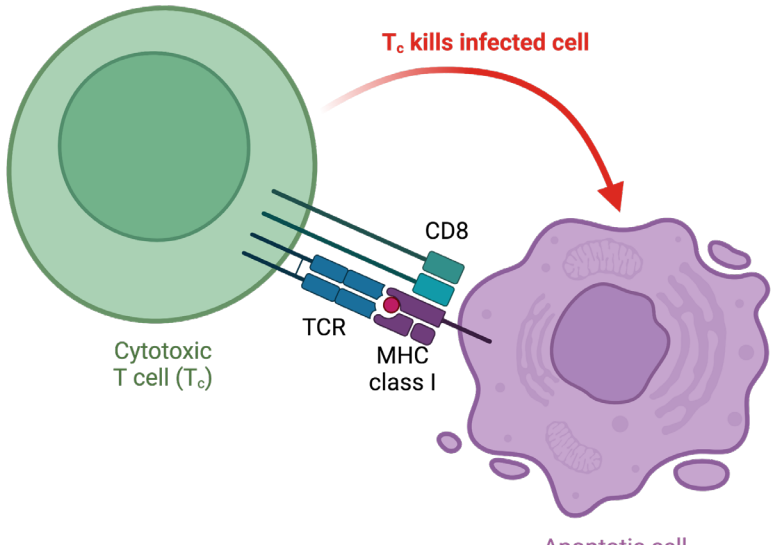
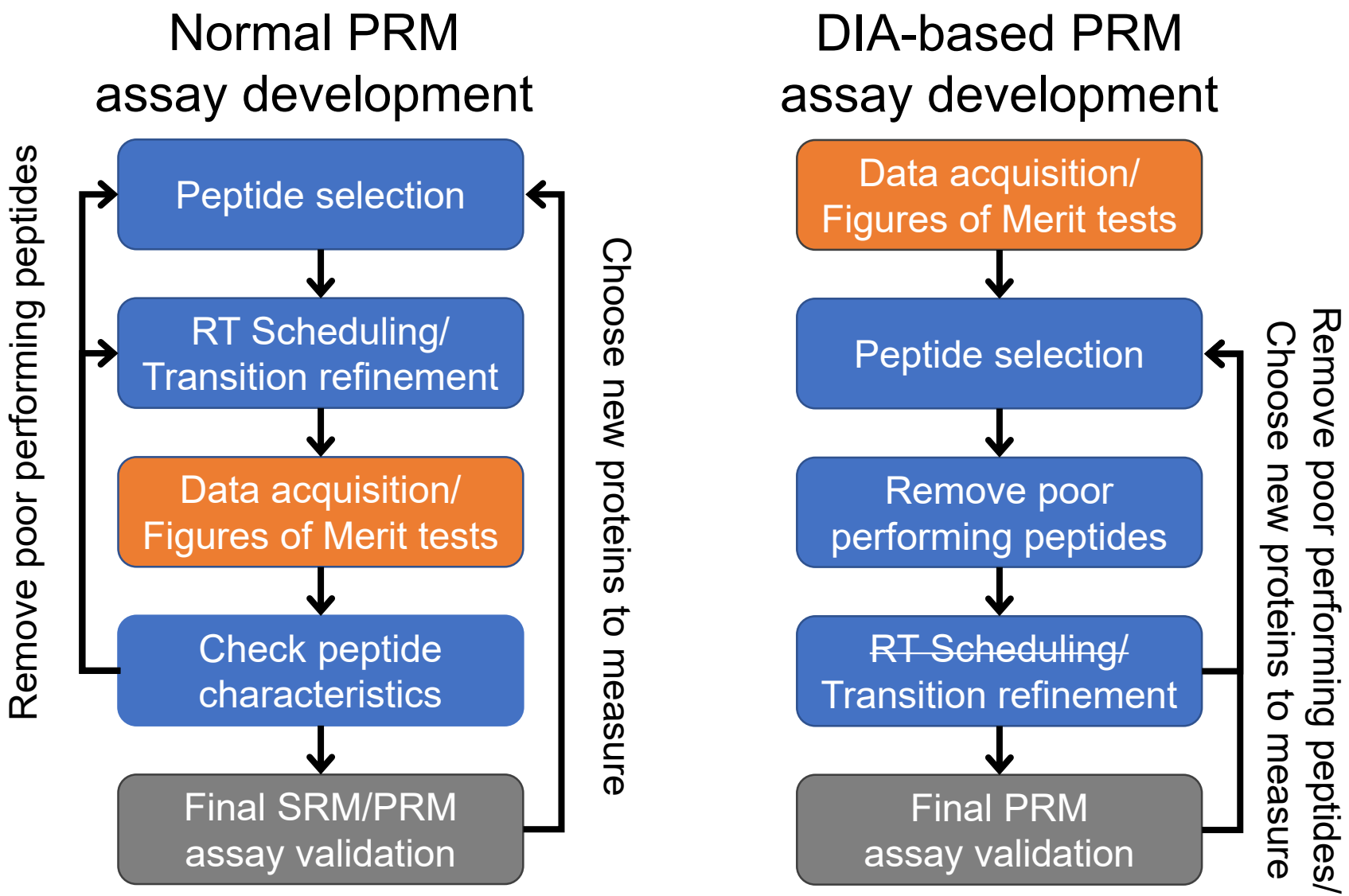
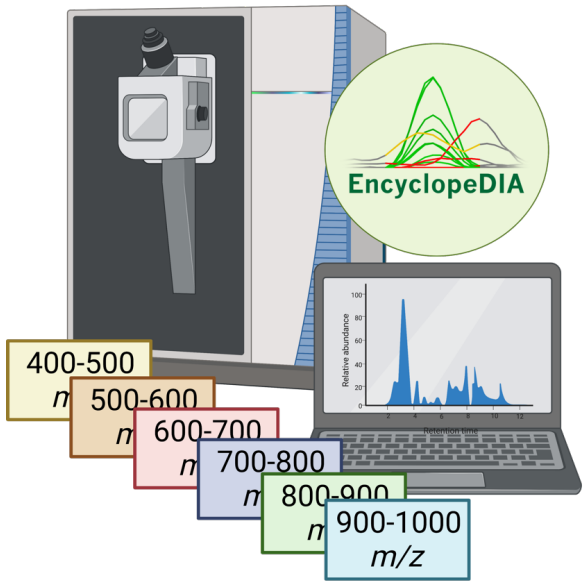
CD8 Cells Kill Virally Infected Cells



