Thermal Stability Analysis (TSA)

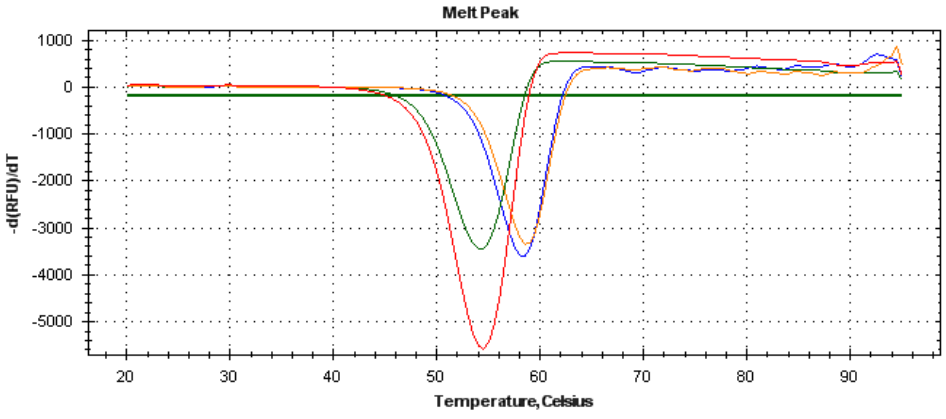
Thermal shift analysis is a useful technique with a broad utility to address important issues in the quality and treatment of proteins and as a biophysical technique for ligand binding studies. This method measures the increase in melting temperature due to the binding of a ligand to the protein. It is a rapid and effective tool for screening hits to identify binding. At Xtal BioStructures we offer this service for a variety of different uses.

This technique measures the temperature, T_m, at which a protein unfolds in the presence and absence of a ligand. Ligands can stabilize and occasionally destabilize the protein, resulting in an increase or decrease in the T_m. Thermal shift can be used for ranking the affinity of a series of ligands, mapping the stability of a protein, pre-formulation, fragment based screening, and many other applications. It is a versatile tool requiring relatively little protein per assy. This technique does not provide an absolute value of binding affinities or kinetics.

Possible uses for this tool include:

- Quality control: each lot of protein produced at Xtal has a T_m as part of the characterization package.
- 'Footprint' Screen: evaluates the pH and ionic strength profile of a protein for protein purification, solubility, and stability
- 'Broadcast' Screen: evaluates parameters that are important for assay design, protein stabilization, and crystallization
- Ligand binding: confirm binding, effect of binding (stabilizing vs. destabilizing), screening ligand libraries or fragment based panels, titrations, etc.

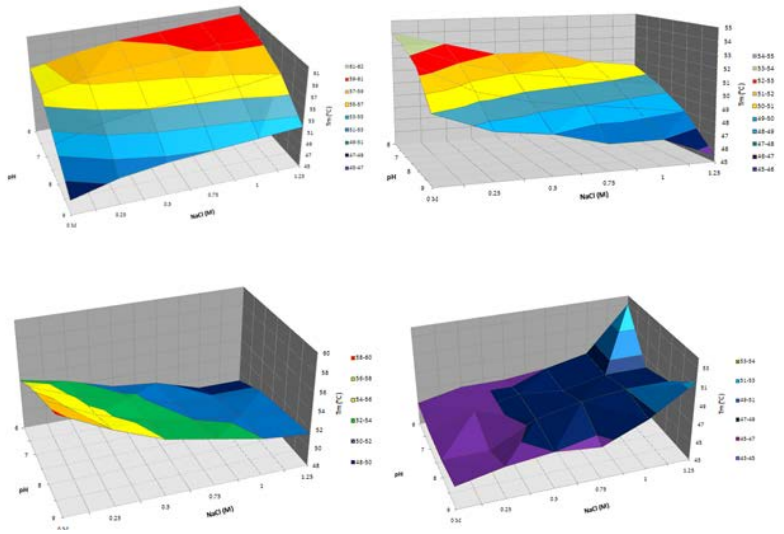
$NADP^+$ Cofactor Binding to Purified Enzyme



Sample	Tm
Protein only	54.5
Protein + $NADP^+$	58.5
Protein + glutathione	54
Protein + $NADP^+$ + glutathione	58.5

This experiment confirmed $NADP^+$ binding to the purified protein, providing a +4.5 °C stability shift. This information provided a batch-to-batch quality control measurement. From this experiment, a system was established for subsequent inhibitor-binding screening studies.

Footprint Screen



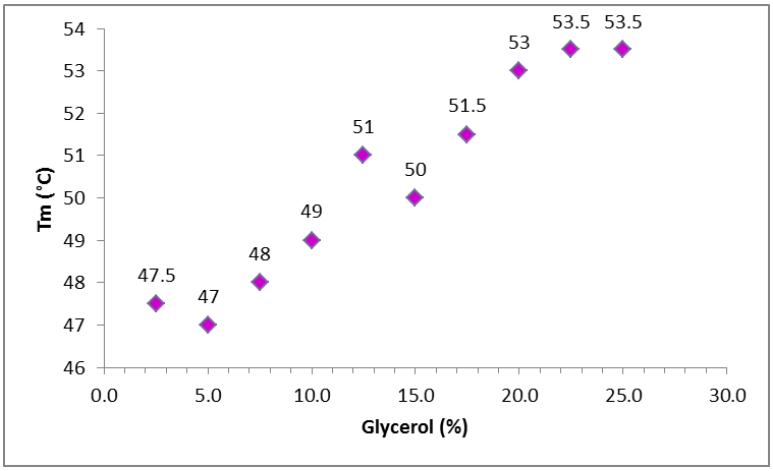
Xtal BioStructures offers a **Footprint Screen** for protein stability characterization profiling. This tool is a 24 condition screen to evaluate protein solubility/stability as a function of pH and ionic strength. The screen is a rapid and efficient method to profile the thermal stability of purified proteins. This provides insight into quality issues, storage, solubility, and stability. (~100 µg protein)

Xtal also offers a **broad-cast screen** for protein stability characterization profiling. This is a 96 condition screen to evaluate parameters relevant to the general stability and solubility of a profile of a protein. This screen requires 0.5 mg of protein.

The screen assists in addressing key biochemical/biophysical parameters that are paramount when developing/optimizing purification protocols, quality controls, and high throughput enzyme assays:

1. Urea unfolding concentration
2. Solubility/stability in ammonium sulfate
3. Effect of DMSO
4. pH profile
5. Buffer interaction (at pH 7.5)
6. Hofmeister ion series
7. Zn, Cu, Mn, Co, Ni
8. Glycerol
9. Misc. additives

Broad-Cast Screen



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