

Target Identification using Thermal Proteome Profiling (TPP)

Scientific Background, Confirmatory Study & Methodology



Scientific Background of Thermal Proteome Profiling

Validated Hypothesis

- Ligand Binding Increases Thermal Stability of the Protein that ligand binds to

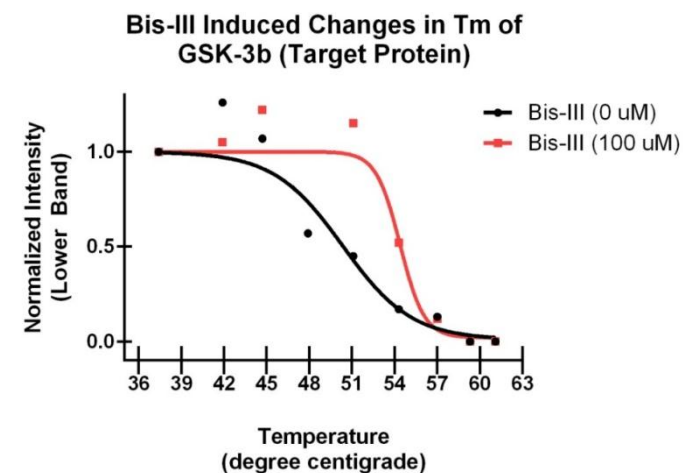
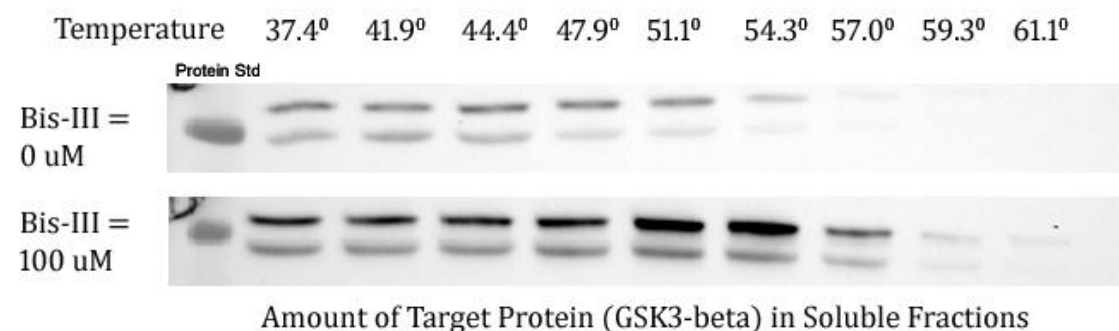
Working Hypothesis for Identifying Targets of Bioactive Ligand(s)

- Characterize thermal denaturation of protein (or many protein = proteome) in presence and absence of ligand and proteins that shows relatively increased thermal stability can be considered as putative targets of the ligand



A Preliminary Confirmatory Study

- Bis-III a small-molecule and is known to interact with GSK3-beta protein.
- Cell-lysate were incubated in presence and absence of Bis-III and heated at different temperatures.
- Precipitated proteins were removed and amount of GSK3-beta in the soluble fraction was measured using western-blot analysis.



Conclusion: Thermal Profiling can be used in confirming Ligand-Target Interaction.

