Introduction

The StarGazer-2 (*Epiphyte3*) instrument is designed for low-volume, 384-well optical bottom microplates used for high throughput data collection.

- Label-free thermo-aggregation assay using differential static light scattering (DSLS) at 620 nm

Protein Applications of the StarGazer-2:

Technical Specifications	
Sample volume	2.5 - 50 µL
Temperature range	10 - 95 °C
Heating rate	0.1 - 5 °C/minute
Protein conc. range	0.05 - 1 mg/mL
DSLS laser wavelengths	620 nm
DSLS resolution	~ 8 kDa mean
Number of Samples	1 - 383

- Temperature gradient and isothermal-based experiments
- Optimal buffer screening
- Compound stabilization / destabilization screening and dose response characterization
- Nucleic acid and peptide binding characterization
- Evaluation of protein refolding conditions
- Chemical fingerprinting
- Formulation development of therapeutic antibodies
- Comparison of stability of SNP proteins

Structural & Biophysical Core Facility

Greg Wasney

Manager, Structural & Biophysical Core Facility
Peter Gilgan Centre for Research & Learning
The Hospital for Sick Children
686 Bay Street, Rm. 21.9708
Toronto, ON. M5G 0A4

Email: *greg.wasney@sickkids.ca*

Office: 416.813.7209
Office Internal Ext. 307209
Lab: 416-813-7654 ext. 309442

http://lab.research.sickkids.ca/sbc-facility/

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StarGazer-2— Epiphyte3

Differential Static Light Scattering (DSLS) Protein Thermo-denaturation

Application Note #2:

Compound library screening and dose response evaluation

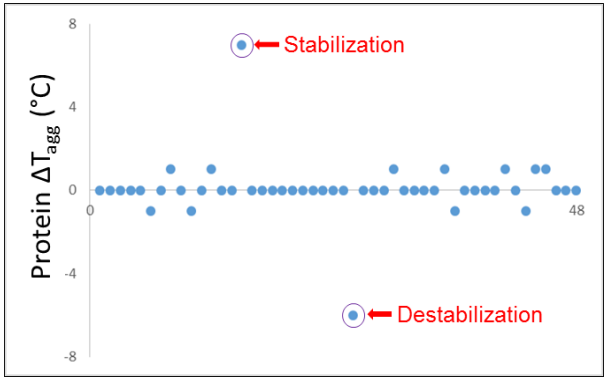
- Compound screening for new binders to enhance protein stability and downstream processes
- Active site characterization and allosteric site discovery / characterization
- K_{agg} — Discover and optimize ligand concentration for co-crystallization
- Promotion of protein co-crystallization

StarGazer-2— Protein Thermo-denaturation Assays

Initial screen of 48 compounds

Protein Sample: 0.4 mg/mL, 10 µL
Compound Concentration: 500 µM
Temperature ramp rate: 1 °C/min, 25 °C to 95 °C
Buffer: 100 mM HEPES pH 8.0, 500 mM NaCl

Performed initial temperature gradient experiments to screen for potential stabilizing / destabilizing compounds.

