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 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 #3:

DNA and peptide stabilization

- Protein-DNA interaction mapping
- Protein-Peptide interaction mapping
- Protein-Polymer interaction mapping

StarGazer-2— Protein Thermo-denaturation Assays

Protein-DNA interaction mapping

Protein Sample: 25 µM, 10 µL
Temperature ramp rate: 1 °C/min, 25 °C to 95 °C
Buffer: 100 mM HEPES pH 8.0, 250 mM NaCl
DNA concentration: 50 µM

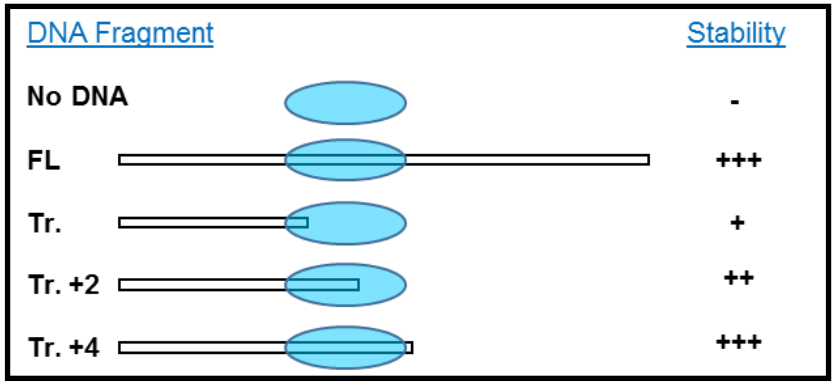
Performed temperature gradient experiments to determine if protein stability is affected by the presence of specifically sized DNA.

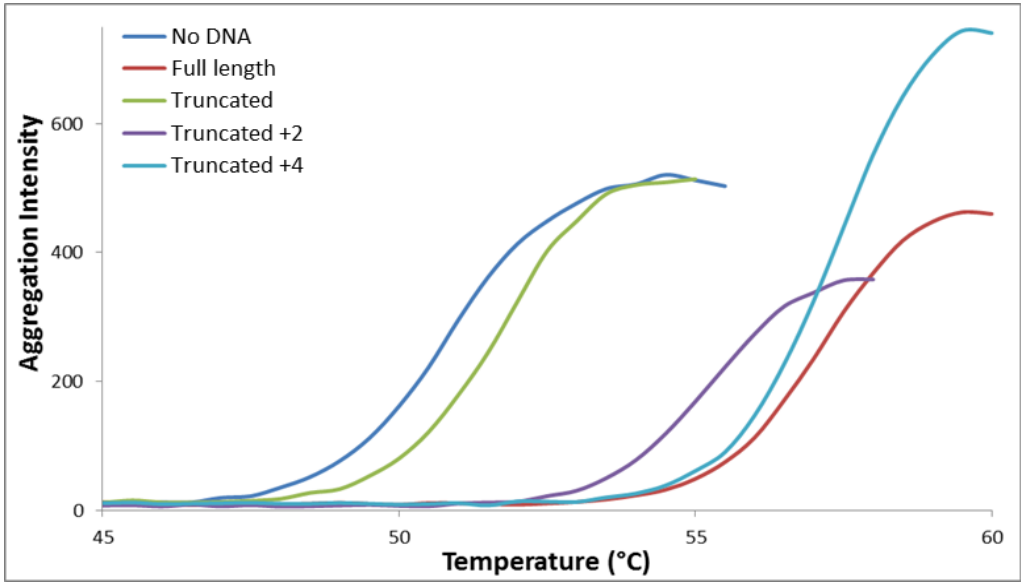
Addition of a full length DNA fragment significantly increased the protein stability due to protein-DNA interaction.

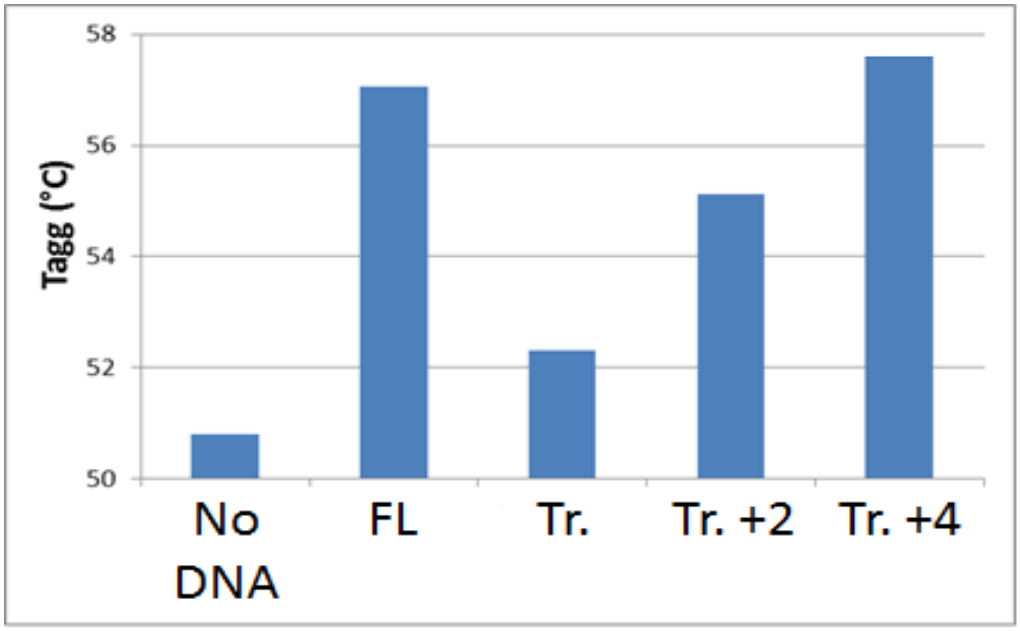
Addition of a largely truncated fragment (Tr.) only slightly increases protein stability. However, as the truncated fragment was elongated by two (Tr. +2) or four (Tr. +4) base pairs, its stabilizing effect on the protein increases.

The truncated fragment elongated by 4 base pairs had a similar stabilizing effect as the full length fragment. Thus, the Protein-DNA interaction can be specific to a region approximately within the Tr. +4 fragment.

This protein thermo-denaturation based interaction mapping strategy can be similarly applied to peptide and polymer binding.







Protein stability is increased in the presence of specifically sized DNA.