Software development for improved sensitivity of mass spectrometry-based thermal shift assays (MS-TSA) for target engagement and drug discovery

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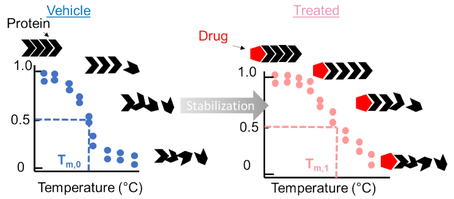
Overview

Thermal shift assays are designed to follow the rate of change in solubility of a protein with increasing temperatures

Thermal Proteome Profiling (TPP) is the first software implemented for mass spectrometry-based assay
-Identifies stabilized protein-drug interactions using melting temperature change (ΔT_m)



$\Delta T_m = T_{m,1} - T_{m,0}$

Possible Scenarios:

$\Delta T_m > 0$ Stabilization

$\Delta T_m < 0$ Destabilization

$\Delta T_m = 0$ No interaction

Figure 1. Schematic showing the melting temperatures (T_m) in a stabilizing event between vehicle and treated samples

Methodology

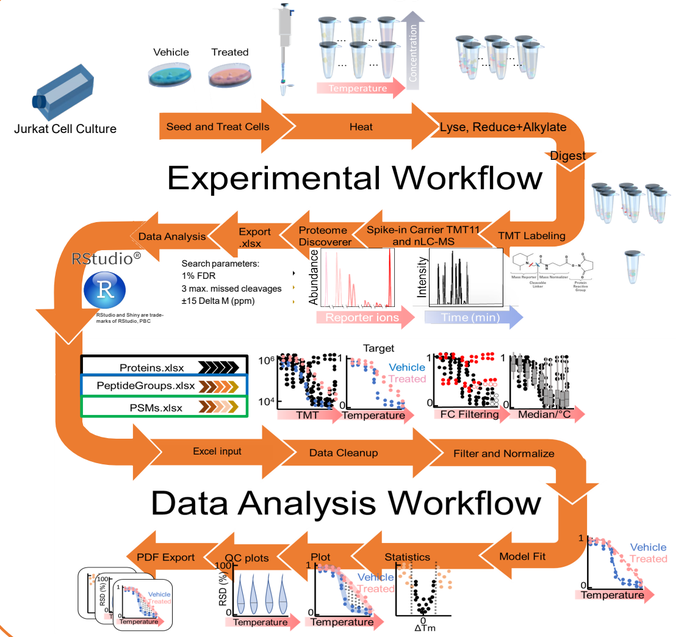


Figure 2. Experimental and data analysis workflows

Thermal shifts detected by hypothesis testing using residual-sum-of-squares (RSS)

H_0: No thermal shift identified, only one spline fitted curve for vehicle and treated

H_1: Thermal shift identified, two fitted splines curves needed

No shift : $RSS_0 \approx RSS_1$

Thermal shift: $RSS_1 << RSS_0$

$$F = \frac{d_2}{d_1}\frac{RSS_0 - RSS_1}{RSS_1}$$

d_1, d_2 : effective degrees of freedom

Results

Additional peptide filtering applied in the data analysis pipeline increases the number of overlaps in fitted curves for proteins with unique peptides

