09/2016

Prometheus™ Series

Product Information

Prometheus™ Instruments
for nanoDSF™

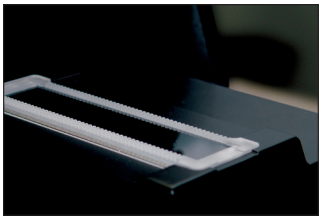
www.nanotemper-technologies.com

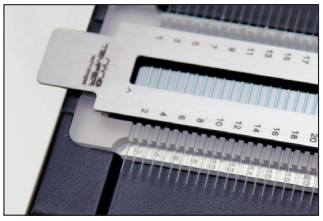
Prometheus™ Series

NanoTemper Technologies offers nanoDSF technology with the Prometheus Series. nanoDSF is the method of choice for easy, rapid and accurate analysis of protein stability and aggregation, with applications in protein engineering, membrane protein research, formulation development and quality control.

Enjoy the benefits of nanoDSF:

- Measure thermal and chemical stability - even for membrane proteins
- See more transitions - ultra-high resolution for antibody engineering
- Detect aggregates - long-term stability and storage of biologics
- Exploit the concentration range - for formulation of biopharmaceuticals
- Integrate into robotic platforms - for fully automated operation





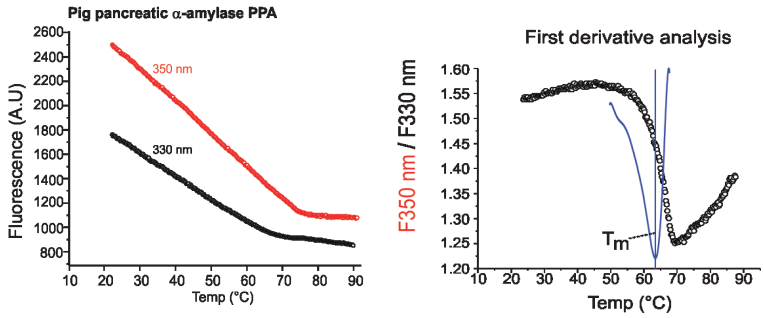
Prometheus NT.48

nanoDSF™

A technology by NanoTemper®

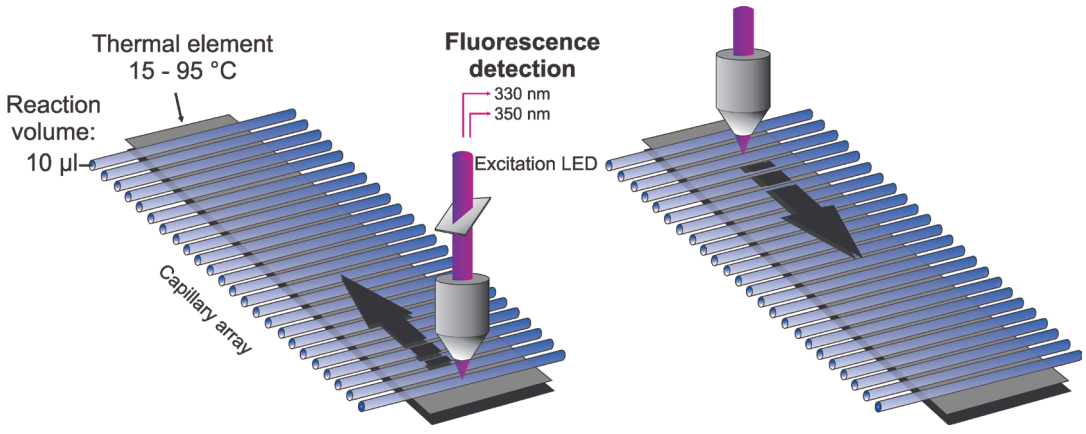
nanoDSF is an advanced Differential Scanning Fluorimetry technology. It detects smallest changes in the fluorescence of tryptophan and tyrosine present in virtually all proteins.

The fluorescence of tryptophans and tyrosines in a protein is strongly dependent on their close surroundings. By following changes in fluorescence, chemical and thermal stability can be assessed in a truly label-free fashion.



