

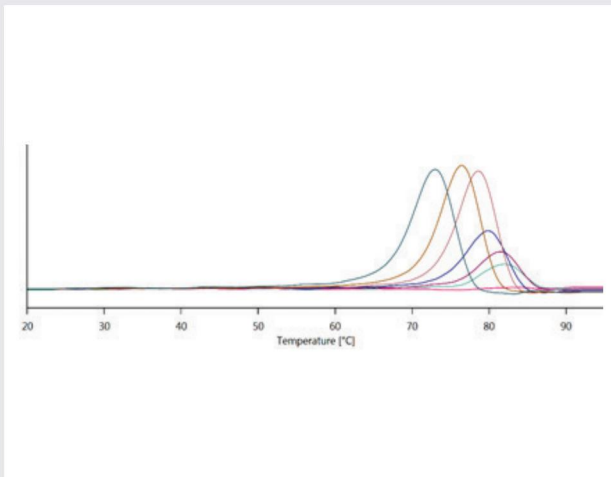
# NanoTemper Prometheus

## Prometheus NT. 48 - Differential scanning fluorimetry (DSF)

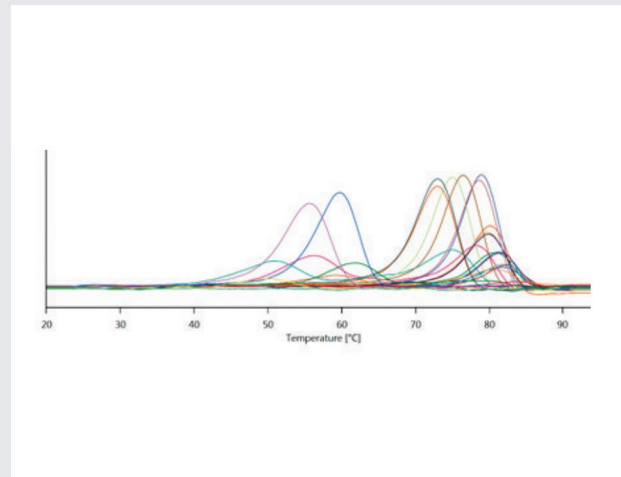
- Prometheus is used to determine **thermal unfolding transition temperatures** and **denaturation midpoints**. Prometheus uses advanced UV-LEDs for excitation of fluorophores. Proprietary UV-detection unit with the ability of rapid and sensitive data acquisition detects changes in fluorescence of the amino acid fluorophores (mainly tryptophan) **over wide range of temperatures**. Concurrent fluorescence detection at two different wavelengths enables discrimination between exposed and buried fluorophore, which corresponds to structural changes.
- The instrument can also detect **chemical unfolding of proteins** upon addition of molecule of interest.
- For thermal unfolding experiments **no assay development** or special sample preparation is needed. In case of chemical experiments it is necessary to prepare series of samples with different concentrations of denaturant mixed with your protein and then incubated for equilibration.

### DSF can be used:

- to determine thermal unfolding transition temperatures
- to determine denaturation midpoints
- to induce thermal or chemical unfolding of proteins
- to detect aggregation of sample



*Different concentration of one additive can make a big difference in protein melting temperature.*



*Up to 48 samples can be measured in one run – curves can be analyze individually or all at once.*

### Features:

- label-free detection system for the study of denaturation midpoints, thermal unfolding temperatures.
- dual UV detector (330 nm and 350 nm) - detection range 0 - 20 000 fluorescence unit
- measuring of broad range of concentration - 3 different sensitivity settings, adjustable LED-excitation
- **samples per run:** analyzing of 48 capillaries at the time
- **protein concentration:** from 5 µg/ml to more than 200 mg/ml
- **sample consumption:** 10 µl per capillary
- temperature range: 20 °C - 110 °C

## ■ Sample requirements - importance of sample preparation

### Differential scanning fluorimetry (DSF) – Prometheus NT.48

- Protein must contain at least one tryptophan (or other fluorescent residue) in order to detect protein unfolding
- **broad concentration range: from 5 µg/ml to more than 200 mg/ml** - suitable concentration is affected by content and surface exposure of tryptophan residues.
- **Prepare at least 20 µl of your samples.** Evaporation or sticking of the sample to the microtube's walls may occur.
- When testing the influence of an additive on sample stability it is advised to prepare series of **samples with different concentrations** of denaturant mixed with your protein and then incubate for equilibration.
- Capillary is filled by capillary forces after dipping in to the sample.
- **Avoid contact between capillary and microtubes during filling - outer surface of capillary has to be dry!**

## ■ Technical specifications

**Instruments: Prometheus NT.48** (NanoTemper Technologies, GmbH)

### Offered consumables:

Standard and high sensitivity capillaries are supplied by BIC facility.

### Operational mode:

DSF measurement is performed manually by the user itself after training

### Data evaluation software:

Data evaluation software is available on PC during/ after measurement. Output evaluated data are in form of chart or in form of raw data for further analyses.



*Prometheus NT.48 (NanoTemper Technologies, GmbH)*

## ■ Provided services

- instrument user training
- basic DSF data evaluation training
- consulting/assistance

**It is recommended to discuss the project and the details of the experiment (sample preparation, sample requirements) with the Core Facility members in advance, especially for non-skilled users.**

## ■ Contacts

### **Biomolecular Interaction and Crystallization CEITEC Core Facility**

[bic@ceitec.muni.cz](mailto:bic@ceitec.muni.cz)

**Core Facility Leader:** MICHAELA WIMMEROVÁ

[michaela.wimmerova@ceitec.cz](mailto:michaela.wimmerova@ceitec.cz)

**MST/DSF Responsible Person** (JOSEF HOUSER, JITKA ŽDÁNSKÁ)

[josef.houser@ceitec.muni.cz](mailto:josef.houser@ceitec.muni.cz)

[jitka.zdanska@ceitec.muni.cz](mailto:jitka.zdanska@ceitec.muni.cz)

### **Instrument Location:**

CEITEC MU Campus Bohunice, pavilion A4/2.19 laboratory, Kamenice 5, 62500 Brno