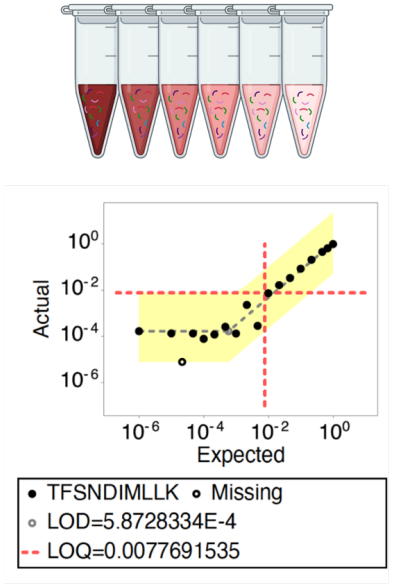

Normal PRM assay development
Peptide selection
RT Scheduling/ Transition refinement
Data acquisition/ Figures of Merit tests
Check peptide characteristics
Final SRM/PRM assay validation
Remove poor performing peptides
Choose new proteins to measure
DIA-based PRM assay development
Data acquisition/ Figures of Merit tests
Peptide selection
Remove poor performing peptides
~~RT Scheduling/~~ Transition refinement
Final PRM assay validation
Remove poor performing peptides/ Choose new proteins to measure

