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Nano differential scanning fluorimetry for comparability studies of therapeutic proteins

Jie Wen*, Harrison Lord, Nicholas Knutson, Mats Wikström**

Higher Order Structure, Attribute Sciences, Thousand Oaks, CA, 91320, United States

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ABSTRACT

Differential scanning calorimetry (DSC) has been extensively used in the biopharmaceutical industry to characterize protein thermal stability and domain folding integrity. Recently, nano differential scanning fluorimetry (nanoDSF) has emerged as a powerful tool for thermal stability analysis and studies of protein domain unfolding. Due to increased interests in the qualification of characterization methods, we are in this study presenting the qualification results for the comparability studies of thermal stability analysis using nanoDSF. The results show that nanoDSF is able to detect thermal transition signals for mAbs, BiTE® molecules, and cytokines at a wide concentration range with high precision, clearly indicating that nanoDSF is suitable for characterization including comparability studies of therapeutic proteins. Compared to the current recognized industry standard DSC, the nanoDSF method enables thermal stability analysis over a much wider concentration range, consumes considerably less materials, and provides significantly higher throughput.

1. Introduction

Since Kohler and Milstein received the Nobel Prize in Medicine for their work on how to produce monoclonal antibodies (mAbs) [1], a large number of antibodies have been approved for human therapy, and an even larger set of antibodies are currently in clinical development worldwide for a variety of diseases [2–7]. Recently different modalities of therapeutic proteins, such as Bispecific T Cell Engagers (BiTE®), have emerged as potential protein therapeutics [8] with different stability profiles and therapeutic dosages as compared to mAbs. The product concentrations can therefore vary significantly due to the different modalities with therapeutic dosages from 0.05 mg/mL up to 200 mg/mL. This presents a significant analytical challenge for most biophysical methods including thermal stability analysis by differential scanning calorimetry (DSC) in comparability studies.

The comparability studies performed, due to changes in the manufacturing process, are described in the International Conference of Harmonization (ICH) guideline Q5E. Comparability studies are executed in order to ensure that a manufacturing process change will not have an adverse impact on the quality, safety, and efficacy of a biopharmaceutical product. Comparability studies provide analytical confirmation that a therapeutic protein product has highly similar quality attributes before and after manufacturing process changes.

Thermal stability of therapeutic protein products could be affected by changes in the manufacturing processes, such as changes in pH, ionic strength, and excipients [9,10]. Lower thermal stability of a product can result in a less stable product and for instance yield higher degree of aggregation, whereas higher thermal stability of a product could in principle decrease the extent of aggregation. Thermal stability analysis is traditionally performed using differential scanning calorimetry (DSC). However, DSC measurements are often limited to a certain concentration range and require large amounts of material.

Recently nano differential scanning fluorimetry (nanoDSF) has been introduced for thermal stability analysis. Compared to DSC, nanoDSF has significant advantages in terms of the applicable concentration range, sample consumption, and throughput. Differential scanning fluorimetry (DSF) is a fluorescence-based assay for thermal stability analysis. DSF was established as a high throughput method to study thermal shifts caused by ligand-binding as a tool for drug discovery by Pantoliano et al. [11]. It was also used in different applications such as the assessment of the stability of proteins [12–17] and DNA [18]. These studies demonstrated the potential uses of the DSF technique. There are two general types of the fluorescence-based assays: intrinsic and extrinsic fluorescence. This paper is focusing on the application of the intrinsic fluorescence-based assay using a Prometheus NT.48 nanoDSF (NanoTemper Technologies).

* Corresponding author. Amgen, Inc. Thousand Oaks, CA, 91320, United States.
** Corresponding author.
E-mail addresses: jwen@amgen.com (J. Wen), matsw@amgen.com (M. Wikström).

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Abbreviations

DSF	differential scanning fluorimetry
nanoDSF	nano differential scanning fluorimetry
DSC	differential scanning calorimetry
mAb	monoclonal antibody
BiTE®	bispecific T cell engagers
T_m	thermal transition temperature or thermal unfolding (melting) temperature

In this paper, nanoDSF is evaluated for its applications in comparability studies for thermal stability analysis. The specificity and precision of the nanoDSF method are accessed, and the equivalence acceptance criteria (EAC) for comparability studies are established. The results obtained by studying different structural classes of therapeutic proteins demonstrate that nanoDSF represents a suitable method for thermal stability comparability studies of therapeutic proteins.

2. Materials and methods

2.1. Materials and sample preparation

Seven different mAbs, four BiTE® molecules, and two cytokines were included in the study. The samples were prepared at the original concentrations or at the desired concentrations by diluting the original samples using the corresponding formulation buffer. The protein sample concentrations were determined by measuring protein absorbance at 280 nm using a calibrated UV–Vis spectrophotometer and applying Beer's Law or directly measured by Solo VPE (C Technologies, Inc) using the individual extinction coefficients. For each sample, at least 100–200 μL of protein was prepared for replicate measurements. nanoDSF can directly measure the thermal stability of proteins at a wide range of concentration.

