University of British Columbia/ Faculty of Pharmaceutical Sciences & Proteomics and Metabolomics Core Facility

A Streamlined Thermal Proteome Profiling Workflow in A Core Facility Using label-free DIA and Mass Dynamics

Danielle Hanke[1], Xiaojing (Jeanne) Yuan[2], Jason C. Rogalski[2], Brent D.G. Page[1]
[1]Faculty of Pharmaceutical Sciences, UBC, Vancouver, BC; [2]UBC Proteomics and Metabolomics Core Facility, Vancouver, BC

Introduction

Thermal protein profiling (TPP) is broadly used to identify protein targets of small molecules at a proteome-wide scale by detecting protein thermal stability shifts upon compound interaction. TMT-DDA is the most traditional method in TPP, offering high proteome depth, efficient MS utilization and direct comparison without imputation.

However, sample prep time, reagent cost and limited experiment size remain significant constraints in TMT-DDA TPP. A recent study (George *et al.*, 2023) compared TMT-DDA and LFQ-DIA in TPP experiments, demonstrating that advances in LFQ-DIA techniques can exchange these constraints with MS time. Our study employed LFQ-DIA to evaluate the feasibility of large-scale TPP in a core facility, from sample preparation to data analysis, making this expertise accessible to non-proteomics experts.

Method and Study Design

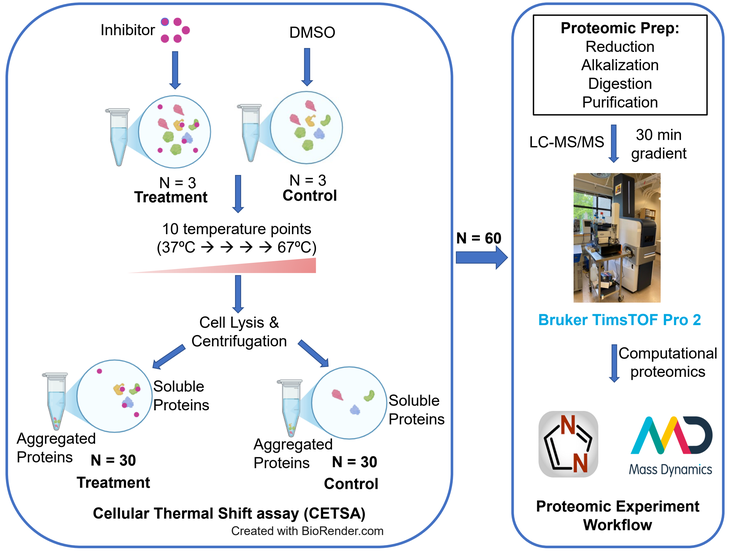

Figure 1. Schematic: from Sample Preparation to Data analysis

Results and Discussion

1. TMT-DDA vs DIA-NN fitted curves

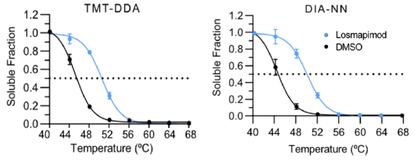

Figure 2. Melting Curve Comparison *THP-1 cells were treated with either losmapimod or DMSO (George et al., 2023). Dashed line marks the melting temperature, where 50% of the protein precipitates. MAPK14 melting curves from TMT-DDA and DIA-NN library-free show similar trend. DIA estimated a melting temperature of 44.6 ± 0.1 °C in controls, consistent with the literature; while TMT-DDA gave a value 0.9 °C higher.*

2. Protein profile & dynamic range changed

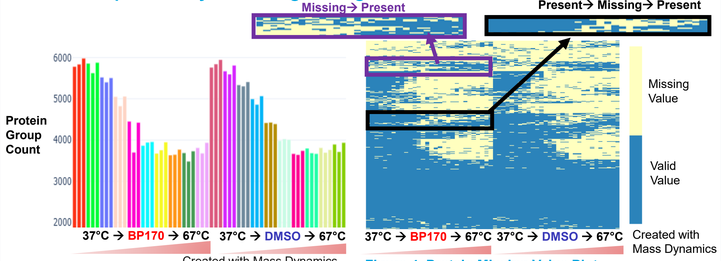

Figure 3. Bar plot *As temperature increases, protein crushed out of the solution, resulting in fewer protein identified.*

Figure 4. Protein Missing Value Plot *As proteome complexity decreases with higher temp., some previously undetectable proteins became quantifiable due to changes in dynamic range (purple). Interesting, some proteins crushed out between 52 – 61 °C, reappeared at higher temperature 64 - 67 °C (**black**).*

