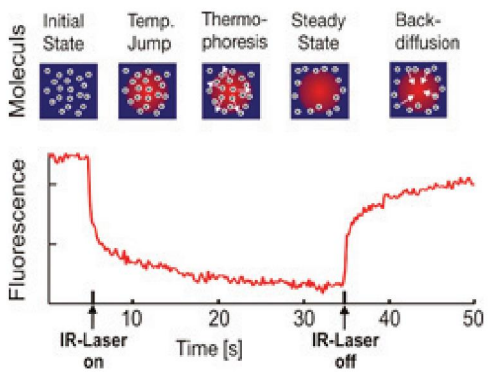
# NanoTemper Monolith

## Monolith NT.115 - Microscale thermophoresis (MST)

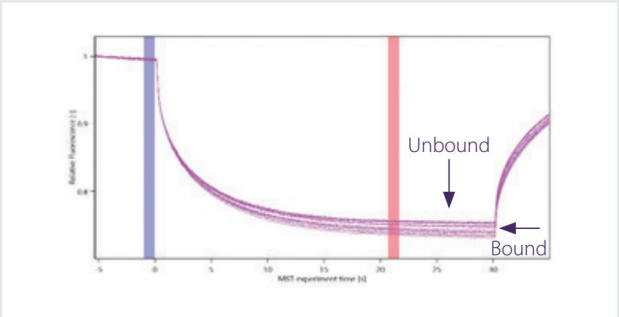
- Microscale thermophoresis is based on physical principle of measuring changes of the mobility of molecules in microscopic temperature gradients. Movement of molecules along the temperature gradient is called thermophoresis.
- The Monolith systems measure equilibrium binding constants for a variety of molecules – thus allows to measure wide range of interactions from ion fragment binding up to interactions of large complexes (liposomes and ribosomes).
- This method allows to detect changes in hydration shell, charge or size of molecules and thus detect biomolecular interaction. Volume of buffer is kept constant in whole dilution series so the observed alterations in the thermophoretic depletion or enrichment can come only from changes in the size, charge or solvation entropy of the fluorescently labeled molecule caused by binding of non-labeled molecule to the labeled one.

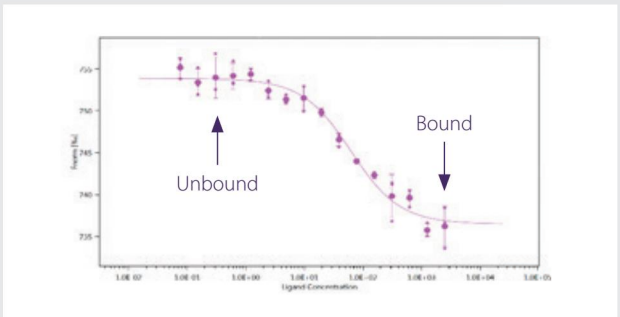



*Monolith NT. 115 (NanoTemper Technologies GmbH)*

## MST can be used:

- to determine the affinity of interaction
- to determine stoichiometry
- to monitor enzyme kinetics
- to measure in close-to-native conditions



**Features:**

- labeling dependent system for the study of interaction
- immobilization-free affinity determination from 1nM to mM range
- broad application range: from ions to ribosomes
- buffer variability: including serum or cell lysate
- purification free measurement possible for : fluorescent fusion proteins (GFP, YFP)
- number of capillaries per run: 16 capillaries
- samples consumption: min 5 µl per capillary – it is highly recommended to use up to 10 µl per capillary
- temperature control: 20 - 45 °C

## Sample requirements - importance of sample preparation

### Microscale Thermophoresis (MST) – Monolith NT.115

- Concentration of fluorescently labeled molecule: 10 nM - 10 mM
- Purity of sample before/after labeling is very important – impure sample may not be labeled correctly and cause unreliable measurement and data analysis. Label pure sample only and remove excessive dye thoroughly.
- Final concentration of unlabeled molecule should be at least an order of magnitude or more above expected dissociation constant ($K_D$). For simulation of binding event and for choosing the right concentration there is "Concentration Finder" software available instrument's control unit.
- Prepare at least 20 µl of your samples. Evaporation or sticking of the sample to the microtube's walls may occur.
- Spin your samples – both labeled and unlabeled molecules for 5 min with 13 000 rpm.
- Avoid contact between capillary and microtubes during pipetting - outer surface of capillary has to be dry!

## Technical specifications

**Instruments: Monolith NT.115** (NanoTemper Technologies GmbH)

**Offered consumables:**
Capillaries are supplied by BIC facility. For MST, standard, premium and hydrophobic capillaries are available. Monolith Assay Kits - Amine-Reactive Labeling Kit and Cysteine-Reactive Kit for your experiment (kit for labeling 4x 100 µg protein) can be purchased from our Core Facility.

**Operational mode:**
MST 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 PDF format or in form of raw data for further analyses.

## Provided services

- instrument user training
- basic MST 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

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