Thermal Unfolding of Antibodies

Application Note NT-PR-006

Comparison of nanoDSF and µDSC for thermal stability assessment during biopharmaceutical formulation development

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Abstract

The assessment of thermal stability parameters of biologics is an integral part of formulation development in biopharmaceutical research. The ever growing number of biologics in the development pipelines worldwide demands rapid and precise methods to quickly screen large sets of conditions in an easy and straight-forward manner.

In our study, we compare two methods for the detection of thermal unfolding transition temperatures (T_m) of a therapeutic monoclonal antibody (mAb): nanoDSF, which analyzes changes in the fluorescence emission properties of proteins, and differential scanning calorimetry (µDSC), which detects changes in the heat capacity of a protein solution upon unfolding. nanoDSF and µDSC both provide precise and consistent data. nanoDSF in addition overcomes several limitations by µDSC, such as low throughput and high sample consumption, and thus represents the ideal technology for rapid and precise thermal stability screening in biopharmaceutical development.

Introduction

The thermal stability of a protein is routinely used as one main indicator for its physical stability which affects long-term storage in a given formulation. Historically, µDSC has been used during formulation development. This approach measures changes in the heat capacity (C_p) of a protein-containing solution relative to a reference solution. µDSC can be used to calculate unfolding transition temperatures as well as thermodynamic stability parameters. Although typically considered the gold standard for thermal stability measurements, µDSC has several drawbacks. Due to technical reasons rigorous equilibration and calibration is required, which preclude a parallelization of measurements so that samples have to be measured one-at-a-time. Moreover, the concentration range is limited to about 0.5 to 5 mg/ml mAb. Sample volumes of typically hundreds of µl per sample are required, which sum up quickly in screening campaigns. Thus, a pre-selection of conditions is often performed to minimize sample consumption. In order to optimize the screening procedure for the identification of ideal formulation conditions, a higher throughput and lower sample consumption are desired while maintaining precise T_m detection.

The Prometheus NT.48 instrument fills this gap by analyzing protein unfolding transitions based on high-precision detection of intrinsic fluorescence changes. This truly label-free approach allows for the parallelized detection of up to 48 samples with concentrations ranging from 10 µg/ml to more than 250 mg/ml without buffer restrictions. It does not require the addition of extrinsic fluorescent dyes like in the classical DSF technique, avoiding potential detrimental interactions of dye and protein or excipients. The innovative dual-UV detection method enables rapid scanning of samples, which results in a very high datapoint density of 20 or more datapoints per °C depending on the steepness of the temperature ramp.

To directly compare the precision and overlap of the T_m-determination by µDSC and nanoDSF, we conducted a small formulation screen using a commercial, therapeutic mAb.

Results

We analyzed thermal unfolding of the mAb in a total of ten different formulations with varying buffers and pH-values. We also analyzed thermal unfolding in presence of polysorbate 20 and 80 (PS20 and PS80, respectively), which are common surfactants for the majority of mAb formulations, but preclude the analysis by orthogonal fluorescence methods such as DSF assays (will be reported elsewhere).