Thermal unfolding with nanoDSF
Two wavelenghts, 330 and 350 nm are recorded. The ratio of the two wavelengths is plotted against the temperature. The 350/330 nm ratio typically yields well-defined transitions, even if the single wavelengths do not exhibit a clear unfolding transition.

The dual-UV technology by NanoTemper allows for rapid fluorescence detection, providing an unmatched scanning speed and data point density. Ultra-high resolution unfolding curves enable detection of even minute unfolding signals.



Since no secondary reporter fluorophores are required, as in conventional DSF, protein solutions can be analyzed independent of buffer compositions, and over a concentration range of 250 mg/ml down to 5 µg/ml. This allows for the analysis of detergent-solubilized membrane proteins, as well as for highly concentrated antibody formulations.

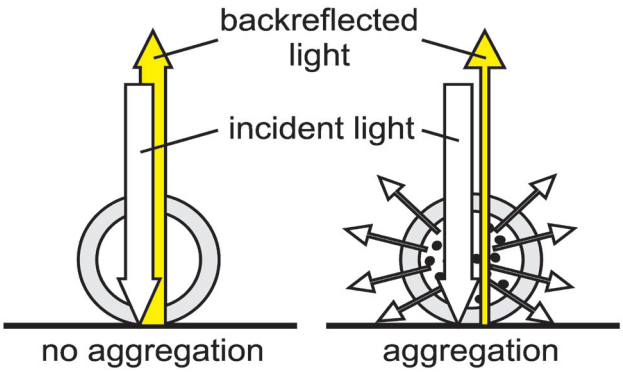
Optional Feature: Backreflection Optics

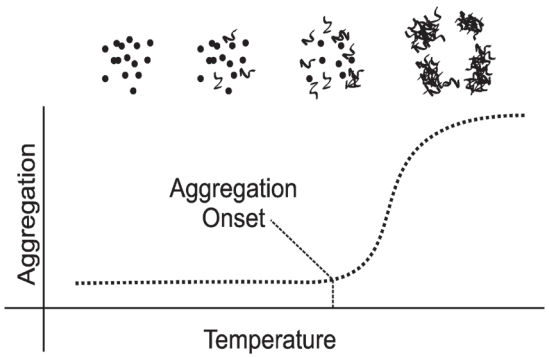
A technology by NanoTemper®

NanoTemper Technologies developed the Backreflection optics to detect aggregation of protein samples such as antibodies.

The detection is based on an aggregation induced scattering of light by particles. Owed to the high precision capillary format of the Prometheus series and automatic internal referencing, reproducibility and sensitivity of the aggregation detection are superior to conventional approaches, while maintaining the high data point density of the simultaneous dual-UV fluorescence detection which allows to monitor protein unfolding without compromising data quality.

The combination of thermal stability detection and determination of aggregation onset temperatures allows for the most rapid, precise and information rich analysis of biologicals, e.g. in formulation screenings or protein engineering projects.





