AAPS Interlaboratory Study

Tier 2 Method: **Nano Differential Scanning Fluorimetry**

Introduction
DSF is a valuable tool for conformational stability assessment. NanoDSF uses intrinsic fluorescence, mainly tryptophan and tyrosine residues, to monitor protein unfolding. A temperature ramp is applied to a protein solution to measure the temperature at which the first conformational changes start to occur (T_{onset}) and the temperature at which 50% of the protein or most temperature-sensitive protein domain is unfolded, i.e. the melting temperature (T_m). In nanoDSF, upon unfolding, the environment of those residues will alter because they become exposed to the solvent and thus, their fluorescence intensity will change. The relationship between fluorescence intensity changes and temperature gradient can be used to obtain the apparent T_m and T_{onset}. MAbs have different T_m values attributed to their different domains, but the most temperature sensitive one is normally the CH2 domain, which is the one that we will be focusing on for the T_m values reported.

This protocol briefly describes a method to measure the apparent melting temperature (T_m) and onset temperature (T_{onset}) by nano differential scanning fluorimetry (nanoDSF) to assess the conformational stability of unstressed proteins. The stir and light stressed samples will not be analyzed with DSF.

Important Notes: This protocol is not a Standard Operating Protocol and assumes the required instrument is in good working order and the analysis is performed by experienced user(s). Required calibration or check standards should be run according to the manufacturer instructions. This protocol does not contain all the details of the analysis. The analyst should rely on their best judgment, routine practices, and knowledge of the technique to conduct the study; this protocol should be used as a guideline for the analysis. Samples should be analyzed immediately after thawing and preparation.

Equipment and Materials List

- NanoDSF device
- Single use sample containers (e.g. capillaries for Prometheus, UNi for UNcle)
- Protein samples and formulation buffers supplied by sample originators
    - Buffers and **aliquots of unstressed samples ONLY** from Shipping #1
- Pipets and pipette tips

Reagents and Solutions

Table 1: The following samples and their buffers will be provided by sample originators. Buffers and aliquots of unstressed samples from Shipping #1 should be used for this analysis. They are nominally at 1 mg/mL.

Proteins	Formulations	Samples
Amgen IgG2	10 mM sodium acetate, pH 5.0	Unstressed, 1 mg/mL
NISTmAb IgG1	12.5 mM L-histidine, 12.5 mMol L-histidine HCl, pH 6.0	Unstressed, 1 mg/mL

Procedures

Instrument Set up and Sample Preparation

1. Thaw the samples from Shipping #1 and aliquot the Tier 2 vials (for the unstressed only) as described in the instructions into smaller aliquots for the various methods and freeze until ready to use. Be sure to follow the procedure for vigorously mixing the sample prior to aliquoting.

2. For this analysis, thaw the aliquots designated for nano-DSF measurements (prepared and frozen in #1) by bringing them to room temperature for no more than 15 minutes.

3. Using a pipette, fill the sample container with the volumes suggested by the manufacturer (e.g. 9μL for Uni, 10μL for capillaries of Prometheus). No dilution is required. Use a maximum of 8 samples in triplicate per run, i.e. up to 24 wells/capillaries per run.

   NOTE: Maximum of 24 wells/capillaries per run to ensure a good and constant data point density. Make sure that there are no bubbles and that the sample order matches the one inserted in the software.

4. Insert the sample container in the support provided by the manufacturer and insert it in the nanoDSF device
5. When setting up the method, use the parameters described in Table 2 and start the measurements.

Data Acquisition

1. Review the **nanoDSF Data** template for the requested information before beginning the measurements and input the set-up information.

2. Use the following experimental parameters to collect the data (default of the UNcle – quick start mode):

Table 2. Data parameters for NanoDSF

Number of samples	Up to 8 samples in triplicate per run, i.e. up to 24 wells/capillaries per run
Temperature ramp	Linear: 1°C/min
Equilibration time	5 min
Temperature range	20-95°C
Sample concentration range	1-150 mg/ml – no dilution required
Fluorescence settings	Ex: 266 nm (or equivalent device default) Em: 300 - 450nm (or equivalent device default)

2. Perform a discovery scan if necessary.
3. Attribute the sample names in the software and proceed to the melting scan.

Understanding Results

1. Data and selected instrument parameters should be entered in the **NanoDSF Data reporting Template.**
2. Use the software default method to calculate the T_{onset} and T_m automatically (e.g. barycentric mean [BCM] in range 300-450nm for the UNcle)
3. Average the temperature of the triplicates to obtain the final values and us the standard deviation to report the measurement variability

Troubleshooting

1) Ensure that there are no bubbles in the UNi/capillaries.
2) Ensure that the sample holder is properly placed in the instrument.

Further Information

For any specific questions regarding this method, please contact Vasco Filipe at Vasco.Filipe@sanofi.com. Please copy aapsinterlab@nist.gov on your email.