2.2. nanoDSF method

There are two types of the fluorescence-based assays using either intrinsic or extrinsic fluorescence. The extrinsic assay, such as Thermal Shift Assay (TSA) (the early version of Differential Scanning Fluorimetry (DSF)), uses an external dye to measure the fluorescence intensity ratio. The nano Differential Scanning Fluorimetry (nanoDSF) is based on the intrinsic fluorescence and is therefore related to the TSA method. Both nanoDSF and TSA methods measure the fluorescence intensity ratio and use about 10 μL per sample, but the nanoDSF method doesn't need to add an external dye. Proteins containing aromatic amino acid residues (tryptophan, tyrosine, and phenylalanine) show intrinsic fluorescence. When a molecule unfolds, the locations of the aromatic amino acid residues change and cause changes in the fluorescence spectra. The fluorescence spectra of the tryptophan residues, which are buried in the hydrophobic core of a protein, can have a 10–20 nm shift compared to those tryptophans on the surface of the protein.

nanoDSF measures the changes in intrinsic fluorescence intensity ratio (350:330 nm) as a function of temperature. During a nanoDSF scan, the intrinsic fluorescence intensity ratio (350:330 nm) are continuously measured and recorded. Plotting the intrinsic fluorescence intensity ratio (or the first derivative of the ratio) as a function of temperature yields a nanoDSF thermogram. The thermal transition (unfolding) temperature (T_m) is obtained in the post-run data analysis. The T_m values can be used to assess the thermal stability of the domains of a protein.

A Prometheus NT.48 nanoDSF (NanoTemper Technologies) was used for the studies in this paper. For each experiment, it is recommended to run the following set in a single run: 2 buffers and at least 3 replicates of each sample. For a comparability study, the samples typically have pre-change and post change lots from manufacturing processes. In such cases, at least five replicates are required for each process (i.e., pre-change process and post change process) in order to perform the statistical analysis. An additional option would be to add a reference standard (RS) for the comparability analysis. In addition, side-by-side tests are required for the comparability studies since different runs may potentially increase the variability in the experimental output.

2.3. nanoDSF data analysis

The nanoDSF data analysis was performed using PR.ThermControl v2.0.4 software (NanoTemper Technologies). The statistical analysis was executed using the JMP 13.0.0 software (SAS Institute).

2.4. DSC method

Differential scanning calorimetry (DSC) measures heat capacity as a function of temperature. During a DSC scan, the heat capacity of a sample cell (containing protein) is compared to that of a reference cell (lacking protein) while the temperature of both cells is gradually increased. Plotting heat capacity as a function of temperature yields what is referred to as a DSC thermogram. The thermal transition (unfolding) temperature (T_m) is obtained in the post-run data analysis. The T_m values can be used to assess the thermal stability of the domains of a protein. The DSC method uses as much as 400 μL per sample depending on the type of DSC instrument.

3. Results and discussion

Different structural classes of proteins, monoclonal antibodies (mAbs), bi-specific T-cell engagers (BiTE®), and cytokines, were evaluated. We first determined the specificity of the nanoDSF method for thermal stability analysis and then we assessed the precision of the method.

3.1. Specificity

The specificity of nanoDSF is defined as the ability of the method to measure analyte (protein) signals accurately in the sample solution and to be able to distinguish the protein from the formulation buffer profile.

The raw thermograms of the formulation buffer and mAb-1 are shown in Fig. 1. The black trace is the formulation buffer alone and the

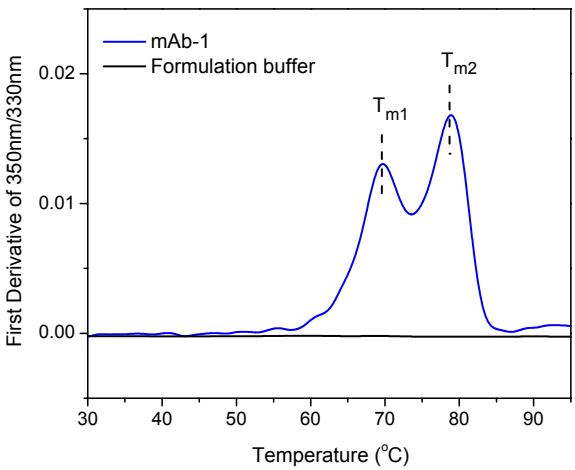

Fig. 1. mAb-1 Raw Thermogram Overlaid with the Formulation Buffer Raw Thermogram. Y-axis represents the first derivative of fluorescence intensity ratio 350 nm/330 nm.

blue trace is mAb-1 in the same formulation buffer. The difference between the blue trace and the black trace shows the distinct signals attributable to the unfolding transitions of the C_H2 domains (T_{m1}) and the Fab/C_H3 domain (T_{m2}) of mAb-1, which is similar to the previously published mAb profiles using DSC [13,19,20]. The specificity of detecting the mAb-1 thermal transition signals by the nanoDSF method is therefore demonstrated.