DIA validation of all targets for on-the-fly PRM

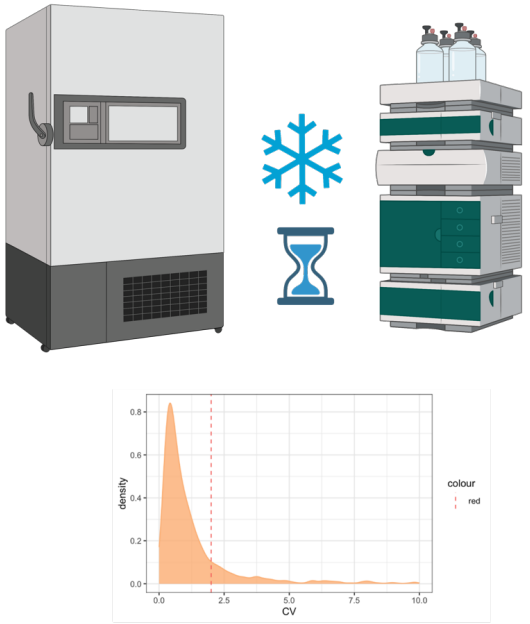
1.Chromatogram Library and Target Generation



2.Matrix-Matched curve for LOD/LOQ of all targets



3. Stability in short and long-term storage



4. Repeatability with and across digestions

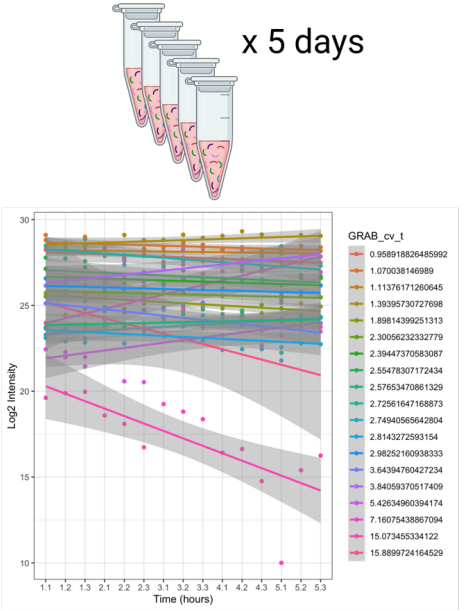
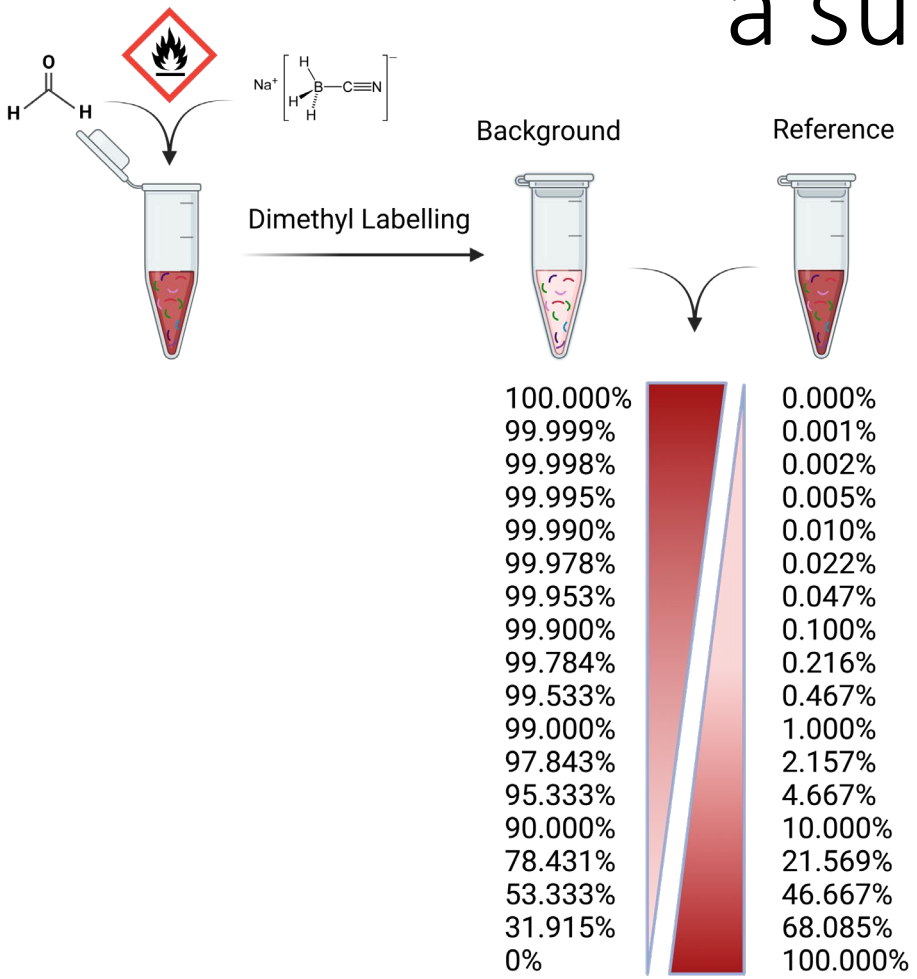
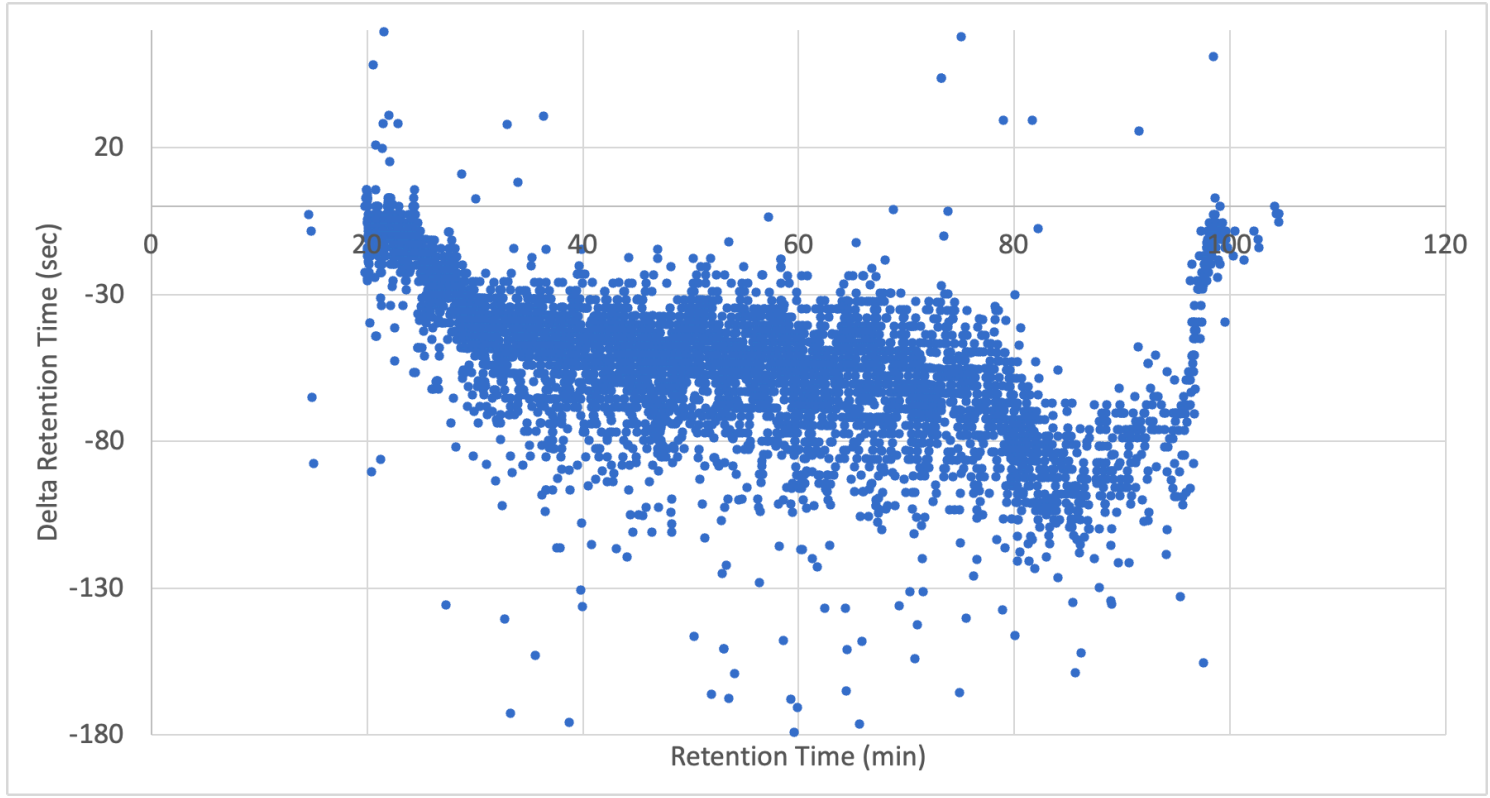