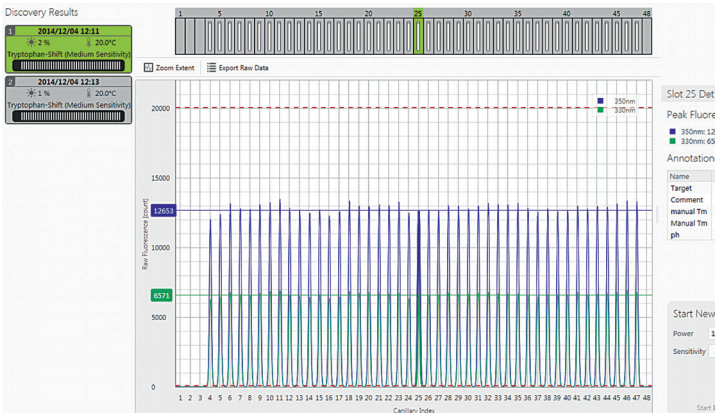
Control and Analysis Software

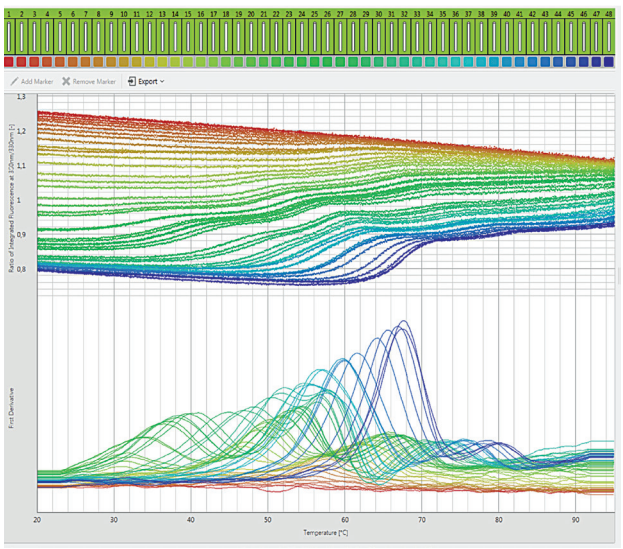
The PR.Control software guides you through all important steps for setting up thermal or chemical unfolding experiments, for data recording and data analysis.

The software provides a user-friendly interface with one-click routines. Data are analyzed automatically using the first and second derivative to ensure highest precision and reproducibility. The software offers publication-ready graphs and raw data can be exported to spreadsheet applications for tabular representation.

The PR.Control software provides optional compliance features for regulated environments.

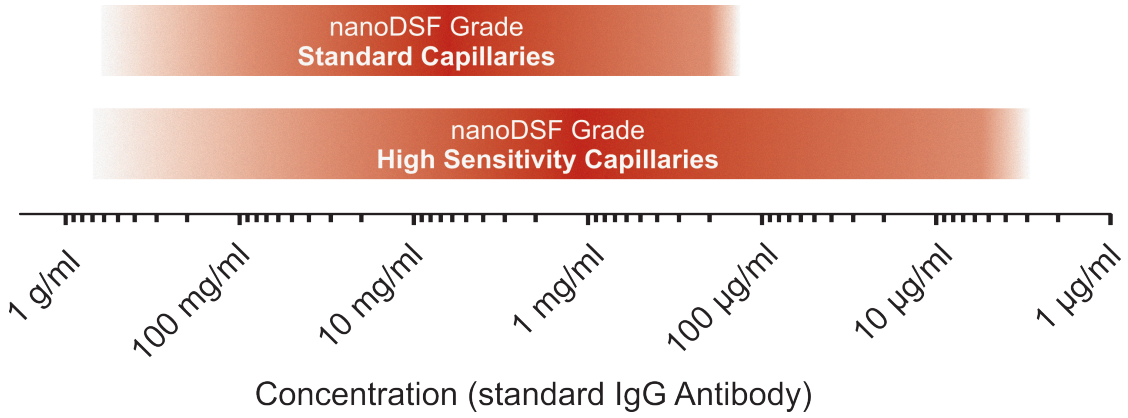
Software updates and upgrades are available to supply users with the latest features and functionality.



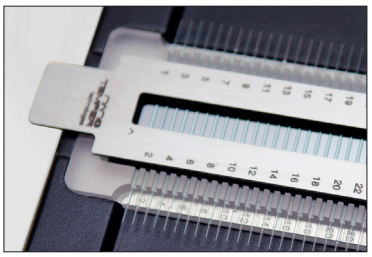


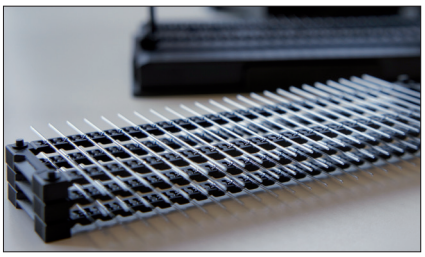
Consumables

NanoTemper Technologies offers consumables of high quality and precision specially designed and optimized for your nanoDSF assay. The capillaries are optimized for nanoDSF measurements provding robust signal detection for a broad range of concentrations. The exceptional reproducibility and glass purity allow you to get high quality nanoDSF data. High-sensitivity capillaries are available to extent the dynamic range to concentrations below 200 µg/ml.



