

# Prometheus Panta

Made for stability characterization.  
Built for confidence.

The Prometheus Panta system is a highly precise and reliable instrument for label-free biophysical characterization of proteins. With Panta, a comprehensive stability profile of, for example, an antibody, an ADC, or an enzyme, can be quickly and easily determined. Due to its versatility, the system is a powerful tool for various analytical applications in discovery, preclinical drug development, and academic research.



Figure 1. Prometheus Panta

## Key benefits

**Reliability.** With high precision, identify subtle differences between molecules. Draw conclusions confidently, without worrying about data quality or having to do several runs to confirm.

**Efficiency.** Measure multiple parameters in less than two hours, using only 10  $\mu$ L sample, with four technologies running in parallel. Corresponding measurements with separate instruments would take more than one day<sup>1</sup> and require additional time to merge data.

**User-friendliness.** Generate meaningful results after only one day of start-up and training. Minimize hassle with an easy experimental set-up, an intuitive data analysis software, and a low maintenance operation.

## System components

The Prometheus Panta system comprises a benchtop instrument, software for control and analysis, and single-use capillaries for sample loading. In the standard configuration up to 48 samples can be analyzed per run. No pumps, valves, or tubing components are included, which minimizes system maintenance. The system is operated from an interactive touchscreen and a laptop supporting the control software. The samples are introduced via a capillary tray gate in the front.

For high-throughput applications, Panta can be configured with a Robotic Autosampler (robotic arm, chassis, control software). This enables fully automated, hands-free analyses on up to 1536 samples.

## Characterization technologies

The core of the Prometheus Panta system consists of four biophysical characterization technologies: nanoDSF, DLS, SLS, and Backreflection. The system can perform both isothermal measurements and acquire data across an entire thermal ramp. The combination makes the system highly versatile and it facilitates direct comparisons and interpretation of data. All measured data points are logged in parallel and no time is required to extract and merge data from different instruments.

### nanoDSF (nano-Differential Scanning Fluorimetry)

The exceptionally sensitive nanoDSF technology is used for thermal and chemical protein stability assessment. nanoDSF is label-free and relies on intrinsic fluorescence of Tyr and Trp amino acids<sup>2</sup>. Under stress inputs, such as temperature change or incubation with chaotropes, the intrinsic fluorescence will change. This is used to determine conformational stability. As nanoDSF measures the native state, potential interference from external dyes is eliminated.

### DLS (Dynamic Light Scattering)

DLS is used to assess the colloidal stability of a sample, i.e. the ability of molecules to remain dispersed in a solution without aggregating or precipitating over time. Particles move at different speeds and scatter different amounts of light depending on their size. This results in light fluctuations that are measured by highly sensitive DLS optics. The resulting data provides information on particle size and size distribution.

### SLS (Static Light Scattering)

In SLS, the intensity of scattered light is used to determine the molecular weight of particles and to provide information on self-interaction propensity, from calculating the second virial coefficient. Molecules with a positive second virial coefficient tend to stay apart and be more stable in solution.

### Backreflection

Backreflection is complementary to light scattering technologies. It measures the amount of light reflected to its source and is used to measure the turbidity of a sample. A highly turbid sample could indicate presence of large aggregates, other particles, or be caused by phase separation. Backreflection is especially useful in cases of highly concentrated or highly turbid samples.

<sup>1</sup> Estimated analysis time to derive the corresponding data output using Panta, compared to using separate single cuvette and plate-based instruments.

<sup>2</sup> This is in contrast with conventional DSF, where samples are labeled with a fluorescent dye.

## Software

The Prometheus Panta system software consists of a control module and an analysis module. The modules are designed with intuitive user interfaces that minimize time required for experimental set up and analysis. System installation and introduction training are performed in just one day. Extensive supporting information is found online at [support.nanotempertech.com](http://support.nanotempertech.com).

### Panta Control

The control software enables design and execution of single and multi-parameter stability experiments, with just a few clicks. The set-up process for the selected type of experiment is automatically guided. Real-time experiment monitoring and alerts are included in case of an unexpected deviation. For high-throughput experiments performed with the automated Robotic Autosampler configuration, a high-capacity software version (Panta AutoControl) is required.

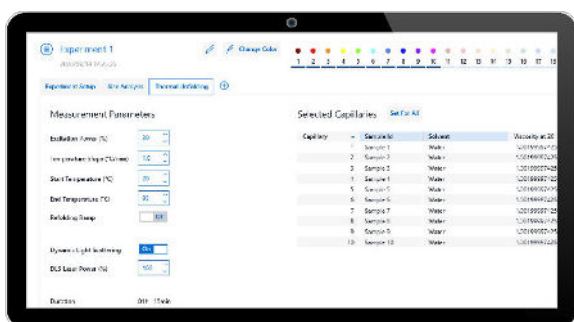


Figure 2. Intuitive experimental set-up with Panta Control.