BiTE® molecules are fusion proteins consisting of two single-chain variable fragments (scFvs) of different antibodies. One scFv of a BiTE® molecule is designed with affinity for the CD3 T-cell binding and the other scFv has affinity for the target-cell binding. The raw thermograms of the formulation buffer and BiTE molecule-1 molecule are shown in Fig. 2. The black trace is the formulation buffer alone and the blue trace represents the BiTE molecule-1 molecule in the same formulation buffer. The difference between the blue trace and the black trace shows the distinct signals attributable to the unfolding transition of the CD3 T-cell binding domain and the target-cell binding domain. The specificity of detecting the BiTE molecule-1 molecule thermal transition signals by the nanoDSF method is therefore demonstrated.

The specificity of six additional mAbs, three additional BiTE® molecules, and two cytokines were also studied (data not shown). For all of the proteins analyzed, the nanoDSF method is able to distinguish the thermal transitions of the proteins from the formulation buffer profiles. The results demonstrate the specificity of the nanoDSF method for accessing thermal transition signals of these mAbs, BiTE® molecules, and cytokines.

3.2. Comparison of DSC and nanoDSF

DSC has previously been qualified and applied for thermal stability analysis [21], and is considered the gold standard method for thermal stability analysis. In order to ensure that the thermal transitions observed when applying nanoDSF are similar to the thermal transitions obtained by DSC, the same sets of samples were also analyzed by DSC. The comparison of the nanoDSF and DSC thermograms of mAb-1 and BiTE molecule-1 are shown in Figs. 3–4, respectively. The results show similar thermal transitions for mAb-1 and BiTE molecule-1 when analyzed by nanoDSF and DSC, respectively.

For most of protein unfolding transitions measured by DSC, the thermal transition signals show positive signals in the DSC thermograms, which indicate that the thermal transitions are endothermic. For nanoDSF, the thermal transition signals can be either positive or negative since nanoDSF measures the intensity ratio of the fluorescence at 350 nm/330 nm. Buried tryptophans result in a red-shift upon unfolding and exposure to buffer, i.e., the maximum emission intensity shifts to longer wavelengths when Tryptophan (Trp) residues are exposed to more polar environments, resulting in positive thermal transition signals in the nanoDSF thermograms. In contrast, surface-exposed tryptophans result in blue-shift or no shift upon unfolding, i.e., maximum emission intensity shifts to shorter wavelengths or doesn't shift when Trp residues are less exposed to polar environments or no significant change in the polar environments, resulting in negative thermal transition signals or no signals in the nanoDSF thermograms. The comparison of the nanoDSF and DSC thermograms of mAb-2 are shown in Fig. 5. The Fab domain of mAb-2 shows a negative thermal transition signal due to the blue-shift as described above. However, there are a total of three thermal transitions for mAb-2 in both the nanoDSF and the DSC thermograms.

Both nanoDSF and DSC can be used for thermal stability analysis in comparability studies. However, there are some advantages of using nanoDSF for thermal stability analysis in terms of the sample concentration range, the sample volume, and the throughput. nanoDSF allows analysis of samples ranging from 0.05 to 200 mg/mL while DSC experiments are usually performed in the concentration range of 0.2–2 mg/mL. Furthermore, nanoDSF uses considerably less volume, 10 µL/per sample, while DSC typically uses 400 µL/per sample. Finally, nanoDSF is able to run 48 samples in about 2 h while DSC can only handle 1 sample in about 2 h.

3.3. Precision

In order to demonstrate that the measurements from the nanoDSF method is precise enough to provide reliable data, the precision of the nanoDSF method was evaluated. The method precision is measured by accessing its repeatability and is estimated as the square root of the residual variance. Different lots of various structural classes of proteins as well as different concentrations were analyzed. The side-by-side testing is recommended for the comparability study since different runs may potentially increase the variability in the experimental output. The repeatability results were obtained by the side-by-side testing method unless otherwise specified.

The precision of mAb-1 (1.0 mg/mL) was studied and the results are shown in Table 1. The T_{m1} represents the unfolding transition of the C_H2 domains and T_{m2} corresponds to the unfolding transition of the Fab/C_H3 domain. The transition of the C_H3 domain is not well resolved from that of the Fab domain. The repeatability of the T_{m1} measurements is 0.2 °C and the repeatability of the T_{m2} measurements is 0.1 °C, suggesting that the nanoDSF can measure the T_m values of this mAb with reliable precisions.

The results of the repeatability values obtained for each domain of mAbs are presented in Figs. 6–8. Fig. 6 shows the repeatability values of the Fab domain transition. In some cases, Fab domain transition may overlap with the C_H2 or C_H3 transitions (Fab/C_H2 or Fab/C_H3). The data in Fig. 6 also includes the repeatability values of the Fab/C_H2 or Fab/C_H3 domains. To establish general repeatability values (or "universal repeatability values" as used in this paper) for the Fab domain, we performed a statistical evaluation of the repeatability data in Fig. 6 applying 95% confidence interval. The repeatability value of 0.2 °C was obtained as a universal repeatability value for the T_m measurements of the Fab domain using the nanoDSF method. The repeatability value is rounded to one decimal place since the nanoDSF data are reported using only one decimal place.

