HTD

BIOSYSTEMS INC.

High Throughput Development



High Throughput Tm analysis using nanoDSF

One of critical formulation parameters in protein stability is determination of the melting temperature (Tm). Various methods exist to perform this analysis, including differential scanning calorimetry (DSC), circular dichroism (CD), and UV analysis. Recently, differential scanning fluorimetry (DSF) has been utilized in Tm analysis where the thermal profile of the protein is monitored in the presence of a dye such as Sypro Orange that binds to the hydrophobic areas of the protein as it unfolds.

At HTD, we have been an early adopter, of performing Tm measurements in a high throughput fashion using a label-free nanoDSF. We were the first company in North America to invest in this technology with the Prometheus N48 in 2015 and have to date performed over 1,000 Tm measurements of various proteins.

Our instrument offers several advantages over other techniques such as

- Small sample requirements (10 uL per run)
- Wide sample concentration (5 ug/ml to 200 mg/ml)
- No external dyes or sample manipulation
- Formulation independency (i.e. no interference from excipients)
- High throughput by analyzing up to 48 samples in parallel
- High data density = High resolution data = data point every 7 seconds.
- High reproducibility (e.g Tm with S.D of ± 0.1 ^{o}C)
- High precision. We can detect transitions not possible by other techniques.

Principle of nanoDSF

The Prometheus N48 measures the intrinsic changes in the tryptophan (and tyrosine) fluorescence as the protein unfolds and this is accurately captured as the F350/F330 fluorescence ratio. Figure 1 shows the DSF profile of a protein.

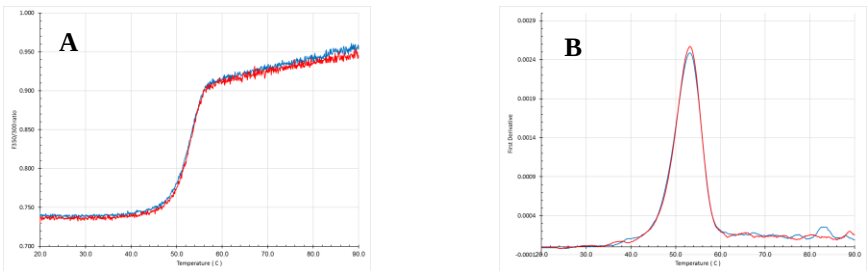
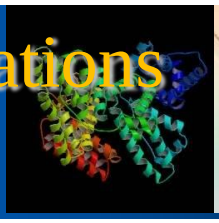


Figure 1. *(A) DSF thermal profile of a therapeutic protein. (B) First derivative of the DSF profile showing the Tm of 53.2 ± 0.0 ^{o}C.*

1061 Serpentine Lane, Suite E, Pleasanton, CA, 94566
Tel. 510.367.0528, www.htdcorp.com

NanoDSF Applications

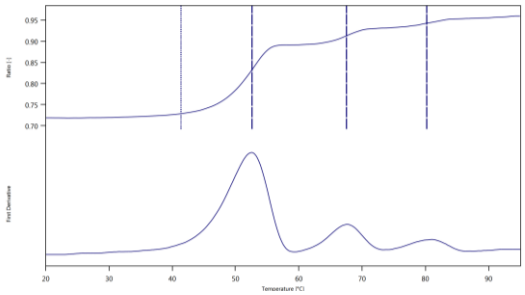


Applications for Formulation development

We have established various protocols for detailed characterization of biotherapeutic proteins. These include

1. **High throughput formulation development using the Prometheus N48 with our iFormulate technologies** to create a stability landscape for a protein. (please see iFormulate application note)

2. **High resolution biophysical characterization of multi-domain unfolding transitions (e.g. antibodies).** Figure 2 shows the different unfolding transitions of an antibody.



IgG1 onset temperature and Tm's of multiple domains.
Onset T = 49.7 oC
Tm1 = 57.9 oC
Tm2 = 68.6 oC
Tm3 = 81.8 oC

Figure 2. *DSF and 1st derivative of IgG1 antibody showing the unfolding of multiple domains.*

3. **Stability screening of research proteins and formulations.**

4. **Quality Control during manufacture and Stability analysis by quantifying amount of unfolded protein.**
   - Long term stability testing
   - Degradation during processing
   - Batch to Batch reproducibility
   - Forced degradation studies

5. **Comparative analysis of**
   - Biosimilars
   - Proteins and their conjugates
   - Antigen complexed with vaccines
   - Nanoparticles entrapped with proteins
   - Lot to lot comparisons

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