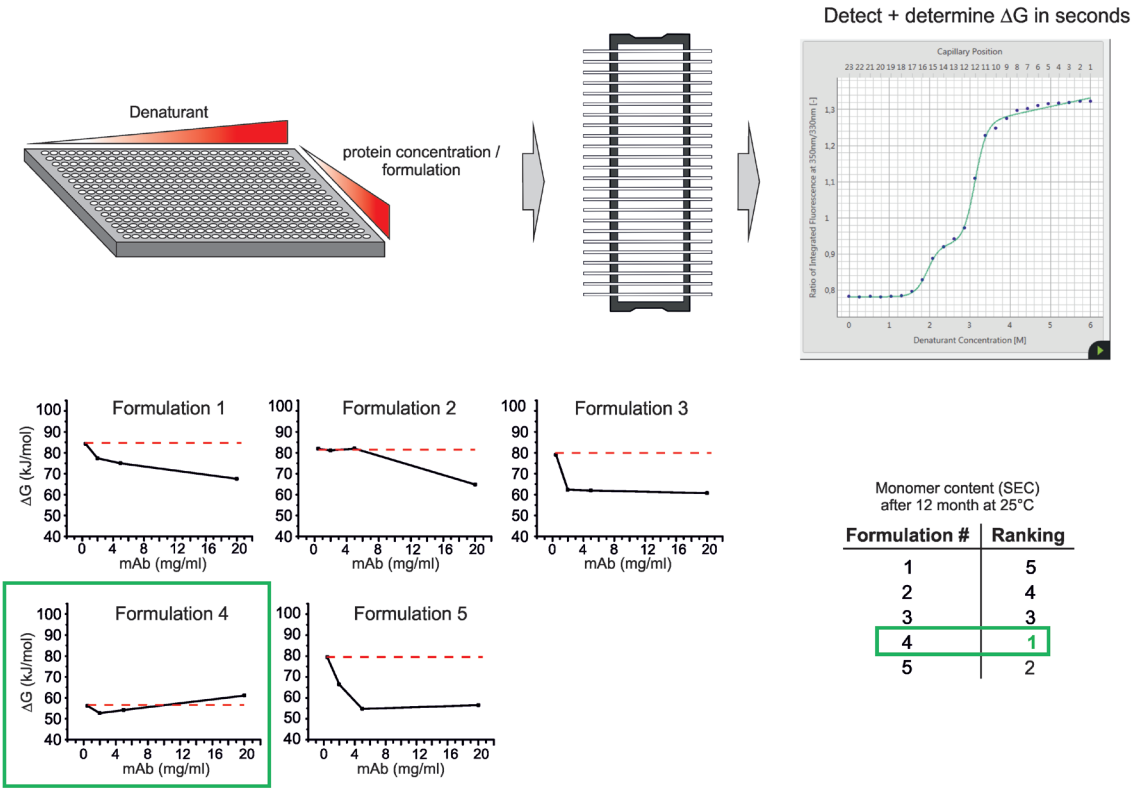
The Prometheus NT.48 is filled with single capillaries for most flexible assay design and easy handling. The Prometheus NT.Plex employs 24-Capillary chips for automated sample loading by robotic platforms.





Chemical Unfolding in Antibody Development

The Prometheus software package detects chemical unfolding and determines ΔG within seconds. Since concentration-dependence of ΔG is a measure for aggregation propensity, nanoDSF is able to predict long-term stability of biopharmaceuticals in different formulations.
The fully automated nanoDSF solution with the Prometheus NT.Plex allows for unattended measurement of hundreds of chemical unfolding reactions per day.



Monomer content (SEC) after 12 month at 25°C

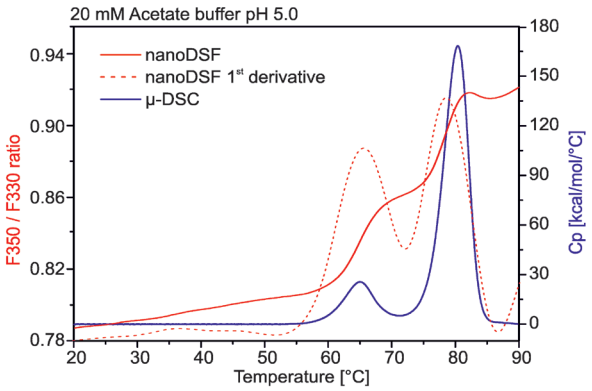
Formulation #	Ranking
1	5
2	4
3	3
4	1
5	2

Determine ΔG of folding to predict aggregation propensities
Upper panel: Schematic representation of chemical unfolding experiments. Denaturant dilutions in formulation buffer are prepared in 384-well MTPs, and loaded into Prometheus capillary chips. Chemical denaturation curves are recorded and analyzed automatically in seconds.
Lower panel: Concentration-dependence of ΔG of a mAb in different formulations. A decrease in ΔG indicates high aggregation propensity of the unfolded state. Formulation 4 shows a constant **Δ**G, and also shows the highest monomer content after 12 months at 25 °C by HPSEC.