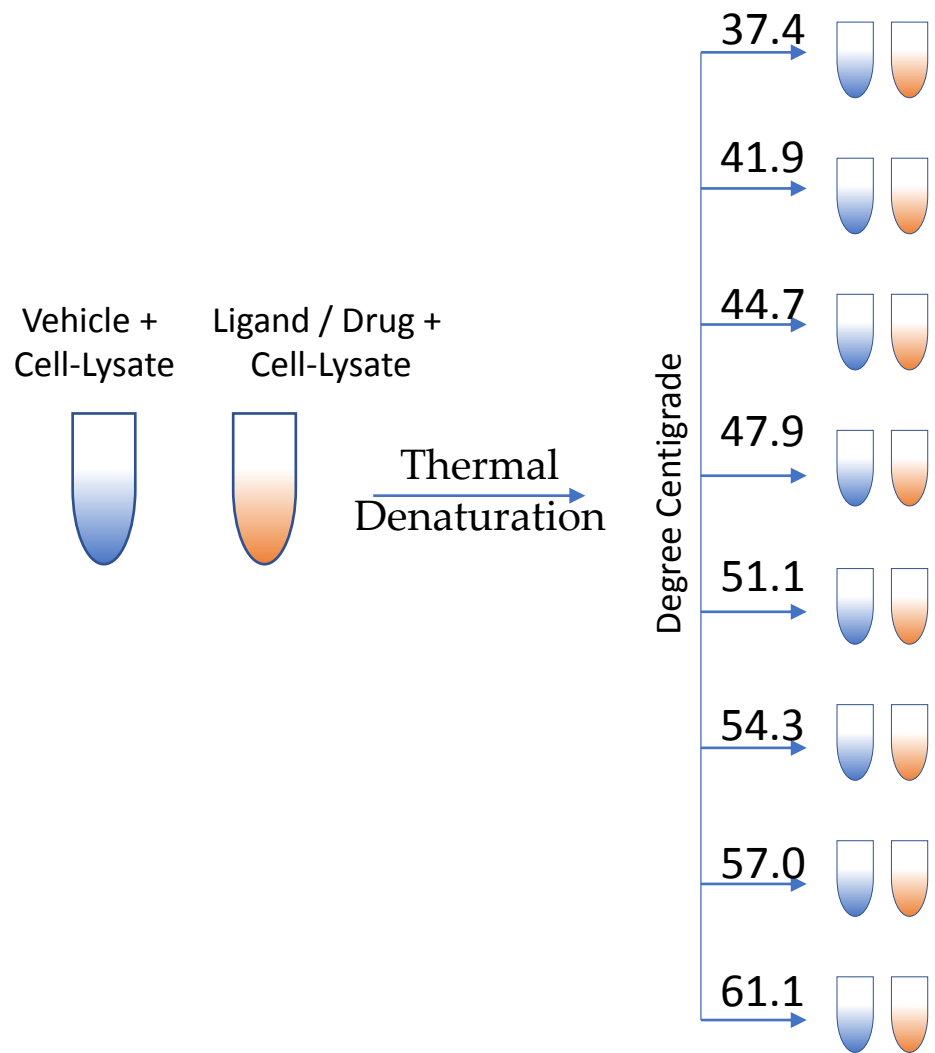
But How To Identify Unknown Target Proteins

Identify and Quantify the proteome in presence and absence of Ligand

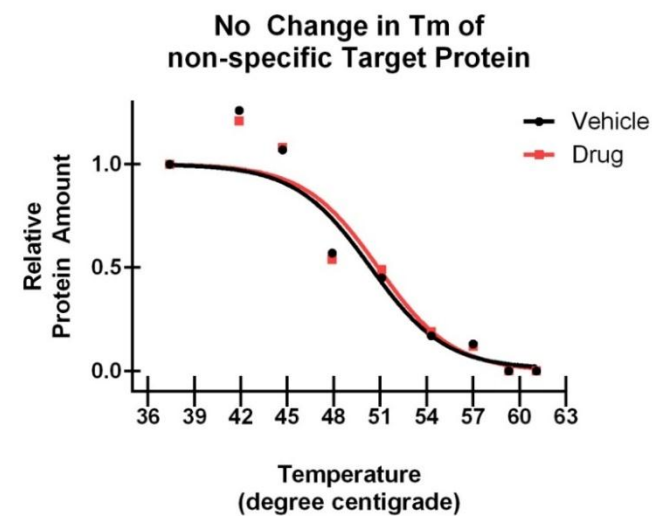
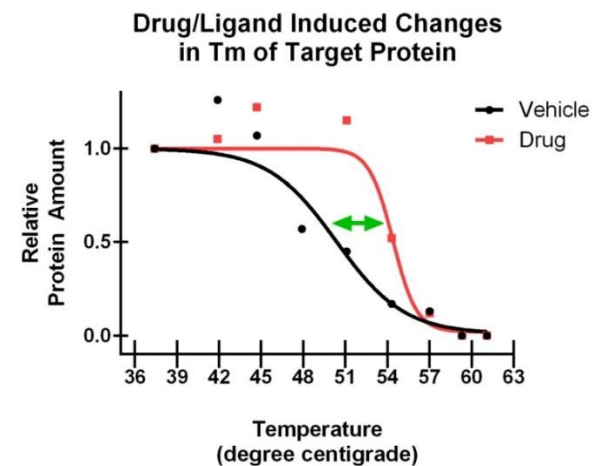


