Introduction

The UNit (*UNchained Labs*) uses up to 3 multi-well cuvettes containing 16 microwells each, monitoring up to 48 samples/conditions in one experiment.

- Determines protein melting temperature (T_m) using tryptophan fluorescence or Sypro Orange and temperature of aggregation (T_{agg}) using static light scattering (SLS) at 266 nm and 472 nm.

Protein Applications of the UNit:

Technical Specifications	
Sample volume	9 µL
Temperature range	15—95 °C
Heating rate	0.01—10 °C/minute
Protein conc. range	0.1—150 mg/mL
SLS laser wavelengths	266 nm, 472 nm
SLS sensitivity	12—22500 kDa · mg/mL
SLS resolution	~ 15 kDa mean
Fluorescence Detector	250—720 nm

- 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

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/

SickKids®
THE HOSPITAL FOR SICK CHILDREN

The Hospital for Sick Children's

Structural & Biophysical Core Facility

UNit — UNchained Labs

Static Light Scattering and Fluorescence Thermodenaturation

Application Note #2:

Compound library screening and dose response evaluation

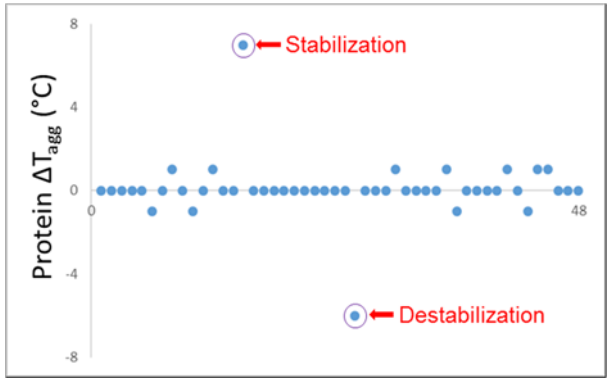
- Compound screening for new binders to enhance protein stability and downstream processes
- Active site characterization and allosteric site discovery / characterization
- K_{agg} — Discover and optimize ligand concentration for co-crystallization
- Promotion of protein co-crystallization

UNit— Protein Thermodenaturation Assays

Initial screen of 48 compounds

Protein Sample: 0.4 mg/mL, 9 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.

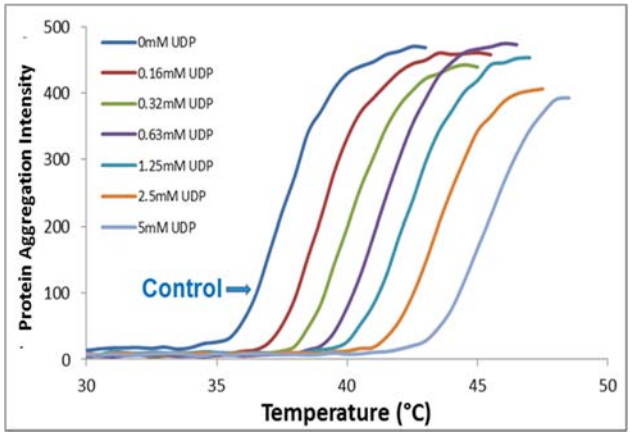


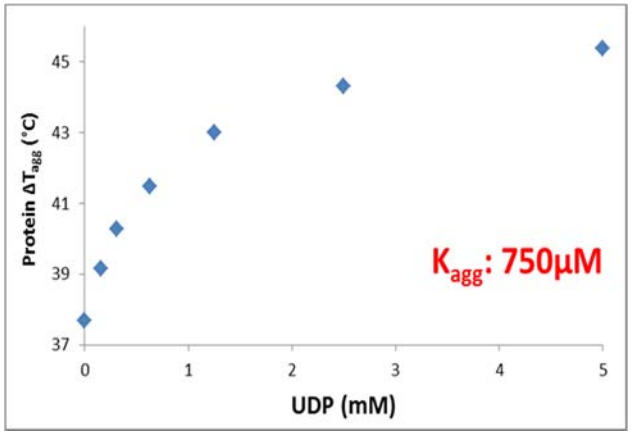
Through the changes in protein stability (ΔT_{agg}), we can identify potential stabilizing and destabilizing compound hits.

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, 9 µL
Temperature ramp rate: 1 °C/min, from 25 °C to 95 °C.
Buffer: 100 mM HEPES pH 8.0, 500 mM NaCl
Test compound: Uridine diphosphate (UDP)

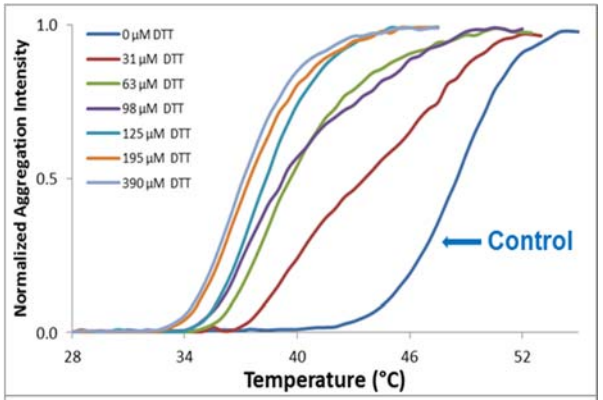


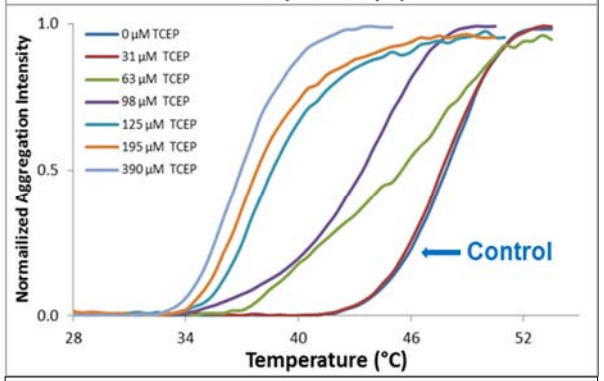


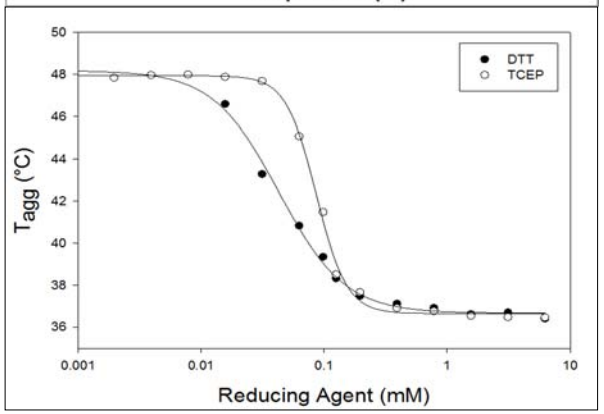
Confirmed UDP dose response stabilization and K_{agg} binding constant determination.

Confirmation of destabilizing compound

Protein Sample: 0.4 mg/mL, 9 µL
Temperature ramp rate: 1 °C/min, from 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.