Figs. 7 and 8 show the repeatability values of the C_H2 and C_H3 domains, respectively. The universal repeatability of the nanoDSF method for the T_m measurements of the C_H2 and C_H3 domains were determined to be 0.2 °C and 0.3 °C, respectively, by applying the same procedures as described above for the Fab domain. Once again, the repeatability value is rounded to one decimal place since the nanoDSF data are reported using only one decimal place.

The precision, in term of repeatability, of BiTE molecule-1 (0.5 mg/

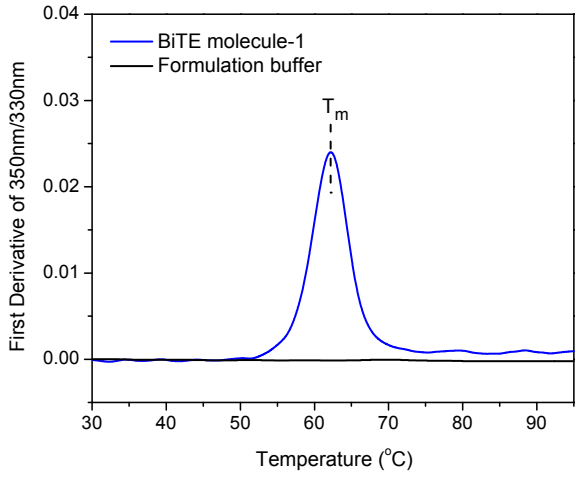

Fig. 2. BiTE Molecule-1 Raw Thermogram Overlaid with the Formulation Buffer Raw Thermogram. Y-axis represents the first derivative of fluorescence intensity ratio 350 nm/330 nm.

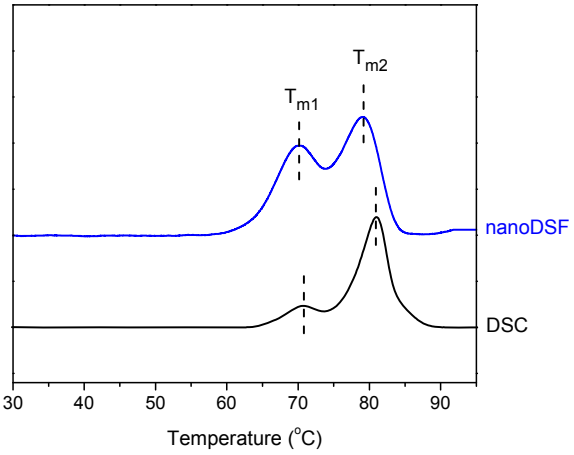

Fig. 3. Comparison of the nanoDSF and DSC Thermograms of mAb-1. Arbitrary Y units (the signals are plotted from figures with two different units). nanoDSF T_{m1} = 69.7 °C and T_{m2} = 79.2 °C; DSC T_{m1} = 71.0 °C and T_{m2} = 81.1 °C.

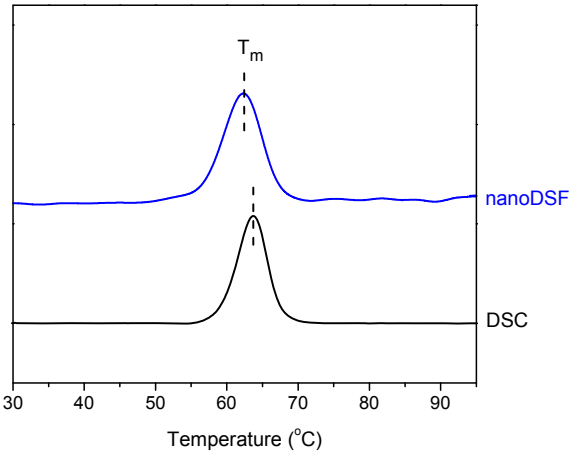

Fig. 4. Comparison of the nanoDSF and DSC Thermograms of BiTE Molecule-1. Arbitrary Y units (the signals are plotted from figures with two different units). nanoDSF T_m = 62.1 °C and DSC T_m = 63.7 °C.

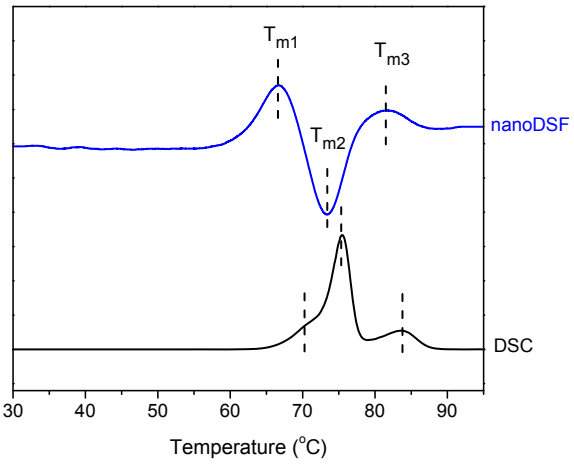

Fig. 5. Comparison of the nanoDSF and DSC Thermograms of mAb-2. Arbitrary Y units (the signals are plotted from figures with two different units). nanoDSF T_{m1} = 66.5 °C, T_{m2} = 73.3 °C and T_{m3} = 81.5 °C; DSC T_{m1} = 70.5 °C, T_{m2} = 75.5 °C and T_{m3} = 83.6 °C.