### Panta Analysis

The analysis software features extensive capabilities to manage data sets post run. This includes auto-merging, statistical analysis, focus on regions of interest, and data export templates. The user interface makes it easy to toggle between parameters, create custom graphs, and rank protein samples. Sample quality is automatically evaluated, and outliers flagged, with additional information provided about the reasons.



Figure 3. The analysis software can manage multiple files for review of large data sets. Replicates are automatically merged.

## Consumables

### Capillaries

The use of single-use capillaries provides several advantages and offers maximum flexibility for the assay. Cross-contamination from re-used sample vessels is avoided and no cleaning is required. Even highly viscous samples can be measured with capillaries. Two different capillary types are available for use with Panta: standard grade, made of borosilicate glass, and high sensitivity grade, made of quartz with a polymer coating to prevent protein adsorption. High sensitivity capillaries are used for low concentration samples (below 200 µg/mL). They provide DLS results with less background noise than standard capillaries.



Figure 4. Easy sample introduction with capillaries.

### Capillary chips

For convenient and fast sample loading directly from 384- or 96- well microtiter plates, capillary chips, with 24 individual capillaries, can be used. The capillaries in the chip are spaced to fit with the wells in the plate (n=2 per well for 96-well plates). For additional convenience, a capillary chip filling station is available as an option for automated analyses with the Robotic Autosampler.

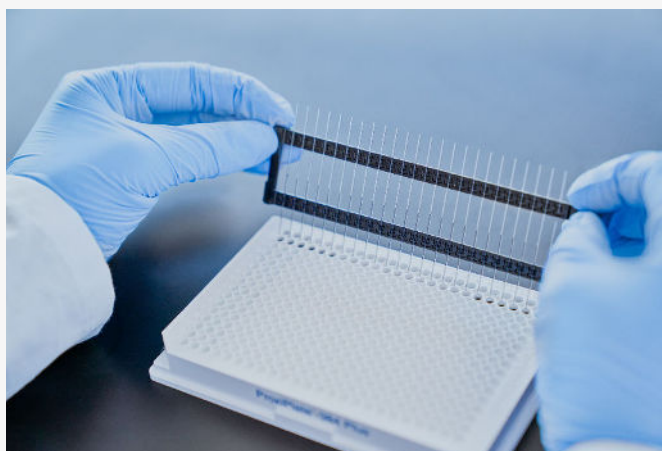


Figure 5. Capillary chips facilitate sample loading from 384-well plates.

### Capillary sealing paste

Sealing of capillaries is only required for measurements that exceed three hours, when experiments are run at temperatures >95°C, or when required for biosafety reasons. The sealing paste is a fast-drying, chemically inert, silicone-free, and water insoluble liquid rubber applied to capillary ends using capillary sealing paste applicators.

# Applications

The Prometheus Panta system is a powerful tool for many biomolecular applications. It provides essential insights into protein stability, aggregation, and size, from a low sample volume, during one single run. With the high sensitivity of Prometheus Panta, subtle differences between molecules and conditions can be identified. The combined approach with four technologies in the same instrument saves time and facilitates interpretation of data post analysis. Examples of use cases are seen below. In-depth application data can be found online in our resource center, resources.nanotempertech.com/application-notes, under the Prometheus tab.

## 1. Protein engineering, candidate selection, and developability

Characterize thermal and colloidal stability to select protein constructs for further development. Screen out poor candidates with undesirable properties early, to minimize development and commercialization risk of your program.

- Identify most thermostable constructs based on melting temperature (T<sub>m</sub>) or melting onset (Tonset).
- Obtain sizing and dispersity data (rH, PDI, and Tsize) to evaluate sample homogeneity and/or purity.
- Measure candidate self-interaction behavior from B22 or kD values, to benchmark molecule liabilities.
- Profile AAV thermal stability to assess vector stability or for AAV serotyping.

## 2. Pre-formulation and formulation research

Screen formulations and evaluate impact of increased temperature. Select pre-formulation conditions that show adequate thermal and colloidal stability.

- Assess potential towards thermal or chemically induced protein aggregation (rH, PDI) over time.
- Identify excipients that protect a protein candidate.
- Conduct comparability studies to ensure that biosimilar formulations have similar stability and biophysical characteristics to the original drug.
- Predict protein candidate half-life at different storage temperatures from calculating the activation energy of unfolding at different heating rates.