Comparison: nanoDSF and µDSC

A collaborative study with a leading CRO demonstrates that nanoDSF overcomes critical drawbacks associated with µDSC since it provides perfect ease of use, is 100 times faster and requires 40 times less sample.

A



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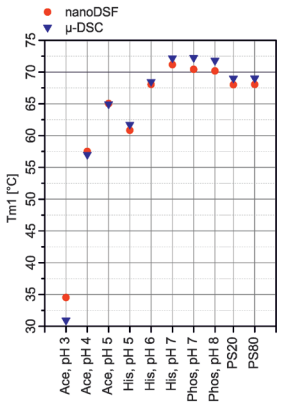

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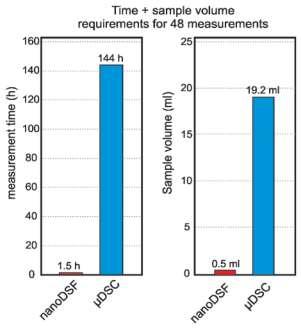


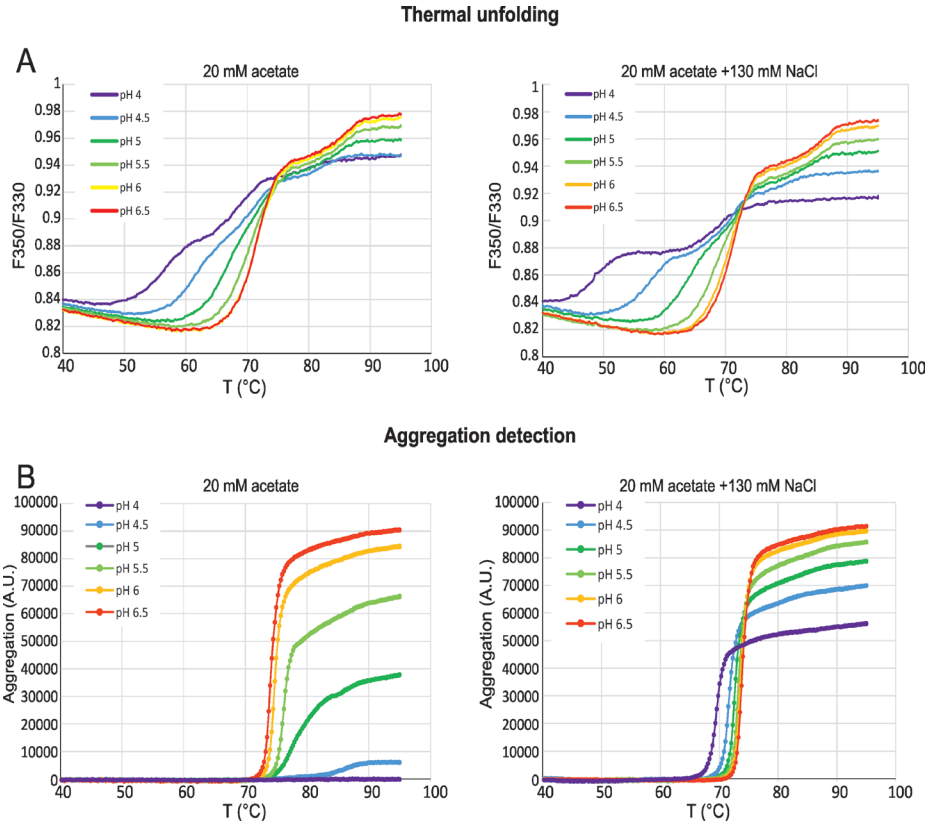
D

	µDSC	nanoDSF
Drawbacks / limitations	• sample and time consumption. • high protein concentrations. • incompatile detergents. • aggregation. • equilibration + maintenance. • sample preparation required	proteins without aromatic amino acids.
Major benefits	• high resolution of all transitions. • thermodynamics. • (optional) full automation.	• parallel measurement of 48 samples. • low sample consumtpion. • high reproducibility. • high resolution of transitions. • calibration + maintenance free. • no buffer restrictions. • (optional) simultaneous detection of aggregaton.

