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

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UNit — UNchained Labs

Static Light Scattering and Fluorescence Thermodenaturation

Application Note #3:

DNA and peptide stabilization

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

UNit— Protein Thermodenaturation Assays

Protein-DNA interaction mapping

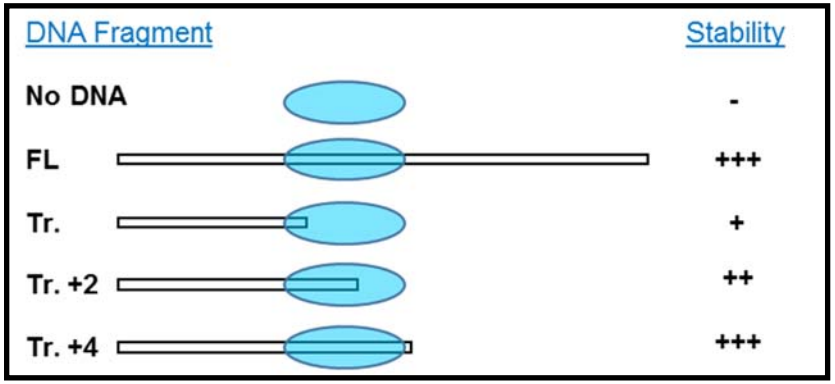
Protein Sample: 25 µM, 9 uL
Temperature ramp rate: 1 °C/min, 25 °C to 95 °C
Buffer: 100 mM HEPES pH 8.0, 250 mM NaCl
DNA concentration: 50 µM

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

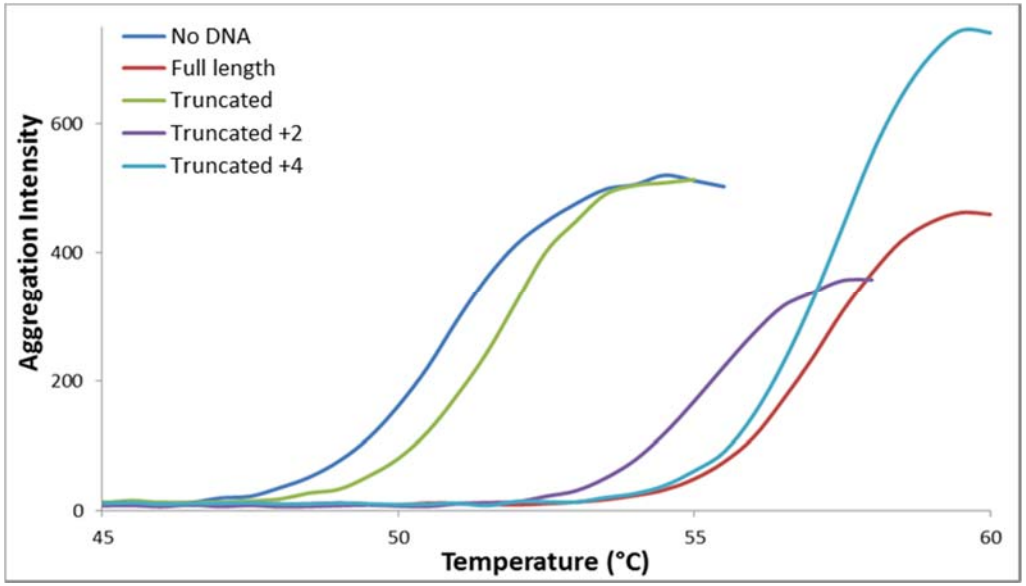
Addition of a full length DNA fragment significantly increased the protein stability due to protein-DNA interaction.

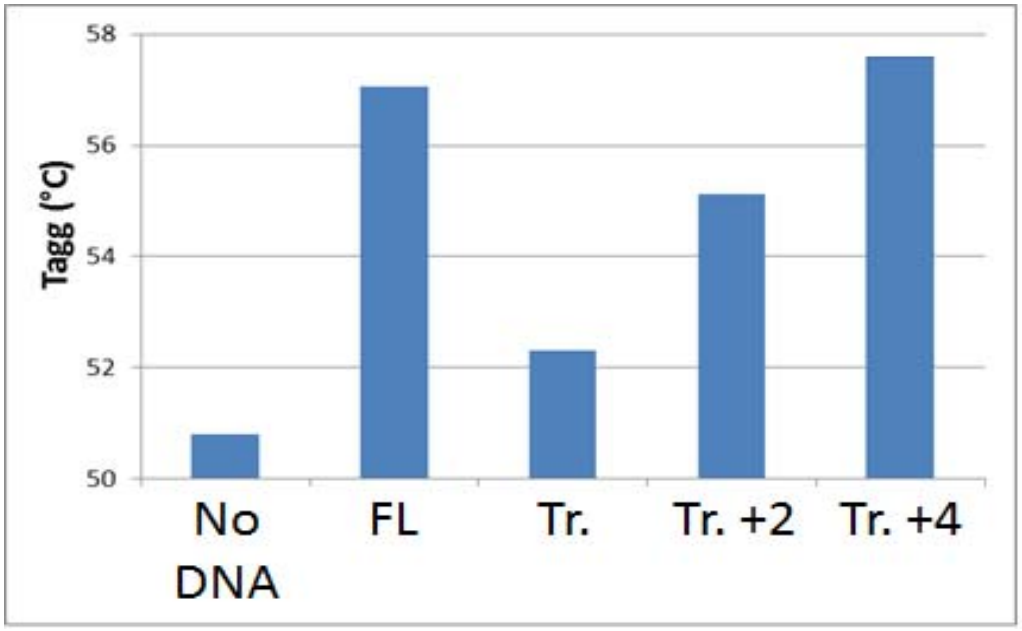
Addition of a largely truncated fragment (Tr.) only slightly increases protein stability. However, as the truncated fragment was elongated by two (Tr. +2) or four (Tr. +4) base pairs, its stabilizing effect on the protein increases.

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 thermodenaturation-based interaction mapping strategy can be similarly applied to peptide and polymer binding.





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