## 3. Additional applications

Thermal Shift Assay. Identify ligands that bind to a protein by comparing protein thermal stability profiles in the presence and absence of ligands.

- Quality Check. Use stability parameters to ensure protein quality after purification steps, or prior to functional assays.
- Structural biology. Optimize buffers prior to structural work, understand folding and unfolding, determine size distribution, polydispersity, and molecular weight.
- Membrane proteins. Profile the stability of membrane proteins in various detergents, liposomes, or nanodiscs.

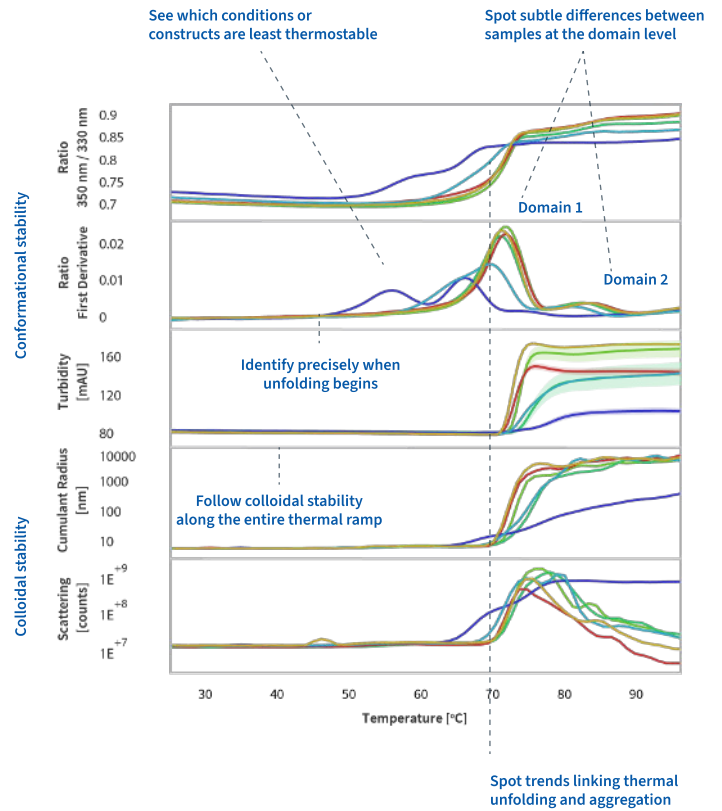


Figure 6. Formulation buffer screening: stability profiles of a monoclonal antibody at different pHs. The high resolution and reproducibility of data of the Prometheus Panta system makes it easy to spot domain level differences between several buffer conditions.

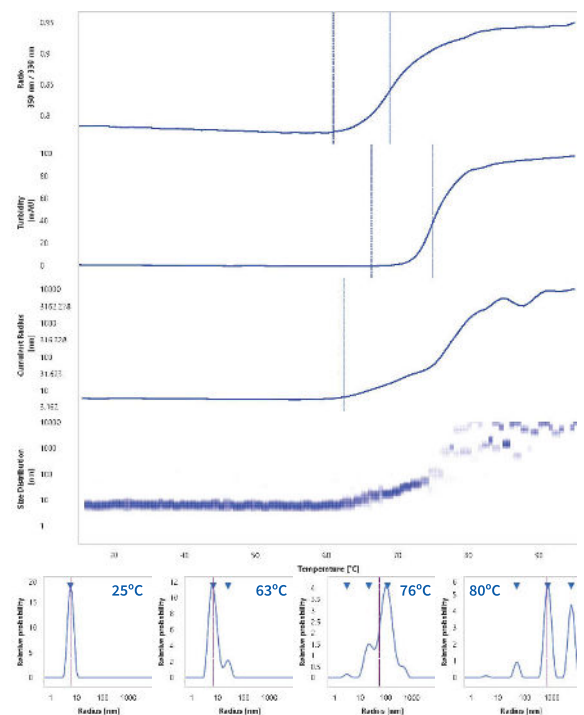


Figure 7. Cumulant radius and size distribution acquisition data from DLS along a thermal ramp. The speed and high resolution of Prometheus Panta's DLS optics enable to follow size distribution changes in real time as shown in the four plots below obtained at 25°C, 63°C, 76°C, and 80°C respectively.