Both nanoDSF and µDSC generate precise and highly comparable T_m values (Figure A and B) in a small formulation screen using a commercially available therapeutic mAb. A total of ten different formulations with varying buffers and pH-values were tested in addition to polysorbate 20 and 80, which are common surfactants in mAb formulations, but preclude the analysis by orthogonal fluorescence methods such as DSF assays.

The integrated nanoDSF protocol and innovative capillary sample format of the Prometheus NT.48 overcomes many key limitations of µDSC (Figure C and D). In addition to its speed, precision and throughput, nanoDSF is a robust method that does not require cumbersome instrument maintenance and time-consuming sample preparation such as dialysis or filtration. Therefore, the Prometheus NT.48 is the ideal instrument for rapid and precise thermal stability screening in biopharmaceutical development.

Thermal and Colloidal Stability of Antibodies

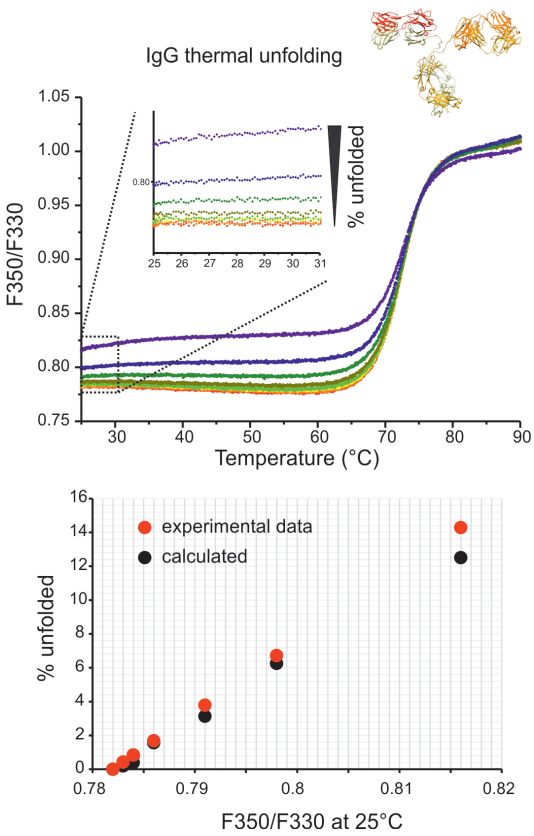


Conformational stability and aggregation of a monoclonal antibody (mAb) under different buffer conditions.
(A) Thermal unfolding monitored by detection of shifts in the fluorescence ratio (F350/F330) in dependence of different buffer pH values and NaCl concentrations.
(B) Aggregation detected by changes in backreflection.

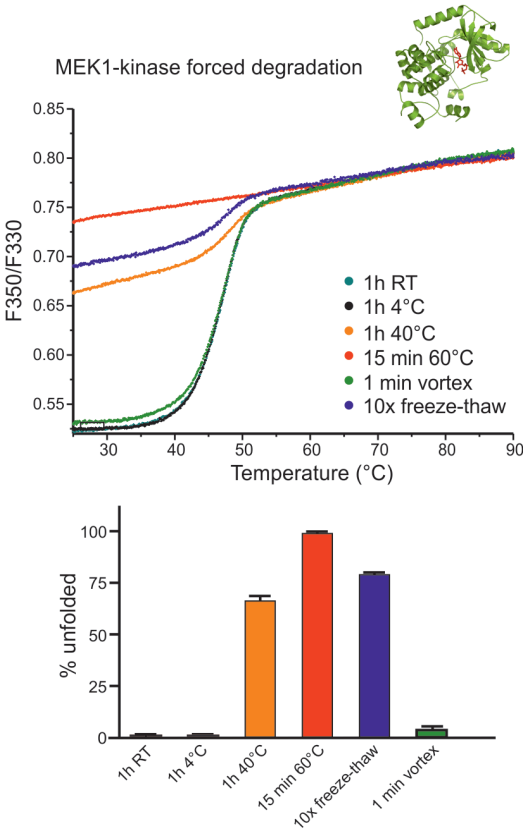
The Prometheus NT.48 delivers high quality thermal unfolding data for antibody buffer screening campaigns and detects unfolding of single antibody domains (A). The large dynamic range of the Prometheus NT.48 allows for analyzing thermal unfolding in solutions containing antibody concentrations between 250 mg/ml down to few µg/ml. In addition, the colloidal stability (B) and aggregation onset temperatures (C) of antibodies can be assessed simultaneously employing the backreflection optics.