Figure Made in Biorender

Dimethyl labelled reference peptides serve as a suitable background



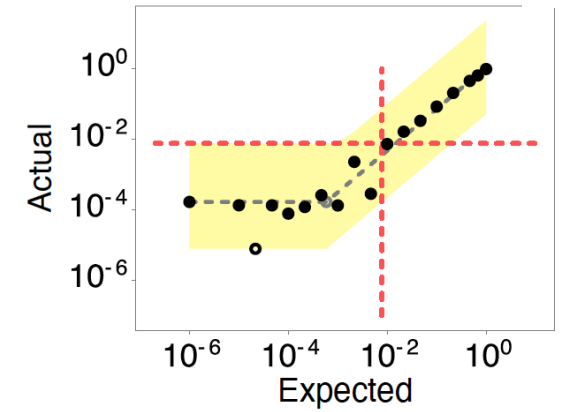
RT deviation between 100% reference (unlabeled) and 10% reference injections



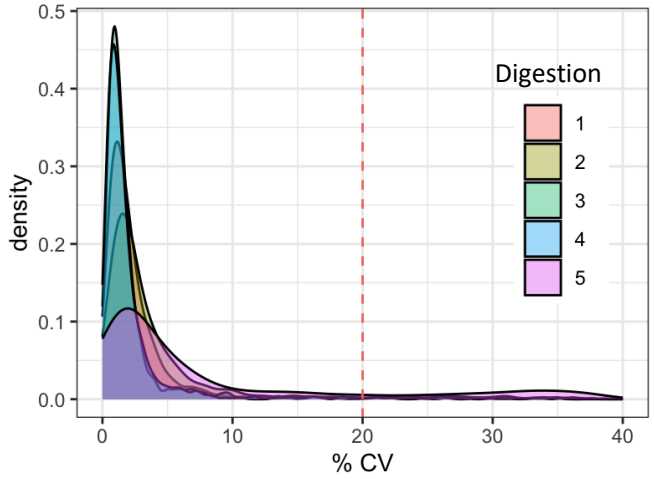
- Deviation of approximately 60 seconds with dimethyl labelled background

Pino et al., 2020, JPR.