Proteins that shows significant change in Thermal Stability = Putative Targets of Ligand

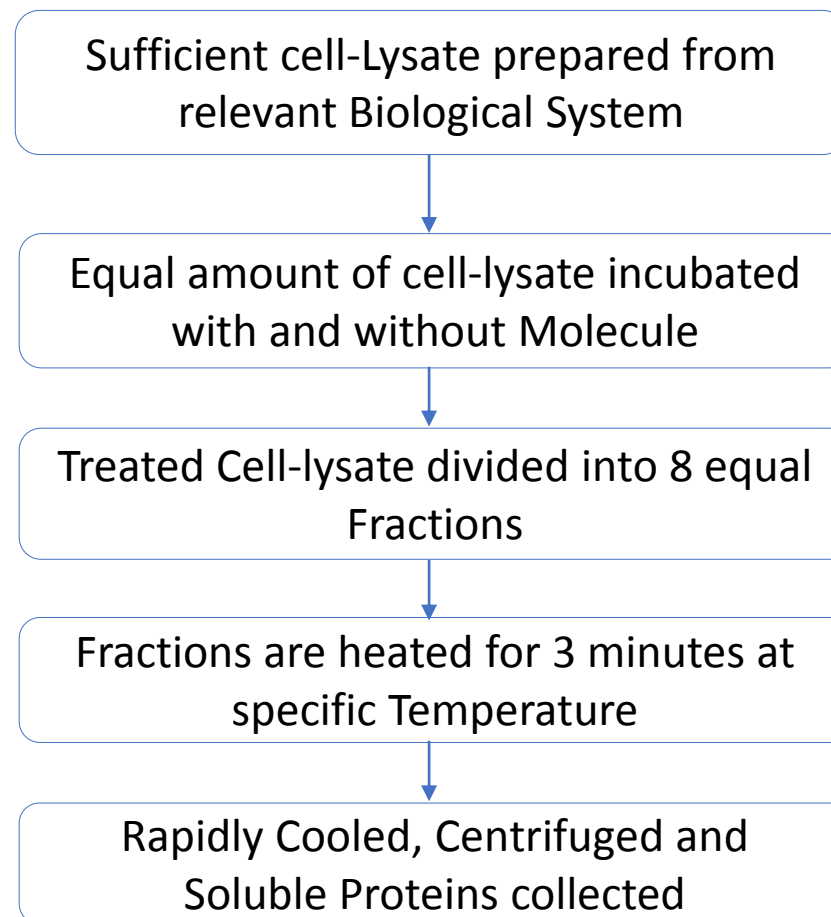