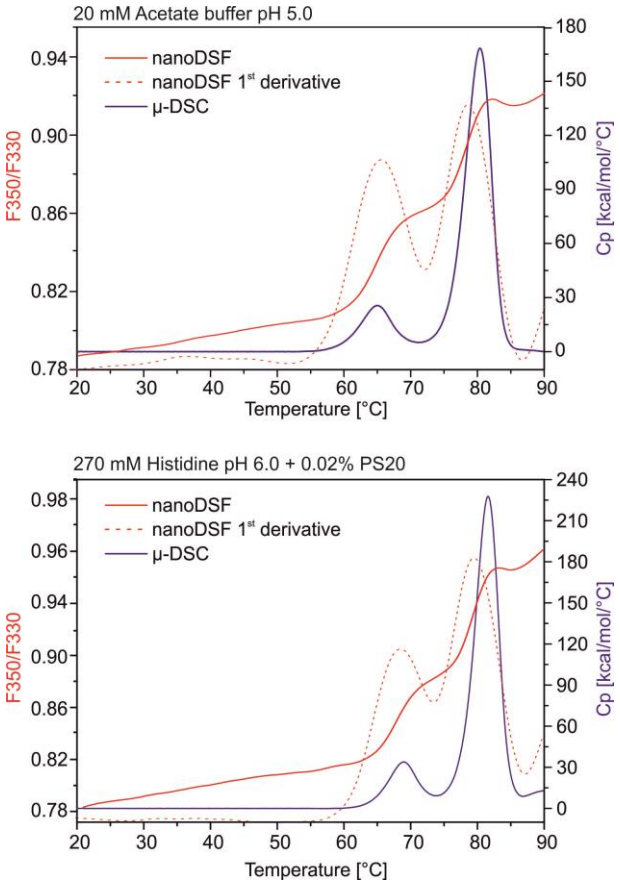
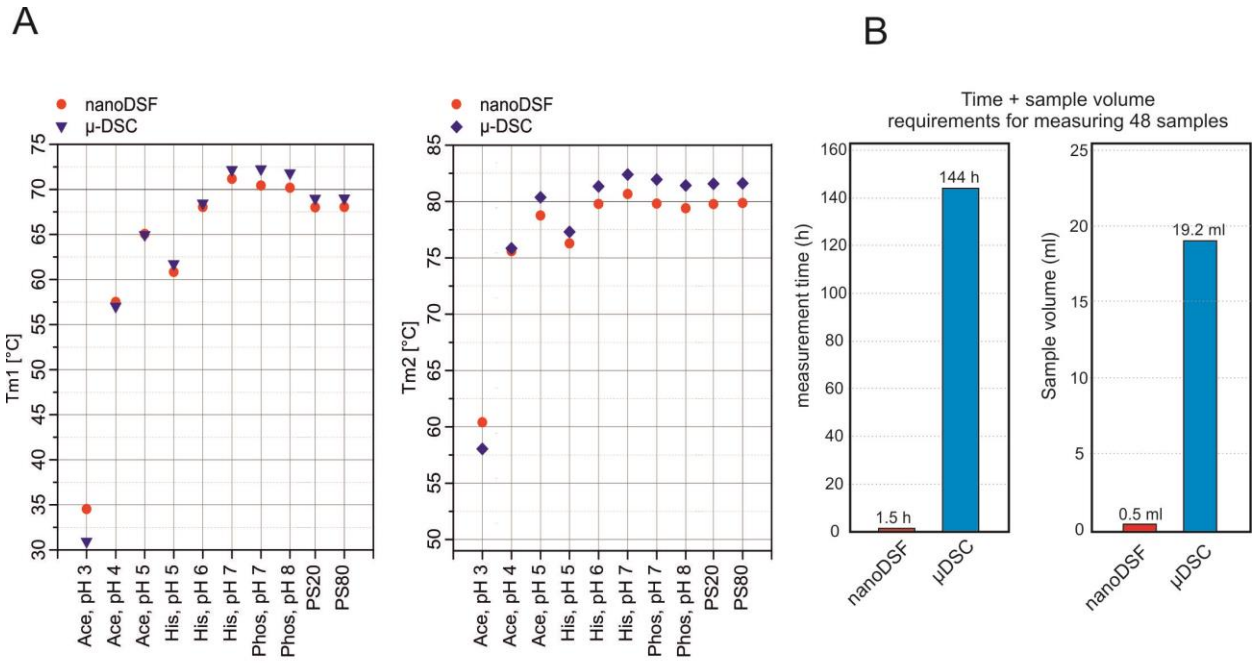


Figure 1: Thermal unfolding data for a mAb at a concentration of 1 mg/ml in two different buffer conditions, recorded by nanoDSF (red) and µDSC (blue). nanoDSF T_m values are determined from the transition midpoints (corresponding to peaks in the first derivative of the F350/F330 data, red dotted line). µDSC T_m values are determined from peaks in the heat capacity C_p.

Representative data for the thermal unfolding experiments by µDSC and nanoDSF are shown in Figure 1. For nanoDSF, samples were measured in triplicates, while samples where measured only once with µDSC to save time and sample material. µDSC detects unfolding transitions in temperature gradients as distinct “peaks” in the heat capacity of the solution in respect to a reference measurement. In contrast, nanoDSF detects unfolding events by recording changes in the emission properties of the environment-sensitive amino acids tryptophan and tyrosine. Typically, exposure of tryptophan residues from the hydrophobic protein core to the aqueous formulation results in a shift of the emission maximum to higher wavelengths, and therefore to an increase in the F350/F330 ratio.

Both methods, nanoDSF and µDSC are very sensitive and allow for detecting multiple unfolding events. mAbs, for example, show multiple unfolding events, which can be attributed to their different domains. In the present case, most likely, the first unfolding transition (T_m1) corresponds to unfolding of the CH2 domain, while the second transition (T_m2) reflects simultaneous unfolding of the CH3 domain and Fab.[1]

Comparison of T_m data from nanoDSF and µDSC data show a good agreement between the methods (Figure 2A). Importantly, the standard deviation of triplicate T_m determination by nanoDSF was on average 0.1 °C for all measurements, highlighting the high precision of the method. Both methods find that the mAb is least stable in acetate buffer pH 3, while its stability substantially increases with increasing pH. µDSC and nanoDSF both confirm that the mAb is thermally most stable in histidine buffer at pH 7, and that the surfactants PS20 and PS80 do not have a positive effect on thermal stability, but instead lower the T_m1 value by ~2.2 °C. Slight differences between the determined unfolding transition temperatures between the methods may be attributed to the different detection methods. In the context of formulation screening it is important to see that the relative correlations between the different formulations are virtually identical. Consequently, stability screenings based on T_m results in the same ranking by nanoDSF and µDSC. Importantly, nanoDSF requires significantly less sample and time for formulation screening projects when compared to µDSC (Figure 2B) while delivering highly comparable T_m-values.