3. Sample clustered by temperature conditions

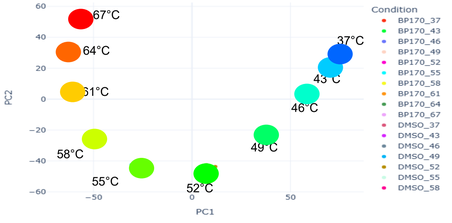

Figure 5. PCA Plot *Samples undergone different heat treatment were grouped together. Image created with Mass Dynamics.*

4. Proteins with different melting points can be differentiated from violin and volcano plots

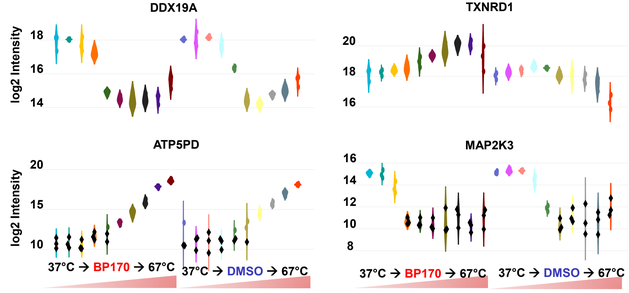

Figure 6. Violin plots of selected proteins *Unlike traditional TMT-DDA-based TPP, library-free DIA based TPP reveals a wider variety of thermal profiles. Black diamond marks the imputed value; all other symbols indicates quantified value. Image created with Mass Dynamics.*

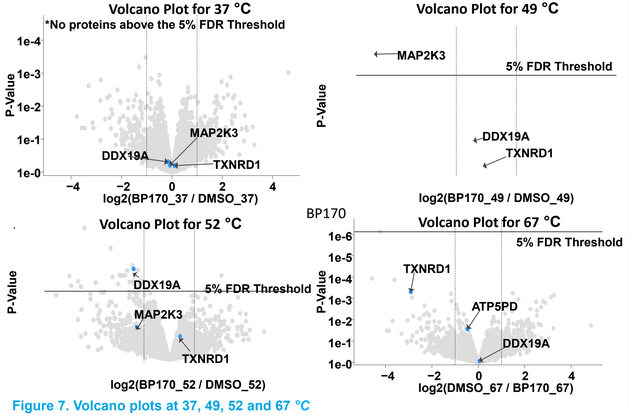

Figure 7. Volcano plots at 37, 49, 52 and 67 °C *At lower temperatures e.g. 37 °C, control and treatment samples show minimal difference. As temperature increases, proteins with altered thermal stability begin to separate significantly ($p < 0.05$), with the most pronounced effects observed between 49 °C and 61 °C. At 67 °C and higher, the difference in thermal stability diminished. Image created with Mass Dynamics.*

5. Difference in protein stability is visible through its position in a volcano plot across condition

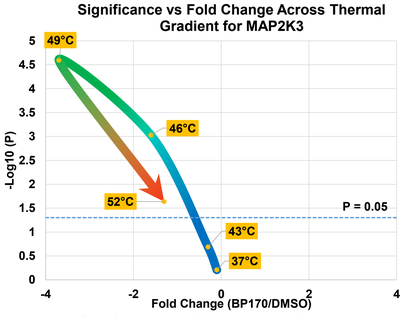

Figure 8. Significance vs. fold change of MAP2K3 across thermal gradient
Control and treatment show little difference at lower temperatures. At 46 °C, MAP2K3 shows a fold change of 2 ($p = 0.0009$), increasing further at 49 °C. Both fold change and significance decline at 52 °C, and MAP2K3 becomes undetectable at 55 °C and above.

Conclusion

TPP, combined with DIA-PASEF and Mass Dynamic, offers a streamlined, cost-effective approach to quickly identify protein interactor in a core facility. Uses can quickly determine interactors and move to validation by traditional western blot or subsequent MS experiments.

Reference

George, A. L., Sidgwick, F. R., Watt, J. E., Martin, M. P., Trost, M., Marín-Rubio, J. L., & Dueñas, M. E. (2023). Comparison of Quantitative Mass Spectrometric Methods for Drug Target Identification by Thermal Proteome Profiling. *Journal of proteome research*, *22*(8), 2629–2640. https://doi.org/10.1021/acs.jproteome.3c00111

Acknowledgement

THE UNIVERSITY OF BRITISH COLUMBIA
Life Sciences Institute
NSERC CRSNG