

Microscale Thermophoresis (MST) User Guide

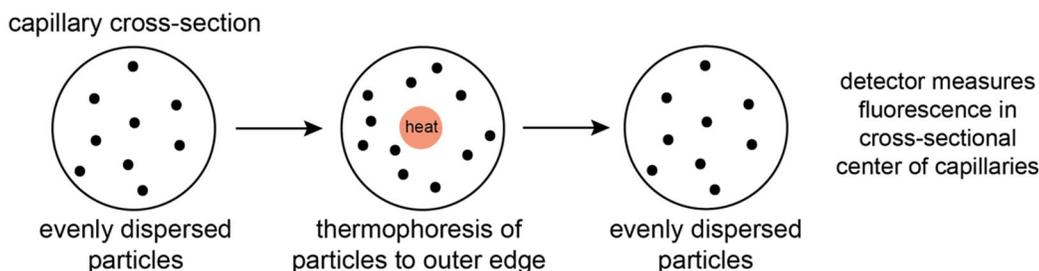
MST BACKGROUND INFORMATION

Microscale thermophoresis (MST) is used to determine binding affinities using fluorescently labeled ligands.

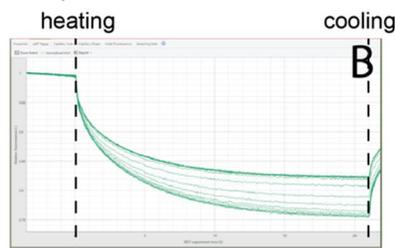
Visualize a cross-section of the capillaries, with particles of interest in a solution inside. During an MST experiment, the capillaries have evenly spaced particles within. Infrared light is used to heat the capillary lengthwise down the center. As heat is applied, particles move outwards toward the edges of the capillary. With a loss of heat, particles return again to their evenly dispersed arrangement. The MST detector measures fluorescence in the center of the capillaries.

The labeled particle (DNA) is present at the same concentration in every capillary, so if there is no binding at all, readings will be equal between all samples.

The unlabeled particle (protein) is added to each sample at differing concentrations. If the labeled particle is bound by the unlabeled particle, migration will be slower and dependent on unlabeled particle concentration.



example MST traces



if binding, migration slows & fluorescence in center increases as add protein

INSTRUMENT BOOKING

Capillary tubes and fluorescent tags are not provided and must be purchased as needed for your experiment. Supplies can be ordered from NanoTemper through their representative:

- sharia.terrell@nanotempertech.com
- 404-997-9811
- nanotempertech.com/monolith

The Monolith NT.115 (NanoTemper) instrument is located in 5147 MRBIII. To schedule instrument time through the iLab Calendar, use the following instructions:

Go to <https://vanderbilt.corefacilities.org/landing/2191#/cores>

- Choose Tab: Vanderbilt Center for Structural Biology Instrumentation Core
- Sign-in using your VU ID
- Choose MST - S10 NanoTemper Monolith NT.115 from the list
- Click View schedule
- Book a desired time in the calendar
- Fill in the information for the payment option
- In case of difficulties contact arina.hadzi@vanderbilt.edu

The access to the instrument needs to be granted, so first time users need to request access ahead of time.

GENERAL OPERATIONAL INFORMATION

The Monolith instrument is equipped with two LED lasers, blue and red, and is good for measurements of the majority of green, blue and red fluorophores with a wavelength within the correct spectrum range:

BLUE . Excitation 465-490 nm; Emission 500-550 nm

RED . Excitation 600-640 nm; Emission 660-725 nm

Consumables can be ordered from NanoTemper, and there are different types of capillaries (standard treat 1000 capillaries cat. no. MO-K002) and labeling kits: <https://nanotempertech.com/monolith-consumables/>. Each lab/user should order capillaries and labeling kits if needed.

The advantage of microscale thermophoresis is that it uses very small quantities and volumes of material. The capillaries need as little as 4 uL of your sample. Typically, 10-11 uL is the volume loaded when the sample is not limiting.

The analysis allows for the measurement of up to 16 samples together and it takes ~10 minutes to have the analysis completed. For statistical purposes, there needs to be at least 3 repetitions (usually 4) for a standard deviation and error bar calculation.

Microscale thermophoresis is a very sensitive method that allows for the measurement of binding affinities between different components, including proteins, nucleic acids, small molecule compounds and many more (for details visit NanoTemper website). **In principle, detection requires one component to be labeled with fluorophore (TARGET) and one unlabeled (LIGAND).**

Check before the experiment if the concentration of the unlabeled molecule is high enough to reach a final concentration at least an order of magnitude (ideally even higher) above the expected dissociation constant (Kd). For details refer to the %Concentration Finder+Tool in the Monolith Software.

