# 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

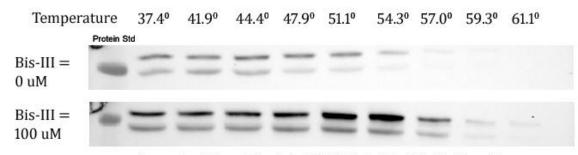
#### Working Hypothesis for Identifying Targets of Bioactive Ligand(s)

• 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

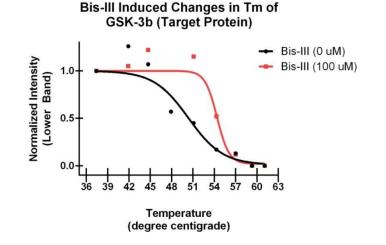


## A Preliminary Confirmatory Study

- 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 westernblot analysis.



Amount of Target Protein (GSK3-beta) in Soluble Fractions



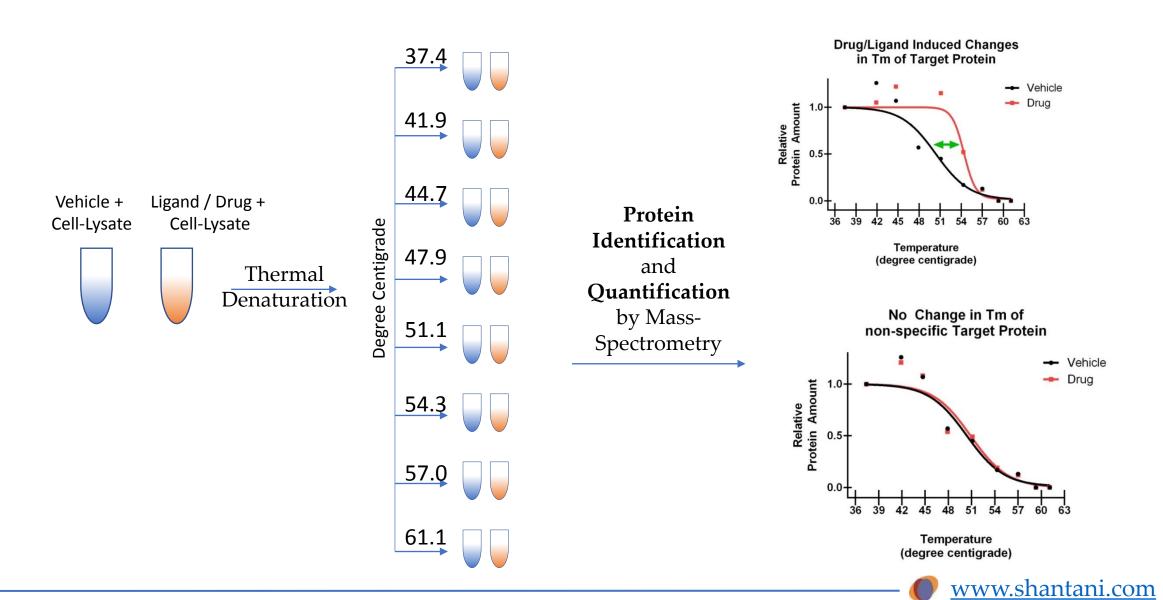
**Conclusion:** Thermal Profiling can be used in confirming Ligand-Target Interaction.



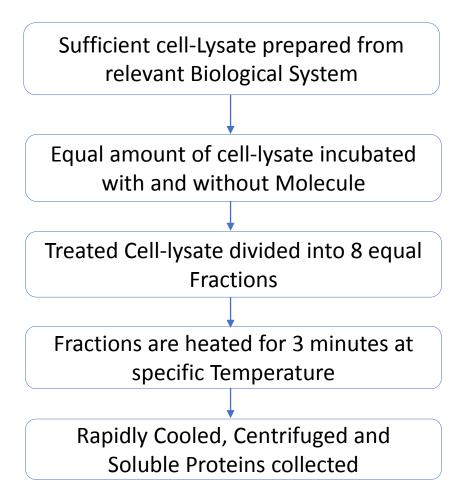
### But How To Identify Unknown Target Proteins