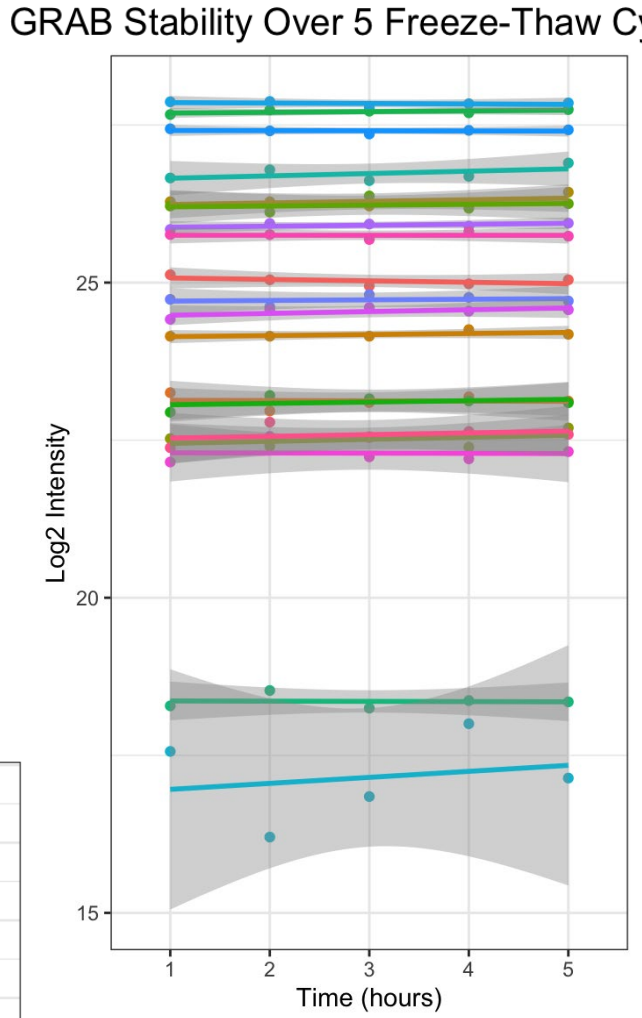
LOD/LOQ

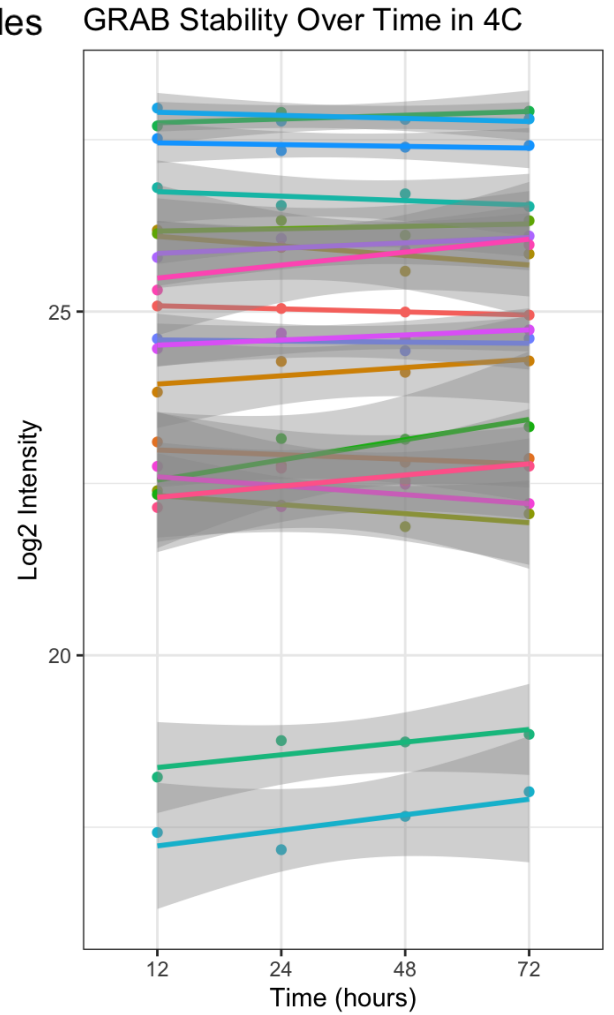


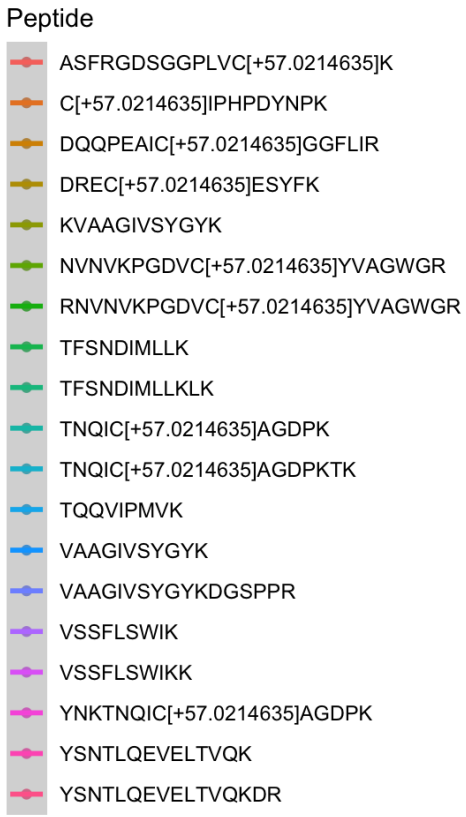
Reproducibility
(5 digestions x 5 days)



Stability





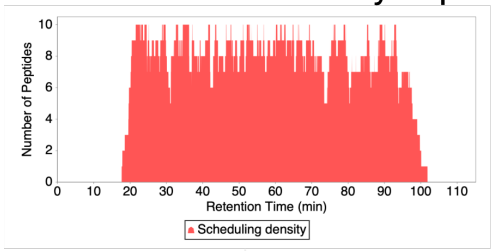


Figures of Merit quantified for 47,717 peptides identified in CD8⁺ T cells after acutely stimiulation. 13,398 peptides passed all validation assays.