LOGIN AND PASSWORD FOR THE MST LAPTOP

After turning on the Monolith instrument (switch in the rear), turn on the laptop and log in using following information:

User: **nanotemper**

Password: **nanotemper**

Each laboratory should have own folder in which each lab member creates their own directory. There are two software types to use for the MST analysis (both with green icons):

MO.Control for experiment control and setup

MO.AffinityAnalysis for data processing post-collection

CHOOSING THE RIGHT PROGRAM FOR THE ANALYSIS

Depending on the user experience and optimization stage for the biological system studied the software offers different levels of control:

- Pretest
- Binding Check
- Binding Affinity
- Expert Mode

You can find a detailed description about each variant/level in the NanoTemper Software Manual manual_mo.control. For the Expert Mode that offers the highest level of control on every single parameter I typically use the accompanying Excel spreadsheet to calculate protein concentration.

PERFORMING THE MST ANALYSIS

- 1) On the computer, start a new project: Folder > File > New Project > Date
- 2) Select Expert Mode
- 3) Fill in required information:
 - a. LED setting
 - b. Concentration of fluorophore
 - c. Dilution
 - d. Table of Runs
 - e. Target
 - f. Ligand
- 4) For one technical replicate at a time, prepare NanoTemper tubes with protein + DNA, and then fill capillaries and load into NanoTemper. Preparing one replicate takes ~ 10 minutes, and reading one replicate also takes ~ 10 minutes.
 - a. Keep all samples on ice until immediately prior to loading tubes and capillaries.
 - b. Always prepare the highest protein concentration last, but load it onto slot #1 of NanoTemper so that it can be read first.
 - c. Place 16 PCR tubes in the black rack (use NanoTemper brand tubes if possible).
 - d. Transfer 10 uL of the protein (LIGAND) to each tube, starting with the highest protein concentration.
 - e. Add 1 uL of the fluorophore-labeled DNA (TARGET) and mix by pipetting. Avoid vortexing as this may trigger adsorption and denaturation.
 - f. Place a single capillary into each of the 16 PCR tubes.
 - g. Make sure that the sample is sucked into each capillary up to the top.
 - h. Place capillaries on the rack, starting with the highest concentration in position #1.
- 5) Place the loaded rack into the Monolith instrument.
- 6) Close the door using the touchscreen down arrow.
- 7) Press Start Measurement on the MST software. Measurement will begin with an initial intensity screen, followed immediately by the MST measurement and the final intensity screen. A full screen of 16 samples takes ~ 10 minutes. The MO.Control software will generate a report on the sample aggregation and adsorption.
- 8) Prepare the capillaries for the next replicate while the former is being measured.
- 9) Perform 4 readings for statistical purposes.

ADDITIONAL OPERATIONAL TIPS

Do not touch the capillaries except at the very ends.

The excitation power should be selected so that fluorescent intensity values stay within 200-2000.

The machine does not have a cooling option, so the analysis can only be performed at room temperature or higher. For standard XPA measurements, perform at room temperature. Keep your samples on ice immediately prior to mixing the substrate and protein, and loading of the capillaries. Because of the small volumes, pre-incubation at room temperature is not needed.

Avoid early mixing of the protein and DNA, as some increased precipitation is observed, as well as adsorption/sticking to the capillary walls.

Measure the highest protein concentration first (in position #1), but prepare this sample last, in order to avoid protein precipitation.

Use magnetic bars to hold previously loaded capillaries in place and avoid misplacing them on the rack.

When you begin the measurement:

- 1) First scan checks fluorescent intensity of all capillaries at room temperature. This provides a good measure of pipetting error.
 - a. Only ~ 10% error is tolerated, anything outside this range should be excluded during analysis.
 - b. Check for symmetric, non-adsorbed peaks, and no aggregation (look at accompanying report).
- 2) Second scan performs thermophoresis with changing temperature.

There are different rules to assess the quality of a signal:

- A signal should have more than 5 response units amplitude (amplitude = difference between bound and unbound state)
- The baseline noise should be at least 3 times less than the amplitude you should always measure at 3 different laser powers and compare results.

DATA PROCESSING

When data collection is completed and the analysis is saved as a .moc file, it can be opened using MO.AffinityAnalysis software. Standard data processing is described well in manual_mo.affinity_analysis tutorial and it will walk you through the data validation and 4 major steps:

- 1) Home . for creating a new analysis
- 2) Data Selection . for selecting runs for the analysis and grouping them in merged sets
- 3) Dose-response . data cleaning and processing
- 4) Compare Results . comparing the results from different sets

TROUBLESHOOTING

At the NanoTemper website, there are multiple tutorials and troubleshooting ideas. A printed hard copy version that covers basics is in the MST drawer under the instrument. The CD with MST software is also there and allows installation of the MO.AffinityAnalysis software on your lab computer. The CD should be ALWAYS in the drawer. If taken should be returned as soon as possible.

In case of any questions not covered by the NanoTemper Tutorial, you may contact Application Specialist Shariya Terrell, PhD (or other person listed on the instrument): shariya.terrell@nanotempertech.com, +1 404 997 9811. They are always happy to help with the experiment design or troubleshooting.