C

	µDSC	nanoDSF
Drawbacks / limitations	• sample and time consumption. • limited protein concentration range (0.5 - 5 mg/ml). • aggregation. • equilibration + maintenance. • sample preparation required.	proteins without aromatic amino acids.
Major benefits	• high resolution of transitions. • high reproducibility. • thermodynamics. • (optional) full automation.	• parallel measurement of 48 samples. • large protein concentration range (0.01 - 200 mg/ml). • low sample consumption. • high reproducibility. • high resolution of transitions. • calibration + maintenance free. • no buffer restrictions. • automatic Tm-determination • (optional) simultaneous detection of aggregaton.

Figure 2: (A) Comparison of T_m1 and T_m2 values determined by nanoDSF (n=3) and µDSC (n=1). nanoDSF data points represent average values. Error bars are smaller than symbol sizes (s.d. <0.1 °C for all measured samples). (B) Comparison of the time- and sample requirements of nanoDSF and µDSC for a formulation screen involving 48 different samples. (C) Summary of benefits and limitations of µDSC and nanoDSF for the determination of protein thermal stability. Protein concentration ranges are given for mAb solutions.

Conclusion

In this comparative study we demonstrate that both methods, nanoDSF and µDSC, provide precise and consistent data. However, µDSC has several limitations, as listed in Figure 2C. nanoDSF integrated in the Prometheus NT.48 overcomes these drawbacks by its innovative capillary format. It allows for easy sample handling, even for highly concentrated and very viscous formulation conditions, and for providing a maintenance-free instrumentation which does not require laborious instrument equilibration and washing. In addition to its speed, precision and throughput, nanoDSF is a particularly robust method which does not request any cumbersome sample preparation such as dialysis or filtration (Figure 2C) and also works in any buffer, even with detergents and high viscosity (> 50 mPa). Therefore, the Prometheus NT.48 is the ideal instrument for rapid and precise thermal stability screening in biopharmaceutical development.

Material and Methods

Protein preparation
A therapeutic IgG1-type mAb was dialyzed into the formulation buffers listed in Table 1. The protein concentration was adjusted to ca. 1 mg/ml.

Table 1: List of formulation buffers.

Buffer substance	pH	Additive
20 mM Acetate	3.0	
20 mM Acetate	4.0	
20 mM Acetate	5.0	
20 mM Histidine	5.0	
20 mM Histidine	6.0	
20 mM Histidine	7.0	
20 mM Phosphate	7.0	
20 mM Phosphate	8.0	
20 mM Histidine	6.0	0.02% PS20
20 mM Histidine	6.0	0.02% PS80

nanoDSF thermal unfolding
Thermal protein unfolding was monitored using the Prometheus NT.48 instrument (NanoTemper Technologies). For each buffer condition, 30 µl a 1 mg/ml mAb solution was prepared, and ~10 µl of sample were filled into 3 nanoDSF Grade Standard Capillaries (NanoTemper Technologies), respectively, and loaded into the instrument. mAb thermal unfolding was monitored in a 1 °C/min thermal ramp from 20 °C to 95 °C. T_m values were determined automatically by the PR.Control software.

µDSC thermal unfolding
Differential scanning calorimetry experiments were performed on a MicroCal Auto VP-Capillary DSC System (Malvern Instruments). For the measurements about 400 µl sample was required.
To determine the background, reference and sample cell were filled with formulation buffer and scanned from 10 °C to 100 °C with a scan rate of 1 °C/min. To determine the T_m of the protein, the sample cell was filled with the protein solution and the reference cell with formulation buffer, and one heating scan from 10 °C to 100 °C at 1 °C/min was performed. After the protein scan, the cells were filled with 10 % Decon 90 solution and scanned with the same settings of the protein sample. Subsequently, the cells were washed extensively with highly purified water to remove the detergent.
The background obtained from the corresponding formulation buffer scan was subtracted from the sample measurement. The T_m values were determined from the peak maxima of the unfolding transitions after baseline subtraction. Data analysis was performed by using the system's Origin 7.0 DSC software.

References

1. Ionescu RM, Vlasak J, Price C, Kirchmeier M. Contribution of variable domains to the stability of humanized IgG1 monoclonal antibodies. J Pharm Sci. 2008; 97(4):1414-26.

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