Table 1
The Repeatability of T_m Measurements of mAb-1 (1 mg/mL).

Sample	Replicate	T_{m1} (°C)	T_{m2} (°C)
mAb-1	Replicate 1	70.0	79.2
mAb-1	Replicate 2	69.8	79.4
mAb-1	Replicate 3	69.6	79.1
mAb-1	Replicate 4	69.6	79.1
mAb-1	Replicate 5	69.5	79.2
mAb-1	Replicate 6	69.8	79.0
mAb-1	Replicate 7	69.6	79.2
Average		69.7	79.2
Repeatability		0.2	0.1

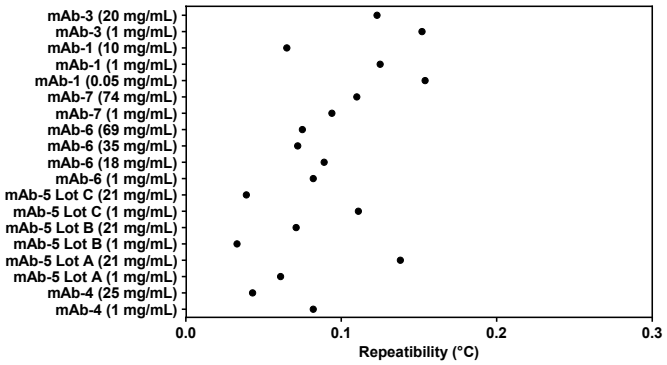

Fig. 6. The Repeatability Values of the Fab Domain of mAbs. Six different mAbs at different concentrations were studied. X-axis represents the repeatability values. Y-axis represents different mAbs at different concentrations (from bottom to top): mAb-4 (1 and 25 mg/mL), mAb-5 Lot A (1 and 21 mg/mL), mAb-5 Lot B (1 and 21 mg/mL), mAb-5 Lot C (1 and 21 mg/mL), mAb-6 (1 and 18 mg/mL), mAb-6 (35 and 69 mg/mL), mAb-7 (1 and 74 mg/mL), mAb-1 (0.05, 1, and 10 mg/mL), and mAb-3 (1 and 20 mg/mL).

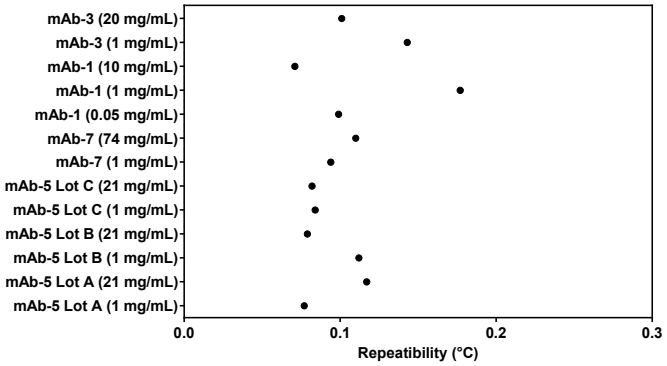

Fig. 7. The Repeatability Values of the C_H2 Domain of mAbs. Four different mAbs at different concentrations were studied. X-axis represents the repeatability values. Y-axis represents different mAbs at different concentrations (from bottom to top): mAb-5 Lot A (1 and 21 mg/mL), mAb-5 Lot B (1 and 21 mg/mL), mAb-5 Lot C (1 and 21 mg/mL), mAb-7 (1 and 74 mg/mL), mAb-1 (0.05, 1, and 10 mg/mL), and mAb-3 (1 and 20 mg/mL).

mL) was studied and the results are shown in Table 2. For this molecule, only one endothermic thermal transition is observed. The calculated repeatability of the T_m measurement is 0.1 °C. This suggests that the nanoDSF can measure the T_m values of this BiTE® molecule with reliable precision.

The results of the repeatability values obtained for different BiTE® molecules are presented in Fig. 9. Four different BiTE® molecules at different concentrations were studied. The universal repeatability of the nanoDSF method for the T_m measurements of the BiTE® molecules are 0.2 °C by the same procedures described above for the Fab domain.