<u>Identify</u> and <u>Quantify</u> the proteome in presence and absence of Ligand

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



#### Method Flow-Chart – Thermal Treatment





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

Proteins in each samples are digested using Trypsin Obtained Tryptic Peptides are labeled with iTRAQ8 isobaric tags and all eight samples are mixed Labelled Peptides are than purified using cation exchange chromatography Three Fractions are collected Proteins in all fractions are concentrated using C18 zip-tips



# Method Flow-Chart – Identification and Quantification of Proteins

Peptides are infused into Q-TOF mass-spectrometer (2 hour gradient) and MS and MS/MS data is acquired

MS and MS/MS data is used to build the peptide sequences

Peptide sequence is searched against redundant protein database to establish the protein identity

Proteins that are identified with at least two unique peptides with FDR < 1% are qualified for further analysis

Corrected reporter ion intensity from iTRAQ labels are used to establish the relative amount of qualified protein present in each sample



#### Data Deconvolution

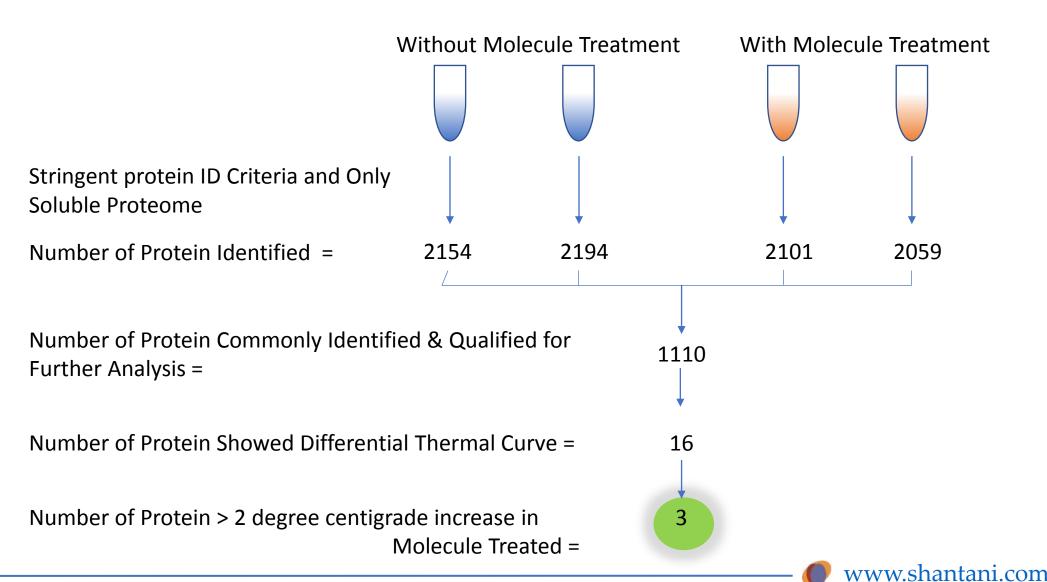
Data is normalized

Relative amount of protein in each temperature condition is fitted with a 4P sigmoidal curve

IC50 value (melting point) of every qualified protein from vehicle treated condition is compared with Molecule treated samples

Proteins that showed > 2 degree centigrade increase in IC50 value were considered as proteins that are stabilized by Molecule = cellular binding partner

### Deconvolution Sample Case Study



### Unique Polymer Technology

#### Thermal Proteome Profiling

#### **Fundamental Differences**

- An affinity based target enrichment method and hence identity of specific binders over control experiments can be easily established.
- Identifies soluble and membrane proteins.

- 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**

- Data deconvolution process is streamlined and analysis of the data is comparatively straight-forward.
- 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**

- UPT experiments can be performed and data can be analyzed in minimum 4 weeks' time.
- Minimum resource utilization helps reduce the total cost.
- 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.



www.shantani.com

#### Thank You !!!

#### Connect for further discussions

Chaitanya Saxena, Ph.D. csaxena@shantani.com +91-9975447489, +91-20-64103918

http://www.shantani.com