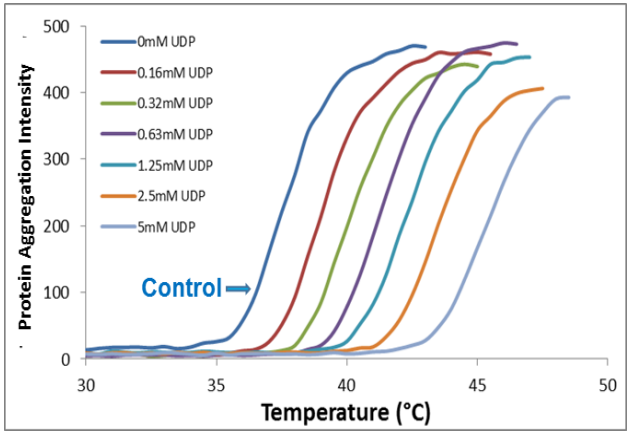


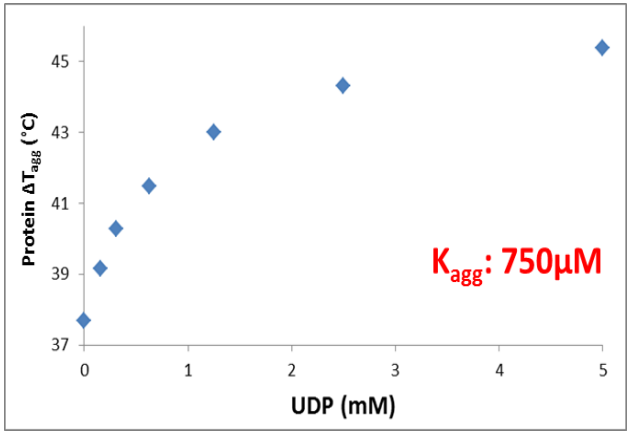
Through the changes in protein stability (ΔT_{agg}), we can identify potential stabilizing and destabilizing compound hits.

To confirm potential hits, a dose response curve should be performed to determine K_{agg}.

Confirmation of a stabilizing compound

Protein Sample: 0.4 mg/mL, 10 µL
Temperature ramp rate: 1 °C/min, 25 °C to 95 °C.
Buffer: 100 mM HEPES pH 8.0, 500 mM NaCl
Test compound: Uridine diphosphate (UDP)

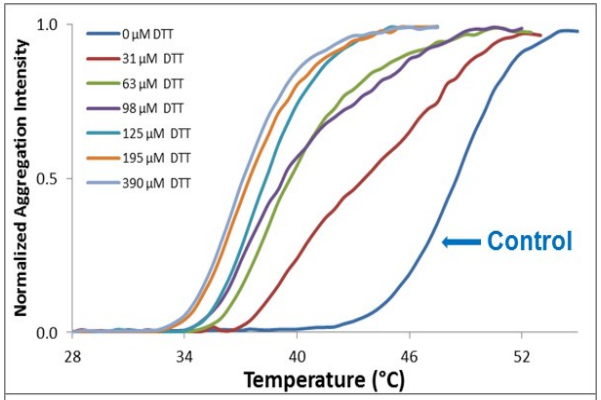


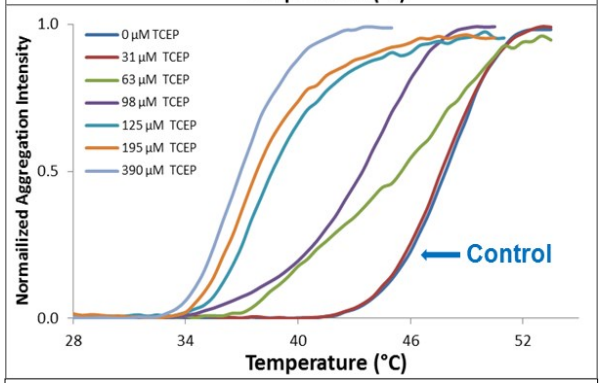


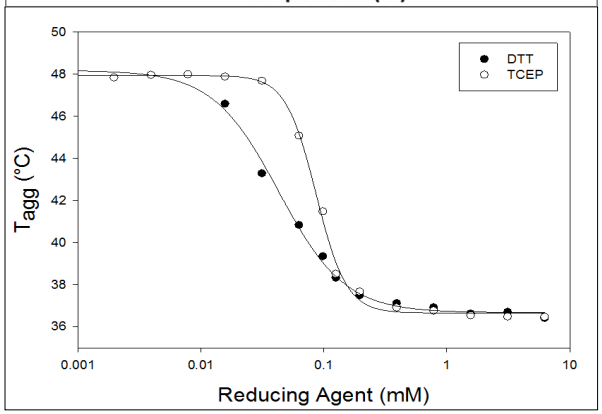
Confirmed UDP dose response stabilization and K_{agg} binding constant determination.

Confirmation of destabilizing compound

Protein Sample: 0.4 mg/mL, 10 µL
Temperature ramp rate: 1 °C/min, 25 °C to 95 °C.
Buffer: 100 mM HEPES pH 8.0, 500 mM NaCl
Test compounds: DTT, TCEP reducing agents







Confirmed DTT and TCEP destabilization.