Introduction

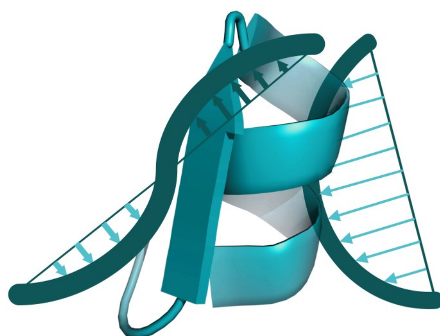
The StarGazer-2 (*Epiphyte3*) instrument is designed for low-volume, 384-well optical bottom microplates used for high throughput data collection.

- 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 #4:

Evaluation of protein refolding conditions

- Evaluate and optimize protein refolding conditions
- Fold and recover inclusion bodies

StarGazer-2— Protein Thermo-denaturation Assays

Evaluation of protein refolding

Protein refolding can be necessary when purifying recombinant proteins from a host system. During expression, the recombinant protein may aggregate into inclusion bodies, which contain mostly inactive and denatured protein. Protein refolding is one step in the purification of active and properly folded protein from inclusion bodies.

After isolation and solubilisation of the inclusion body, the unfolded protein sample can be introduced to a series of new buffer conditions in an attempt to refold it.

Some factors affecting refolding are:

- Buffer composition (pH, ionic strength)
- Other additives (e.g. detergents, chaotropic agents, sugars, L-arginine)
- Redox environment (addition of reducing and oxidizing agents, DTT, glutathione)
- Final protein concentration, incubation temperature and time

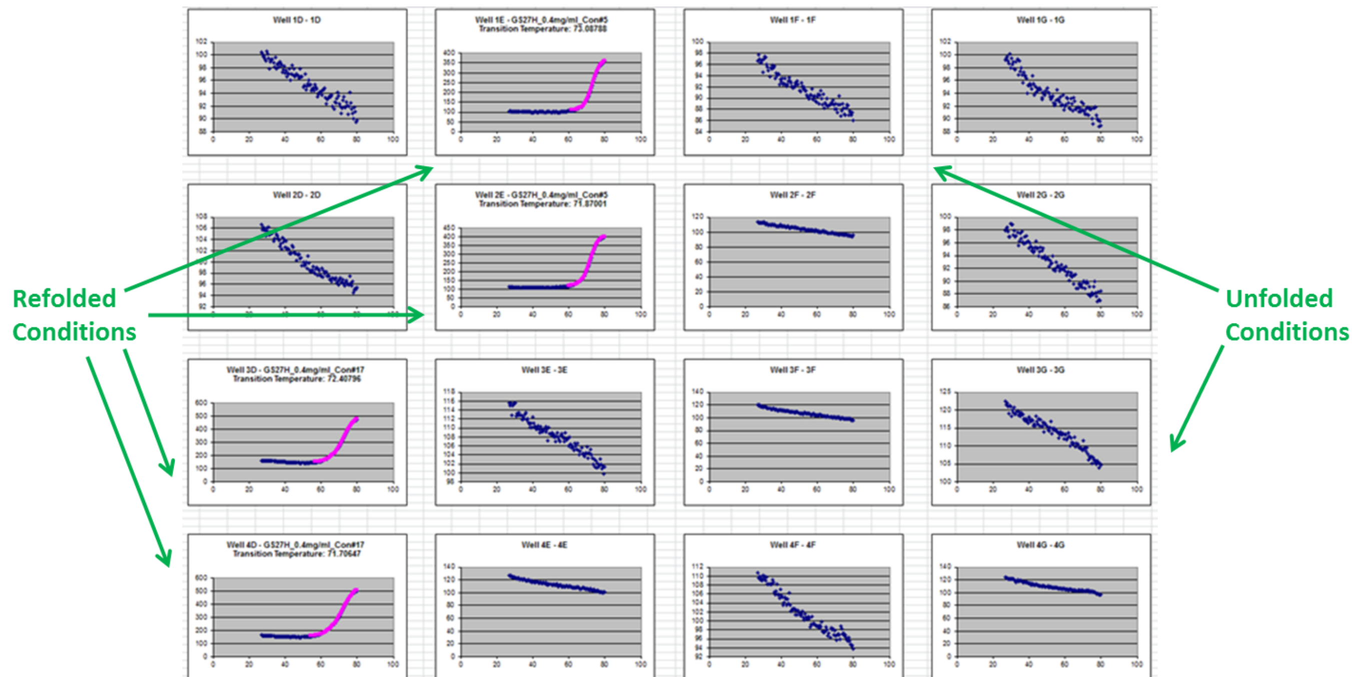
Using the StarGazer-2, many refolding conditions can be screened to verify if the protein has been refolded. Only folded protein can unfold and produce a denaturation curve.

The ideal conditions for refolding are unique for each protein and can be empirically determined using a protein refolding buffer screen.

Several commercial kits are available to screen for optimal protein refolding conditions.

General experimental set-up for protein refolding buffer evaluation

1. Dispense the refolding buffers into tubes or wells of a multi-well plate.
2. Add the unfolded protein sample to each tube or well and incubate. Incubation conditions can include temperature, time, final protein concentration and other factors. Small aliquots of unfolded protein can be added to the refolding buffer over time or the entire amount can be added at once.
3. Analyze protein for refolding by using the StarGazer-2 thermo-denaturation assay with 10 µL sample volumes and a temperature ramp rate of 1 ^{o}C/min from 25 ^{o}C to 95 ^{o}C.
4. Buffer conditions that show a protein denaturation curve indicate that the buffer successfully acts as a refolding condition.



Only folded proteins can unfold!

If there is a denaturation curve, then the protein was refolded in that condition.