The repeatability of nanoDSF when applied to two different

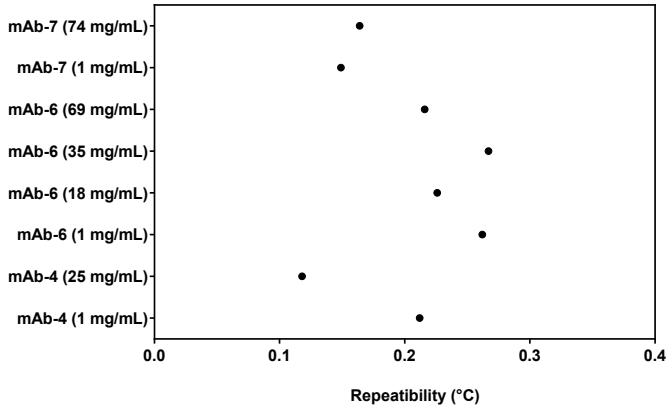

Fig. 8. The Repeatability Values of the C_H3 Domain of mAbs. Three different mAbs at different concentrations were studied. X-axis represents the repeatability values. Y-axis represents different mAbs at different concentrations (from bottom to top): mAb-4 (1 and 25 mg/mL), mAb-6 (1 and 18 mg/mL), mAb-6 (35 and 69 mg/mL), and mAb-7 (1 and 74 mg/mL).

Table 2
The repeatability of T_m measurements of BiTE Molecule-1 (0.5 mg/mL).

Sample	Replicate	T_{m1} (°C)
BiTE molecule-1	Replicate 1	62.2
BiTE molecule-1	Replicate 2	62.2
BiTE molecule-1	Replicate 3	62.1
BiTE molecule-1	Replicate 4	62.2
BiTE molecule-1	Replicate 5	62.1
BiTE molecule-1	Replicate 6	62.1
BiTE molecule-1	Replicate 7	62.1
Average		62.1
Repeatability		0.1

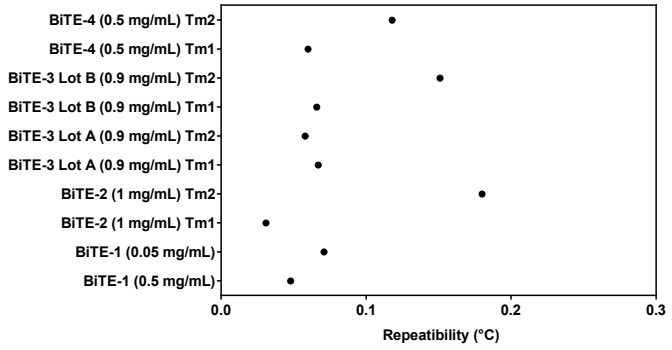

Fig. 9. The Repeatability Values of the Unfolding Domain(s) of BiTE® molecules. Four different BiTE® molecules at different concentrations were studied. X-axis represents the repeatability values. Y-axis represents different BiTE® molecules at different concentrations (from bottom to top): BiTE molecule-1 (0.5 and 0.05 mg/mL), BiTE molecule-2 (1 mg/mL), BiTE molecule-3 Lot A (0.9 mg/mL), BiTE molecule-3 Lot B (0.9 mg/mL), and BiTE molecule-4 (0.5 mg/mL).

therapeutic cytokines was also investigated. The repeatability of cytokine-1 is 0.4 °C and repeatability of cytokine-2 is 0.1 °C. The values vary significantly for this class of molecules compared to mAbs and BiTE® molecules. This may be due to that the primary sequences and the presence of aromatic amino acid residues (tryptophan, tyrosine, and phenylalanine) of cytokines display a high degree of variation. Therefore, the universal repeatability and EACs of cytokines are not further discussed in this paper. When the nanoDSF method is used for studying cytokines, we recommend qualifying each molecule individually. The summary of the repeatability values of the unfolding domains of mAbs, BiTE® molecules, and two individual cytokines are presented in Table 4.

Table 3
The Repeatability and EAC of the nanoDSF Method.

Domain Class	T_m Repeatability (°C)	T_m EAC (°C)
Fab domain (or Fab/C_H2 or Fab/C_H3)	0.2	± 0.6
C_H2 domain	0.2	± 0.6
C_H3 domain	0.3	± 0.9
Unfolding domain(s) of BiTE® molecules	0.2	± 0.6
Cytokine-1[a]	0.4	± 1.2
Cytokine-2[a]	0.1	± 0.3

[a] The repeatability and EAC values of cytokines in this table are the individual values. See the previous section for the details of the discussions.

Table 4
nanoDSF T_m Results of mAb-3.

Sample Lot	Replicate	T_{m1} (°C)	T_{m2} (°C)
Pre-change Lot 1	Replicate 1	69.2	80.7
Pre-change Lot 1	Replicate 2	69.1	80.9
Pre-change Lot 2	Replicate 1	69.2	80.9
Pre-change Lot 2	Replicate 2	69.2	80.7
Pre-change Lot 3	Replicate 1	69.0	80.7
Pre-change Lot 3	Replicate 2	69.3	80.6
Post-change Lot 1	Replicate 1	69.1	80.6
Post-change Lot 1	Replicate 2	69.0	80.8
Post-change Lot 2	Replicate 1	69.3	80.7
Post-change Lot 2	Replicate 2	69.3	80.8
Post-change Lot 3	Replicate 1	69.2	80.7
Post-change Lot 3	Replicate 2	69.1	80.7

3.4. Equivalence acceptance criteria

In order to perform an equivalence testing, the two one-sided *t*-test (TOST) approach were used. To apply the TOST equivalence approach for comparability study, an equivalence acceptance criterion (EAC) need to be established. If the confidence interval on the difference in means between the two samples (or processes) evaluated is contained completely within the EAC, then the two samples are considered to be equivalent.