# Specifications

## General specifications

Detection technology	nanoDSF, DLS, SLS, Backreflection
Information obtained <sup>3</sup>	nanoDSF: $T_m$ , $T_{onset}$ , $\Delta G$ DLS: $r_H$ , PDI, $k_D$ , $T_{size}$ SLS: MW, $B_{22}$ , $T_{scattering}$ Backreflection: $T_{turb}$
Data presentation	Graphics and tables. Data export format: Excel, CSV, png, PDF
Analysis cycle time	<2 h for typical applications
Sample type <sup>4</sup>	mAb, mAb fragments, ADC, bioconjugate peptide <sup>5</sup> , enzyme, VLP, viral vector.
Sample volume	10 $\mu$ L
Sample format	Single-use capillaries of borosilicate glass or quartz
Sample capacity	48 (single capillaries) 1536 (64 chips with 16 chips x 24 capillaries, autosampler config.)
DLS/SLS Laser wavelength	405 $\pm$ 5 nm
DLS detection angle <sup>6</sup>	140°
nanoDSF™ fluorescence	Excitation: 280 $\pm$ 10 nm Detection: 330 nm, 350 nm $\pm$ 10 nm
System dimensions (W x H x D)	Standard: 35 x 51 x 52 cm Autosampler: 110 x 188 x 90 cm
System weight	Standard: 35 kg Autosampler: 200 kg
Power supply	100-240 VAC, 50/60 Hz, 230 VA max
Electrical input	24 VDC, 10 A

## Typical working ranges

Temperature control range	15 – 110 °C
Heating rate	0.1 – 7 °C/min
Temperature control accuracy	$\pm$ 0.1 °C
Precision of $T_m$ Streptavidin ref. <sup>7</sup>	$\pm$ 0.1 °C
Sample viscosity	Up to 50 Pa.s
Sample concentration range IgG ref. <sup>8</sup>	nanoDSF: 5 $\mu$ g/mL – 250 mg/mL DLS/SLS: 0.08 mg/mL – 40% w/v.
Particle size range	DLS/SLS: ~0.5 nm to 1 $\mu$ m radius Backreflection: $\geq$ 12.5 nm radius

## Computer requirements

Operating system	Windows 10 64 bit or higher, English language
CPU	12 <sup>th</sup> Gen Intel Core i5 or better
RAM	$\geq$ 8 GB
Hard drive	$\geq$ 60 GB free disk space
Display resolution	1920 x 1080 or better
Software	Microsoft.NET 4.7.0 & Microsoft.NET Core 3.1
Network	1000 Mbps Ethernet connection

## Compliance

Compliant with	CE, CB, NRTL/UL, CSA
Safety	IEC 61010-1:2010/AMD1 :2016 Part 1, IEC 61010-2-010:2019 Part 2-010, IEC 60825-1:2014, 21 CFR 1040.10 and 1040.119
Electromagnetic compatibility (EMC)	IEC 61326-1:2012 EMC
Overvoltage category	CAT I
Laser classification	Laser Product Class I
Environmental	Pollution degree 2

## Ordering information

Product	Code
Prometheus Panta	PR013
<i>incl. Dell Mobile Precision Workstation, SW license Panta Control and Panta Analysis</i>	
Prometheus Panta with Robotic Autosampler	PR-014001
<i>incl. Dell Mobile Precision Workstation, SW license Panta AutoControl and Panta Analysis</i>	
Standard capillaries	PR-C002
High sensitivity capillaries	PR-C006
Standard capillary chips	PR-AC002
High sensitivity capillary chips	PR-AC006
Capillary sealing paste	PR-P001
Capillary sealing paste applicators	PR-P002
Capillary chip filling station	NT-AT100

<sup>3</sup> The parameters  $T_{size}$ ,  $T_{scattering}$ , and  $T_{turb}$  are all representing the onset temperature at which changes start to occur.

<sup>4</sup> Illustrative, nonexhaustive examples of common applications.

<sup>5</sup> Peptide must contain Trp or Tyr for use of nanoDSF technology.

<sup>6</sup> After diffraction, the light reaches the detector back at an angle of 147°.

<sup>7</sup> Precision is calculated based on the difference between  $T_m$  measurements data points across 48 individual capillaries.

<sup>8</sup> Smaller proteins will have a different DLS/SLS detection limit. For a protein of 15 kDa the lower limit will be in the range of 0.5 mg/mL.

<sup>9</sup> Exception: conformance with IEC 60825-1 Ed 3, as described in Laser Notice No 56, May 8, 2019.



Scan the QR code to open the NanoTemper webshop for consumables and software.

[shop.nanotempertech.com](https://shop.nanotempertech.com)

For more information on Prometheus Panta, visit [nanotempertech.com/prometheus](https://nanotempertech.com/prometheus).