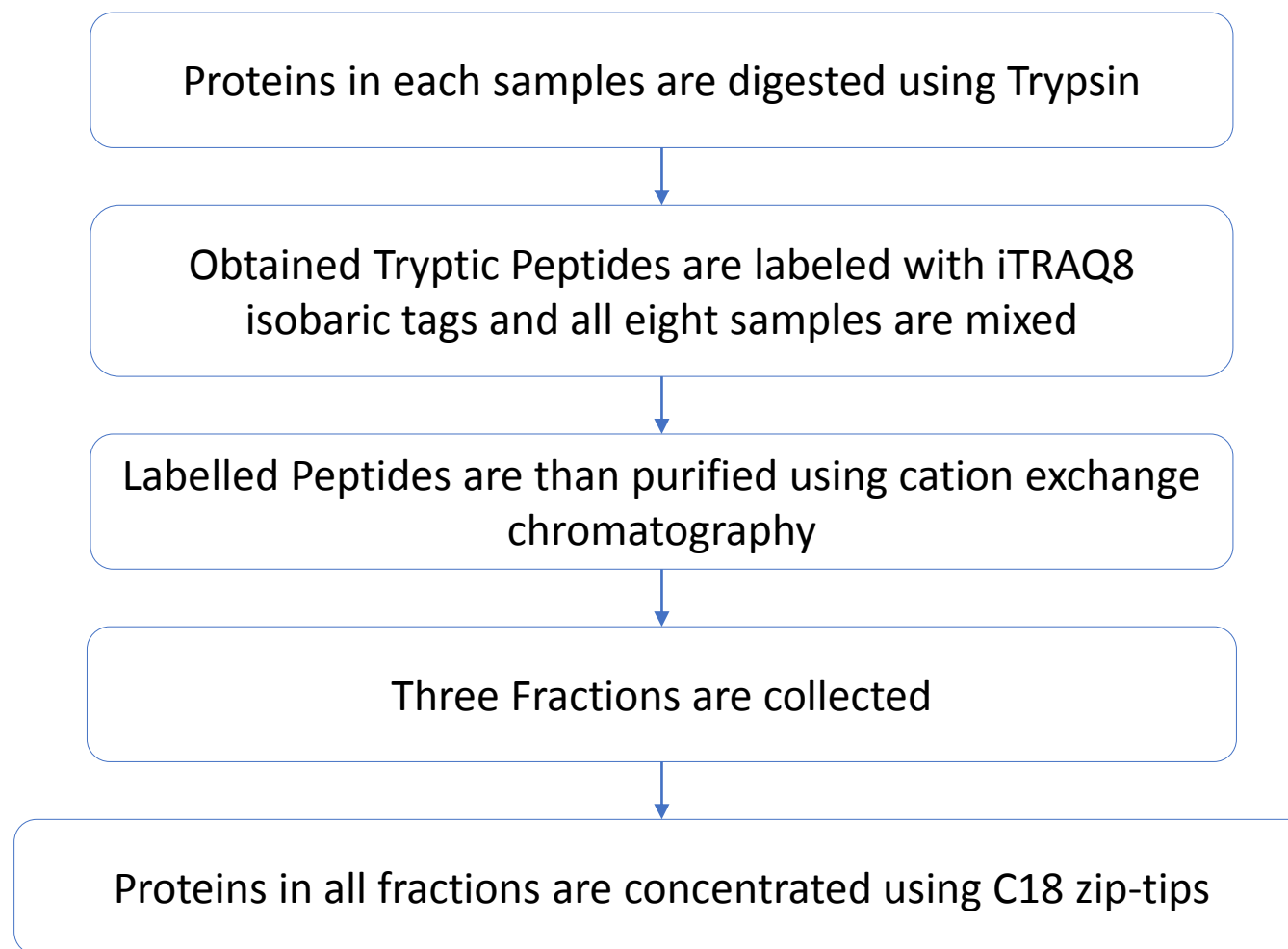
Protein Identification and Quantification by Mass-Spectrometry