The results from our parallel investigation of thermal unfolding and aggregation using the Prometheus NT.48 suggest that a slight thermal instability might be acceptable and even favourable for long-term stability of the antibody, due to reduced aggregation under these conditions (D). Future screening approaches could therefore be designed to find excipients which thermally stabilize the mAb at lower pH values while maintaining low aggregation of the unfolded state.

Quality Control



Establishing a protein unfolding standard.
Unfolded IgG at different concentrations was mixed with folded IgG and subjected to thermal unfolding. The percentage of unfolded IgG in the solution was quantified based on the F350/F330 ratio measured at 25 °C.



Forced-degradation stress-test on MEK1
MEK1 protein was subjected to the indicated stresses, and the fraction of unfolded protein was calculated based on the F350/F330 ratio at 25 °C. Error bars are s.d. from three measurements.

nanoDSF can be employed to quickly detect and quantify unfolded proteins for quality control purposes with unmatched speed, at the same time offering unique ease of use.

The presented quality control experiments can be performed by filling capillaries directly from stock solutions without laborious sample preparation. F350/F330 values of up to 48 samples are then recorded in parallel using a one-button routine, providing stability data within seconds.

Customer Statements

Dr. Michaela Blech, Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach, Germany

“The Prometheus instrument allows for label-free analysis of 48 samples simultaneously independent of their protein concentrations (high dynamic range) and selected solution conditions and/or –compositions. Unlike other techniques the Prometheus NT.48 measurements remained unaffected by any excipient, sugar, detergent or additive. Altogether, the Prometheus instrument enables for very flexible experimental design and provides maintenance-free instrumentation. In addition, our obtained data demonstrate very high reproducibility, consistency, the robustness and precision of this particular technology. The outstanding construction design allows for on-the-fly detection of fluorescence intensity resulting in impressive data point density that there is virtually no need for data fitting.”

Dr. Alexey Rak, Sanofi, France

“Native, intrinsic fluorescence based nanoDSF technology has been quickly integrated in our standard operations and is applied nowadays in every project we are currently working on, including small molecules and biologics modalities. We are using Prometheus for initial screening as well as for lead optimization profiling of small molecules and fragments and for stability characterization of therapeutic proteins. Furthermore, nanoDSF is an invaluable tool for quality control since the fraction of unfolded protein can be quantified just within a few seconds.
The Prometheus NT.48 instrument is maintenance-free and provides an easy to use handling platform. The capillary format allows us to even measure highly viscous formulation conditions.

We experienced that nanoDSF technology is superior to standard DSF regarding its application range as well as in terms of precision, reproducibility, wider applicability and greater potential for new applications development.”

Prof. Dr. Thomas Müller, University of Wuerzburg, Germany

“Commonly used methods to determine protein stability suffer from various drawbacks requiring either rather large amounts (DSC), specific conditions (CD), or otherwise cannot be applied to all biomolecules species (ThermoFlour). In particular very few methods exist for membrane proteins, as the requirement of detergent-containing buffers very often impedes the use of CD spectroscopy (due to strong light absorption by the detergent) and the application of the usually preferred ThermoFluor methodology (due to binding of the dye to the detergent micelles). The capabilities of the new Prometheus NT.48 in measuring thermal unfolding now allows us to quickly determine the ideal buffer conditions and the detergent best suited for crystallization trials. Fast measurements, very low material consumption, label-free capabilities, and low background noise make the Prometheus NT.48 the best current solution for screening membrane protein buffer conditions.”

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