The EAC values applied to the nanoDSF method are established using the universal repeatability of the nanoDSF method and are defined as three times the repeatability. The EAC results of different thermal unfolding domains are shown in Table 4. Statistical power calculations were performed for the T_m statistical analysis. A minimum of 5 replicates per process should be collected, which provide sufficient statistical power for the use the EACs to establish equivalence. Six replicates are usually recommended when performing comparability studies.

3.5. Method verification or qualification for new products

In order to demonstrate that the EACs defined in Table 3 apply to new products, a streamlined verification approach is suggested. We recommend running at least 6 replicates in the verification approach. The repeatability values of the new product should be compared to the verification criteria, i.e., the repeatability values listed in Table 3. If the repeatability values of each unfolding domain of the new product are equal or less than the repeatability values in Table 3, the new product has then passed the verification criteria, indicating that the universal EACs in Table 3 can be applied to the product in future studies.

However, if any repeatability values of the unfolding domains of the new product are greater than the verification criteria in Table 3, repeated experiments need be performed to ensure data reliability. In such cases, all of data acquired should be analyzed to determine the applicable repeatability value as described in the Precision section.

Table 5
Tabular Summary of DSF Equivalence Results (Pre-Change vs. Post-Change).

Parameter (°C)	Pre-Change Mean	Post-Change Mean	Difference in Means	Lower 95% Confidence Bound on Difference in Means	Upper 95% Confidence Bound on Difference in Means	EAC (°C)	Conclusion
T_{m1}	69.1882	69.1725	−0.0157	−0.1267	0.0953	± 0.6	Statistically Equivalent
T_{m2}	80.7417	80.7115	−0.0302	−0.1349	0.0746	± 0.6	Statistically Equivalent