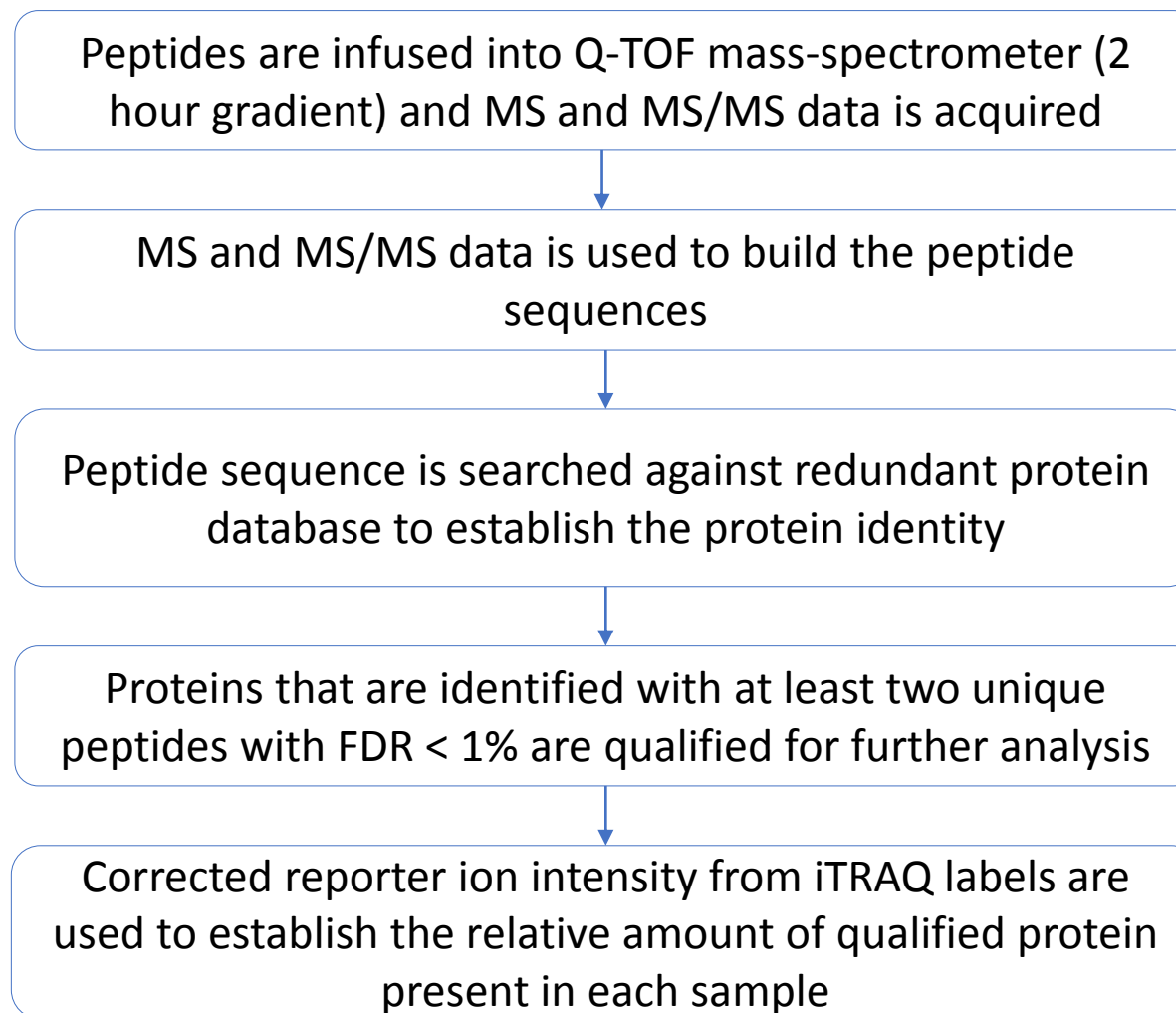
Method Flow-Chart – Thermal Treatment



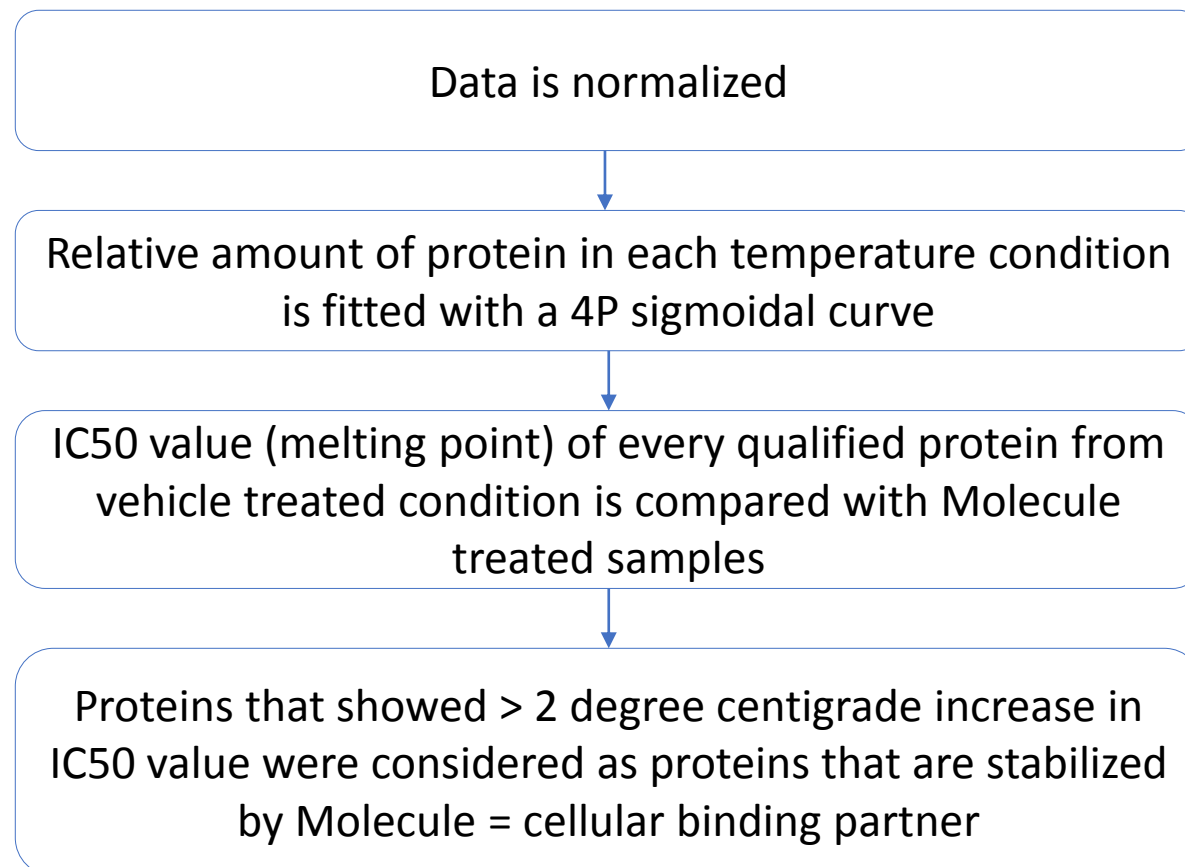
Method Flow-Chart – Protein Digestion and Labelling of Peptides with Isobaric Tags