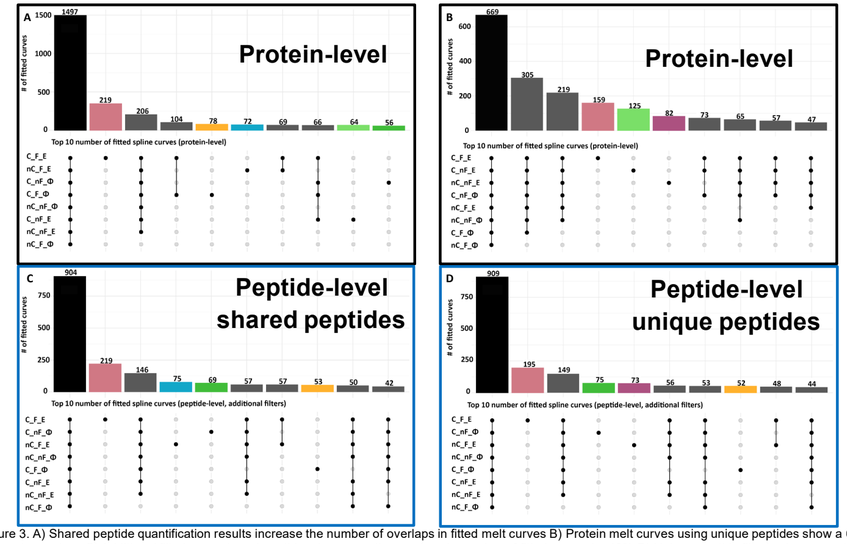


Figure 3. A) Shared peptide quantification results increase the number of overlaps in fitted melt curves B) Protein melt curves using unique peptides show a 65% decrease in overlaps C) Additional filtering on shared peptides decreases the overlaps in number of fitted curves by 40% D) Unique peptide filtering increases the melt curves by 27% in overlaps from 8 different approaches

Quality Control

Missing values, signal-to-noise ratios and variability in reporter ion abundance channels are key components that affect melt curve quality

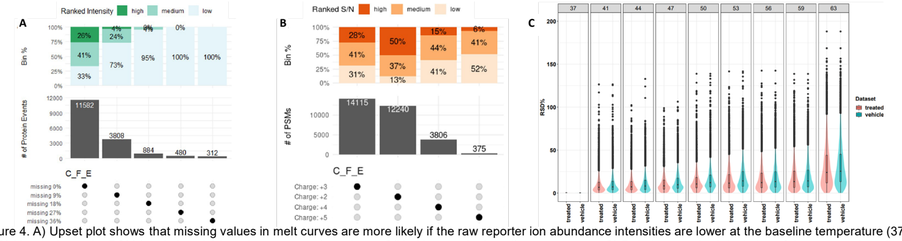


Figure 4. A) Upset plot shows that missing values in melt curves are more likely if the raw reporter ion abundance intensities are lower at the baseline temperature (37° C) B) Upset plot at the PSM-level show higher charge states have lower S/N values at the baseline temperature C) Relative standard deviation of the reporter ion abundances increase with higher temperatures due to a decrease in reporter ion abundance

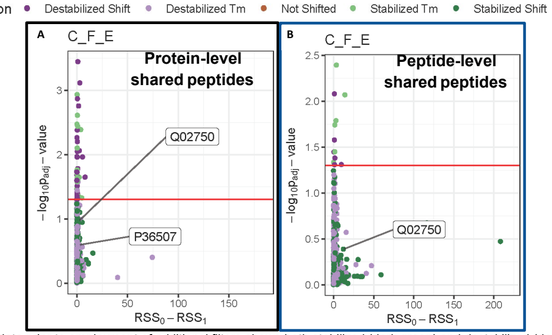


Figure 5. A) Protein-level data prior to employment of additional filters shows both stabilized (dark green) and destabilized (dark purple) shifts as proteins that have shown thermal shifting ability using the F-statistic and ΔT_m values B) Peptide-level data using shared peptides after filtering shows that MEK2 was filtered out with the filters implemented as well as other possible targets MEK 1 and MEK 2 targets in this study are labeled and horizontal red lines depict –log(0.05) value threshold

Conclusions

The software pipeline developed can analyze from 1 to 8+ experiments to record overlaps as well as outliers for thermal shift assays

Novel peptide filtering approaches help increase the number of fitted curves for proteins with unique peptides by 27%

Quality control experiments aid in identification of missing values, signal-to-noise and variability to diagnose their effect on the number of fitted melt curves

Future work

Analyze external data to evaluate potential target identification improvements

Develop receiver operating characteristic (ROC) curves to evaluate sensitivity using STRINGdb

Develop a software package in R to share with the scientific community

References

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