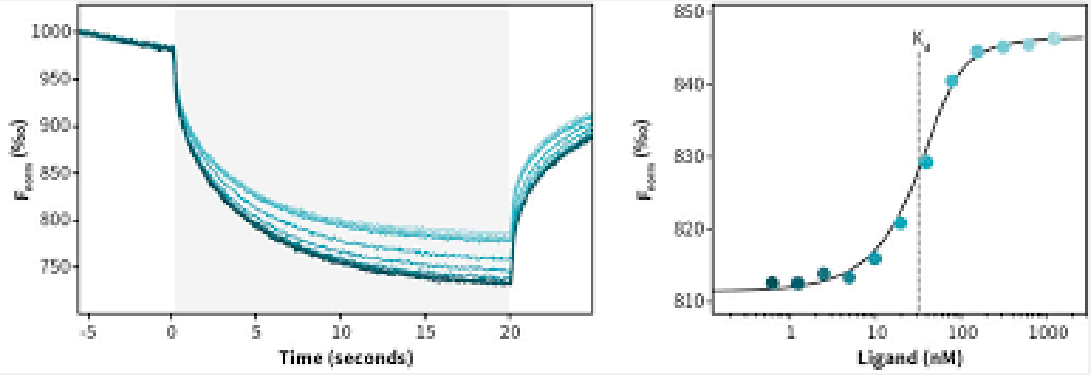
Monolith NT.115

Microscale thermophoresis (MST)

Microscale thermophoresis is based on a well-known phenomenon when molecules move along the temperature gradient = thermophoresis. The Monolith system uses this principle to measure the binding affinity of a wide range of interactions. The thermophoretic movement strongly depends on the properties of the measured molecule, such as size, charge, or hydration shell. Upon binding with a ligand, the properties of the molecular complex differ from the unbound state, leading to a different thermophoretic movement. It is necessary to fluorescently label one of the binding partners. The monolith system detects the resulting change in fluorescence of bound/unbound state. The equilibrium binding constants can be measured for a variety of molecules – from small compounds up to large complexes (liposomes and ribosomes).

MST can be used for:

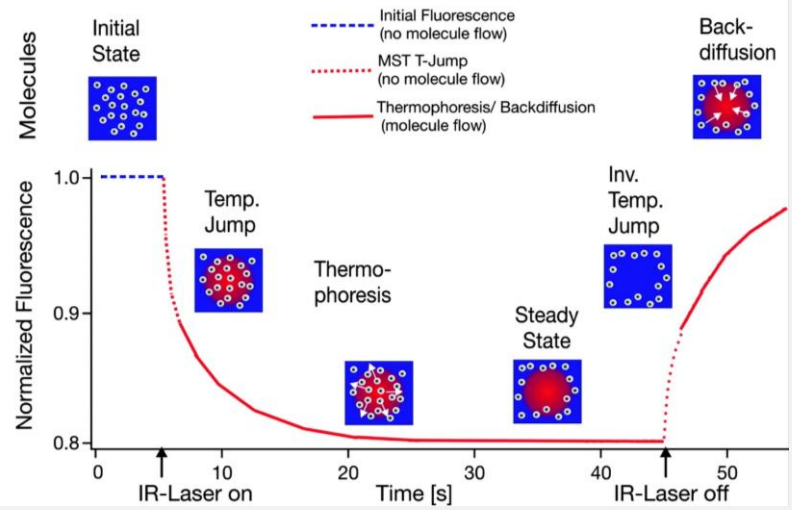
- Determination of the binding affinity constant of interaction.
- Stoichiometry determination.
- Measurement in close-to native conditions.



CEITEC – Central European Institute of Technology
Masaryk University
Kamenice 753/5, 625 00 Brno, Czech Republic
www.ceitec.eu

Technical specifications

Instrument: **Monolith NT.115** (NanoTemper technologies, GmbH)



Features:

- Labeling-dependent system for the study of interactions.
- Work in solution.
- An accessible range of affinity constants (K_D) 1 nM – mM.
- Broad application range: from ions to ribosomes.
- Detected molecule range: 10^1 – 10^7 Daltons.
- Buffer variability: including serum or cell lysate.
- Purification free measurement possible for his-tagged proteins, or fluorescent fusion proteins.
- Low sample consumption – minimum 7 µl per capillary (10 - 20 µl optimal).
- 16 capillaries used per one measurement.
- Temperature range 20 - 45 °C (in special cases down to 10 °C)
- Red and blue lasers available – examples of usable dyes: Red-NHS 2nd generation, Cy5, Alexa647, NT-647 (Red), FITC, GFP, Alexa488.

CEITEC – Central European Institute of Technology
Masaryk University
Kamenice 753/5, 625 00 Brno, Czech Republic
www.ceitec.eu

Instrument operation

Accesories:

- Available capillaries: standard, premium and hydrophobic.
- Available dyes: Red-NHS, Red-NHS 2nd generation, Red-tris-NTA (his-tag), Red-Maleimide.

Data evaluation SW:

- MO.Affinity Analysis software is installed on the machine-operating PC.

Operational mode:

- MST measurement is performed by the users themselves after training.

Provided services:

- Instrument user training
- Basic MST data evaluation training
- Consulting/assistance

Sample requirements

- The purity of the 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.
- Spin your samples, both labeled and unlabeled molecules for 5 min at 13 000 rpm.
- The **precise pipetting** is crucial when preparing a dilution series.
- Avoid contact between capillary and microtubes – the outer surface of the capillary has to be dry.
- Typical MST experiment is performed as a dilution series in 16 capilaries.
- The concentration of the **labeled molecule (=target)** is kept constant and has to be **below the expected K_D** (typically 20 nM). For one experiment - **160 µl** of target molecule at twice the selected concentration is required.
- The concentration of **unlabeled molecule (=ligand)** is changing in the measurement, the highest concentration should be **20x above the expected K_D**. For one experiment, **20 µl** of ligand molecule at twice the selected concentration is required.
- The sample volume can be halved in case of precious samples.

CEITEC – Central European Institute of Technology
Masaryk University
Kamenice 753/5, 625 00 Brno, Czech Republic
www.ceitec.eu

It is recommended to discuss the project and the details of the experiment (sample requirements and preparation, method of immobilization) with the Core Facility members in advance.

Contacts

Biomolecular Interactions and Crystallography Core Facility

bic@ceitec.cz

Core Facility Leader: JOSEF HOUSER

josef.houser@ceitec.cz

Responsible Person: EVA PAULENOVÁ

eva.paulenova@ceitec.muni.cz

Instrument Location:

CEITEC MU Campus Bohunice, pavilion C04/221 laboratory, Kamenice 5, 62500 Brno