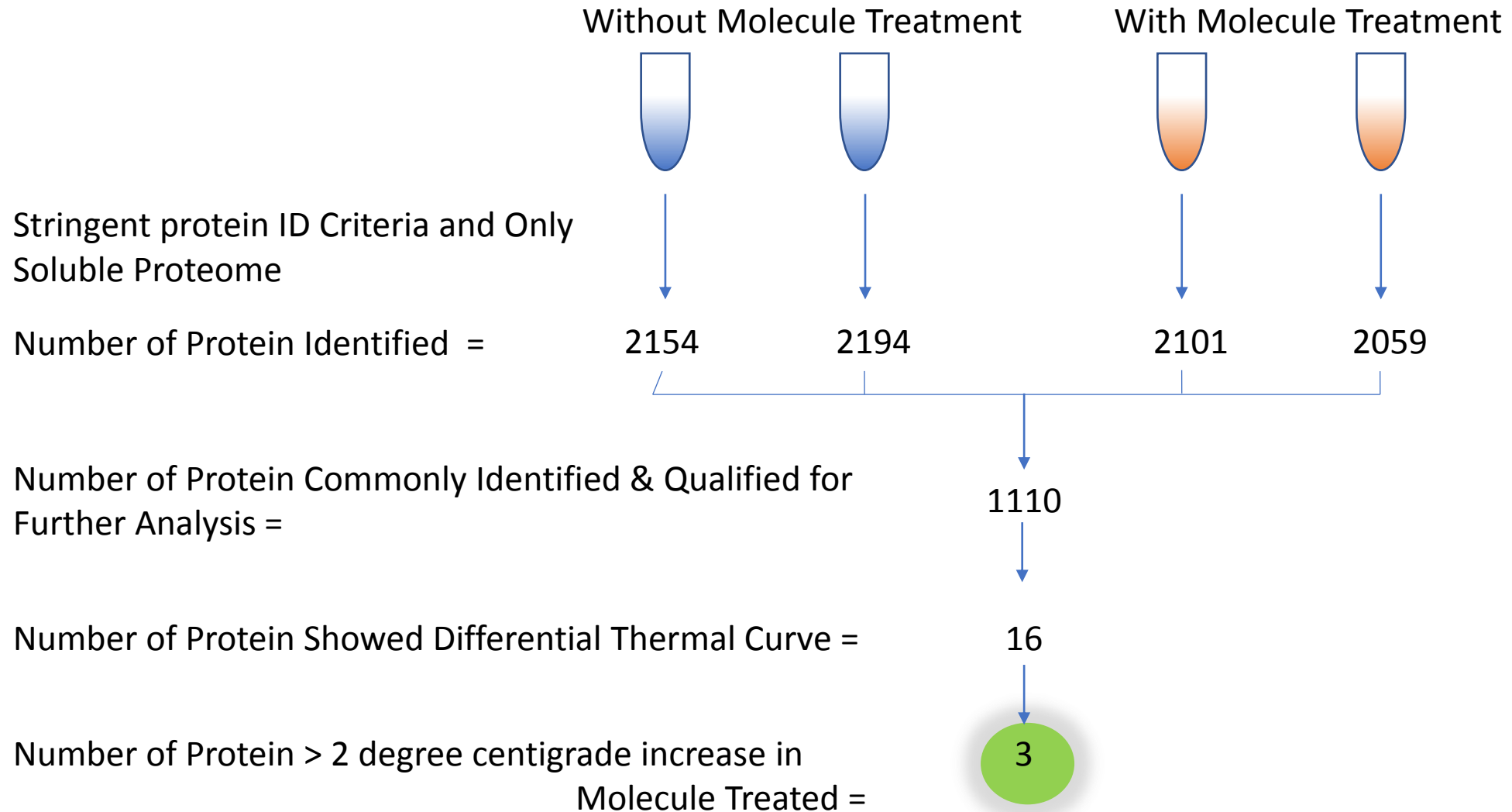
Method Flow-Chart – Identification and Quantification of Proteins



Data Deconvolution



Deconvolution Sample Case Study



Unique Polymer Technology	Thermal Proteome Profiling
Fundamental Differences	
<ul style="list-style-type: none"> • An affinity based target enrichment method and hence identity of specific binders over control experiments can be easily established. • Identifies soluble and membrane proteins. 	<ul style="list-style-type: none"> • Based on the extent of thermal stabilization/ destabilization induced by ligand. If the molecule does not alter the thermal stability of its bonafide target then the target_id efforts may not be successful. • Limited to soluble proteins only.
Data Analysis	
<ul style="list-style-type: none"> • Data deconvolution process is streamlined and analysis of the data is comparatively straight-forward. 	<ul style="list-style-type: none"> • Profiling the whole proteome at multiple temperatures generates a huge amount of data. • Multiple rational but yet 'assumptions' are used in analyzing and extract valuable information from huge dataset.
Cost and Timeline	
<ul style="list-style-type: none"> • UPT experiments can be performed and data can be analyzed in minimum 4 weeks' time. • Minimum resource utilization helps reduce the total cost. 	<ul style="list-style-type: none"> • TPP Experiments and data analysis require relatively more time and can be completed in minimum 10 weeks'. • Utilization of expensive reagents and resources leads to higher cost.



Thank You !!!

Connect for further discussions

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Advancing Technologies and Applications of Proteome Analysis