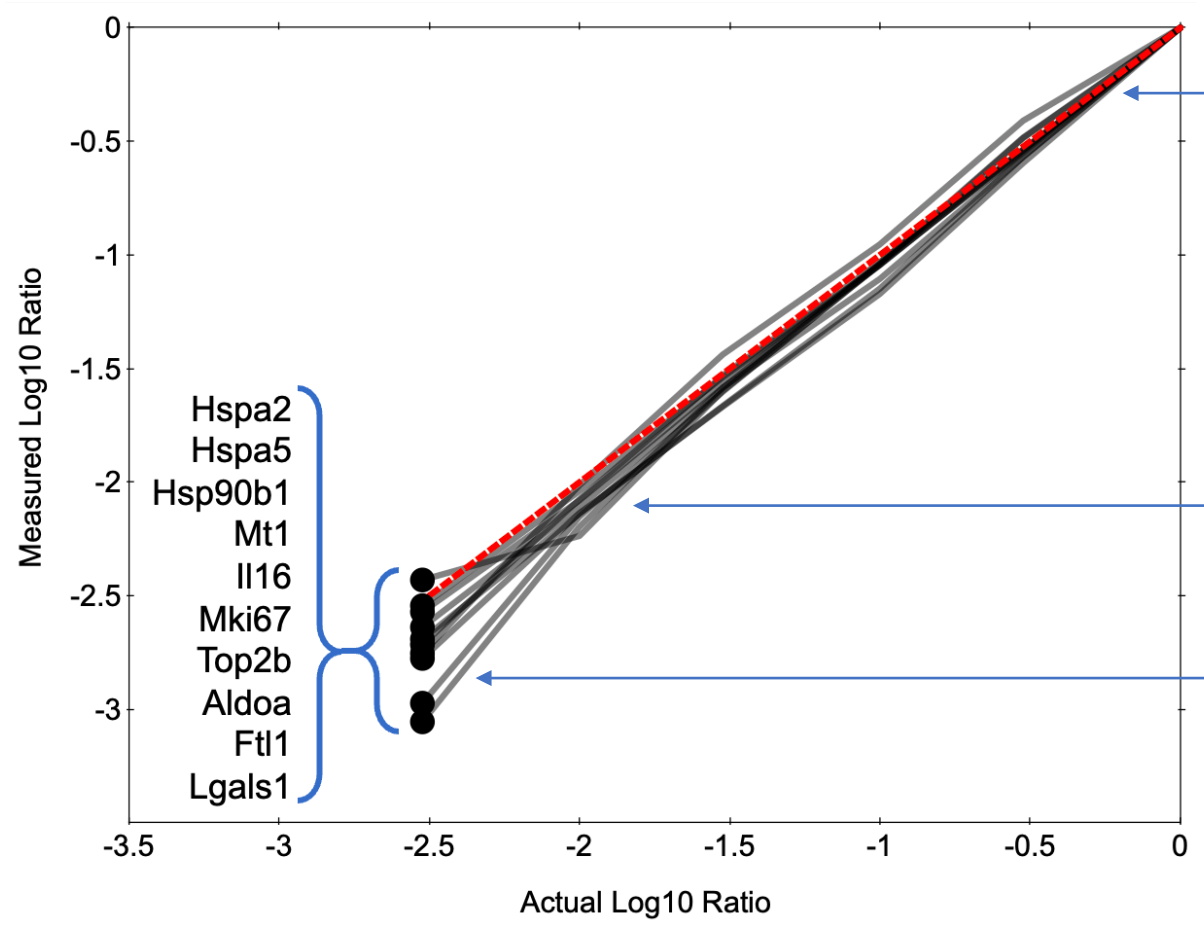
PRM with validated targets

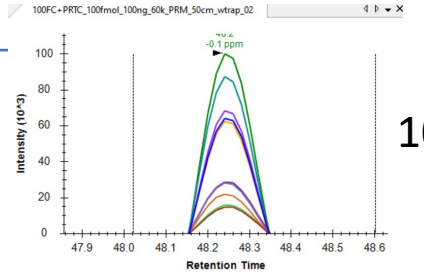
Peptide selection with validated targets

Scheduled PRM with EncyclopeDIA

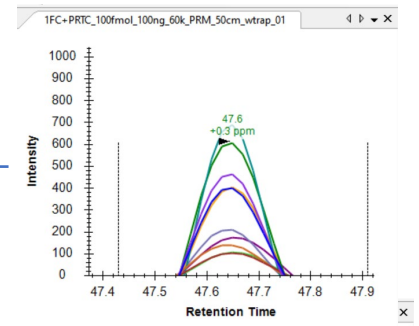


Manual integration in Skyline

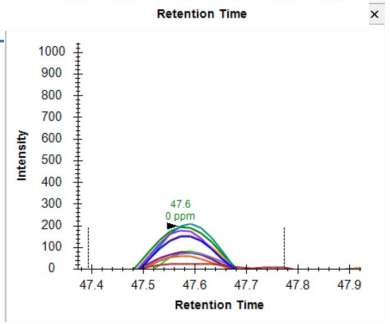




100 ng



1 ng



0.3 ng

25 proteins were detected at 0.3 ng level on the Exploris 480, equivalent to 10 T cells or 1 HeLa cell.

Conclusions

- Dimethyl peptides can serve as a suitable background for matrix-matched calibration curves.
- Validating thousands of peptides from a DIA experiment allows for the generation of quick, preliminary PRM assays prior to acquiring stably isotopic labelled peptides.
- Mass spectrometry assays can be sensitive enough to compete with flow cytometry for monitoring protein abundance.

Acknowledgements

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