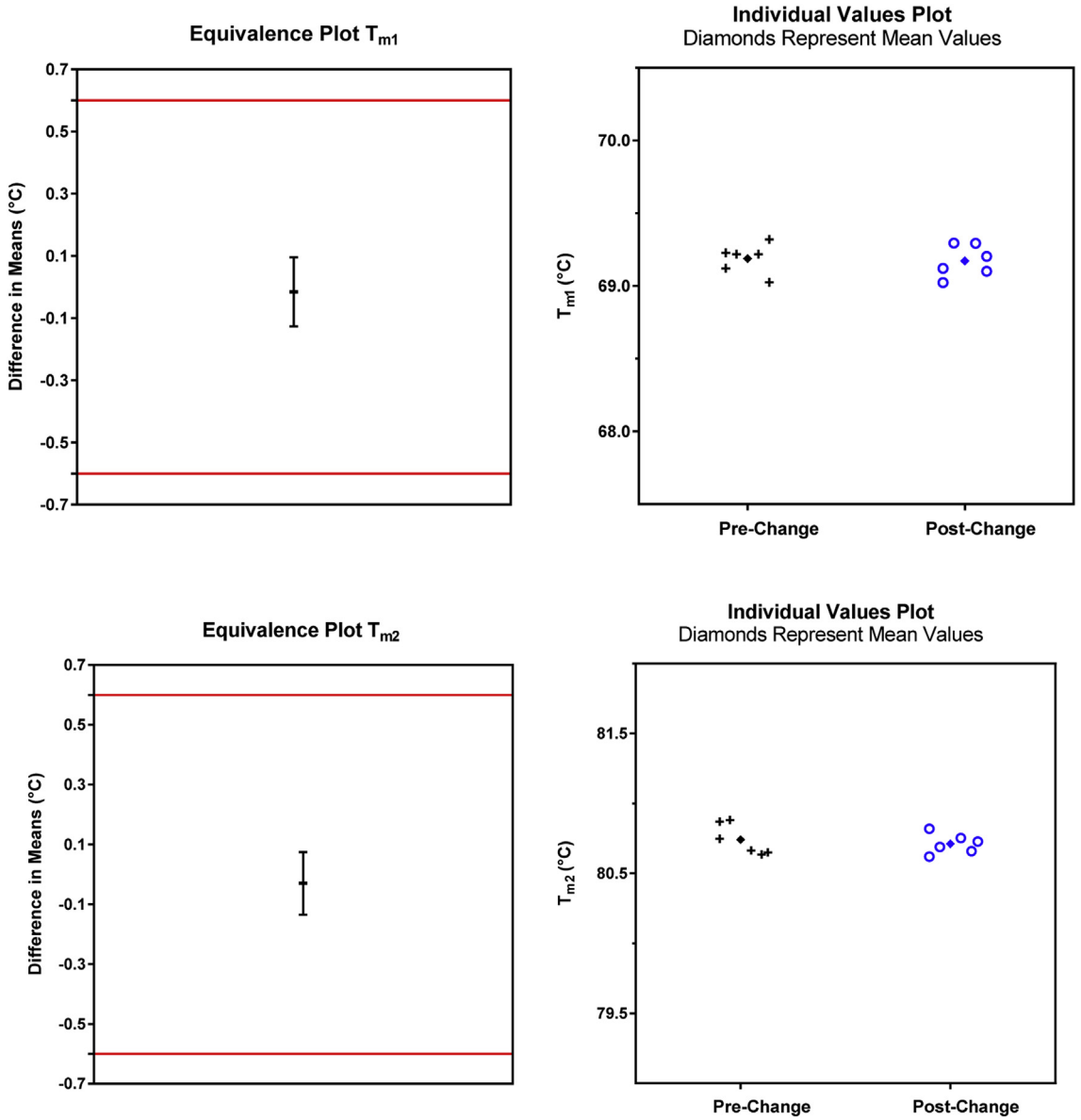

Fig. 10. Graphical Summary of nanoDSF T_{m1} and T_{m2} Equivalence Results (Pre-Change vs. Post-Change). Left plots in the figure: The EAC limits are shown by the red horizontal traces and the confidence interval for the mean difference is shown by the black traces. Right plots in the figure: The individual T_m values of the pre-change process and the post-change process are indicated by + and o symbols, respectively, and the ♦ symbols represent the mean values of each process. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

3.6. Low concentration consideration

Small differences in the formulation conditions between different samples may cause a significant difference in the observed T_m values. If the protein samples under study are at high concentrations, they can be diluted using the same formulation buffer to match the buffer conditions for the different samples. However, this approach is not feasible in situations where the concentrations of the samples are too low to dilute, resulting in different formulation conditions. In such cases, the difference of the means of the observed T_m may increase to 0.1–1.0 °C due to the differences in buffer compositions between the samples and therefore not related to the differences between the thermal stability of the protein samples. To take this effect into account, in the cases that the buffer conditions cannot be matched, we suggest that an additional range of 0.1–1.0 °C may be added to the EACs.

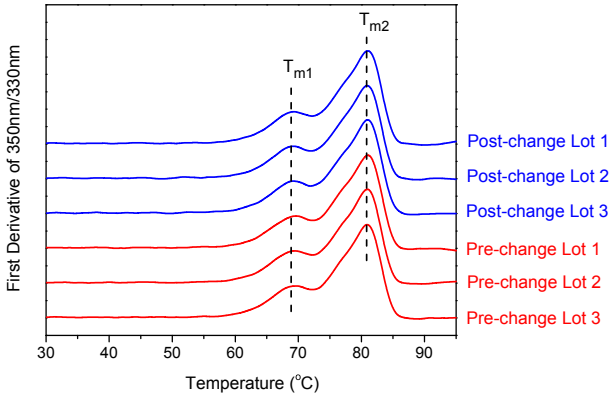

Fig. 11. nanoDSF thermograms of the mAb-3 comparability study. The traces of the pre-change lots are shown in red and the traces of the post-change lots are shown in blue. Y-axis represents the first derivative of fluorescence intensity ratio 350 nm/330 nm. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

3.7. Case study: nanoDSF for the comparability study of mAb-3

A site change is shown here as an example using the nanoDSF method for a comparability study. The mAb-3 was initially manufactured at site 1 and later transferred to manufacturing site 2. The nanoDSF method was used as a part of the complete comparability assessment package. The pre-change lots were from site 1 and the post change lots were from site 2. Six measurements were collected from each process (or each site in this case).

The T_m results of the nanoDSF study are listed in Table 4. T_{m1} represents the unfolding transition of the C_H2 domain and T_{m2} corresponds to the unfolding transition of the Fab/C_H3 domain. The unfolding transition of the C_H3 domain is not well resolved from the unfolding transition of the Fab domain. The acceptance criteria (EAC) described in Table 3 are used in this study and the summary of the equivalence results for the lots are shown in Table 5. In addition, the results are also shown graphically in the left-hand plot in Fig. 10. In these plots, the EAC limits are shown by the red horizontal traces, and the confidence interval for the mean difference is shown by the black traces, which are within the EAC. The plots on the right-hand side of the figures show the raw data for the pre-change and post-change lots. The thermograms are shown in Fig. 11, indicating that the profiles are visually similar. The nanoDSF data demonstrate that the two one-sided 95% confidence bounds on the difference between the mean values of the pre-change and the post-change lots fall entirely within the predefined EAC limits (T_m: ± 0.6 °C). Therefore, the thermal stability of the pre-change and post-change lots is comparable.

4. Conclusions

nanoDSF has emerged as a powerful tool for thermal stability analysis and domain folding integrity of therapeutic proteins. We have qualified nanoDSF for thermal stability analysis and exemplified its utility in comparability studies. Our qualification results demonstrate that nanoDSF is able to detect thermal transition signals for mAbs, BiTE® molecules, and cytokines at a wide concentration range with high precision. Compared to the current industry standard DSC, the nanoDSF method enables thermal stability analysis for a much wider concentration range, consumes considerably less materials, and provides significantly higher throughput. Based on our qualification results, we conclude that the nanoDSF method is suitable for the thermal stability analysis for all studied protein modalities and its applications in comparability studies.

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