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 StarGazer2:

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 monoclonal antibodies
- Comparison of stability of SNP proteins

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StarGazer-2— Epiphyte3

Differential Static Light Scattering (DSLS) Protein Thermo-denaturation

Application Note #1:

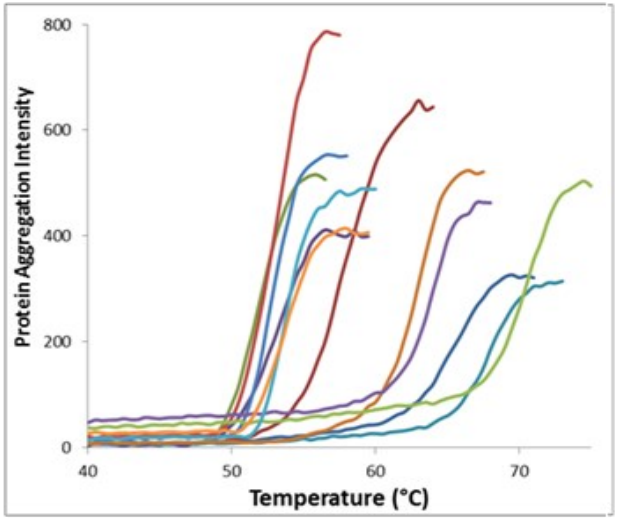
Optimal protein buffer screen

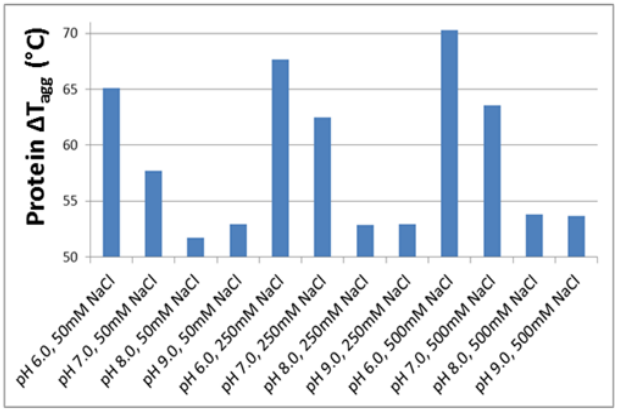
- Greater long/short-term stability
- Reach higher concentration, recover low solubility proteins
- Better binding / functional activity
- Promotion of protein crystallization

StarGazer-2— Protein Thermodenaturation Assays

Experiment Type 1: Temperature Gradient Denaturation

Protein Sample: 0.5 mg/mL, 10 µL
Temperature ramp rate: 1 °C/min, 25 °C to 95 °C
Buffers: 100 mM MES pH 6.0; 100 mM HEPES pH 7.0, 8.0; 100 mM Glycine pH 9.0
Salt: 50, 250, 500 mM NaCl





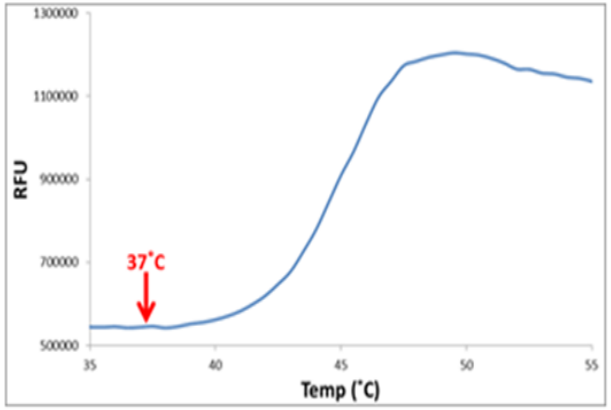
Best: Low pH, High [NaCl]

The protein is stabilized by buffers with lower pH and higher NaCl concentrations.

Experiment Type 2: Isothermal Denaturation

Protein Sample: 0.5 mg/mL, 10 µL
Isothermal denaturation @ 37 °C for 200 min
Buffers: 100 mM MES pH 6.0; 100 mM HEPES pH 7.0, 8.0; 100 mM Glycine pH 9.0
Salt: 50, 250, 500 mM NaCl
Sypro Orange: 5X

Performed initial temperature gradient experiment using DSF (differential scanning fluorometry) to determine temperature of denaturation using a midrange buffer of 100 mM HEPES, pH 7.5, 250 mM NaCl.

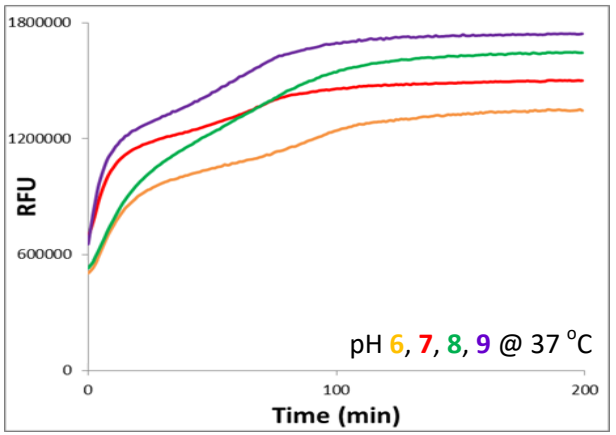


Based on the protein thermo-denaturation gradient curve above, an isothermal denaturation temperature of 37 °C should be used (2 °C below the onset of thermos-denaturation at 39 °C).

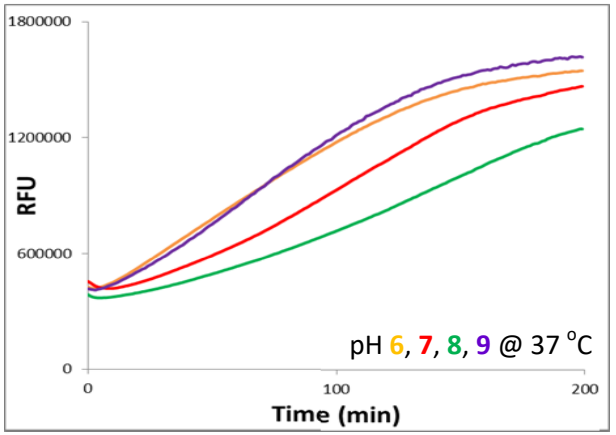
Therefore, the isothermal temperature was held at 37 °C for 200 minutes.

The protein is stabilized by buffers with higher NaCl concentrations while no significant changes are observed with varying pH.

Fast Denaturation (50mM NaCl)



Slower Denaturation (250mM NaCl)



Little Denaturation (500mM NaCl)