Introduction

The NanoTemper Monolith NT.Automated uses microscale thermophoresis (MST) of analytes in solution to obtain information on biomolecular interactions like K_d or EC_{50}. MST has been used to study proteins that are present in complex buffers like serum and can be used for competition assays as well.

The Monolith comes equipped with a PicoRED detector and a LabelFree detector and with its high throughput processing capabilities can be used for target-ligand library screening without concerns about molecule size or molecular weight of the sample and its ligand.

Features of the Monolith NT.Automated

Determination of K_d or EC_{50} values

Detectability between 10 pM to mM

Large experimental flexibility

Laser power and MST durations can be adjusted on the fly to obtain thermophoresis results that work best for your sample set.

Two protein detection methods

Protein detection can be done using PicoRED fluorescent detection channel (for protein-protein and protein-ligand interactions) or with LabelFree detector channel (protein-ligand interactions).

Minimal sample required

Sensitivity down to pM amounts of protein, with minimum sample volumes of 15 µL. The experiments can be set up in 384 -well plates.

User friendly software

Software guides user from the beginning of the experiment and how best to design and prepare an experiment, to the end of data analysis.

Structural & Biophysical Core Facility

Greg Wasney

Manager, Structural & Biophysical Core Facility
Peter Gilgan Centre for Research & Learning
The Hospital for Sick Children
686 Bay Street, Rm. 21.9708
Toronto, ON. M5G 0A4

Email: *greg.wasney@sickkids.ca*

Office: 416.813.7209
Office Internal Ext. 307209
Lab: 416-813-7654 ext. 309442

http://lab.research.sickkids.ca/sbc-facility/

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Structural and Biophysical Core Facility

NanoTemper Monolith NT.Automated for MST

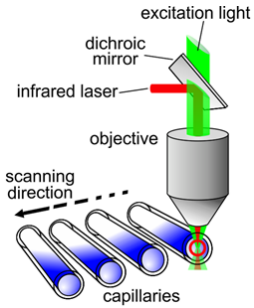
Microscale Thermophoresis for biomolecular interactions

- Measure molecular interaction kinetics (K_d or EC_{50}) for protein-protein or protein-ligand systems. Can determine K_d values ranging from 10 pM to mM.
- High throughput analysis allows for screening of 96 samples in 30 minutes.
- Immobilization free technology that can be used with proteins in native conformation.
- Sample volumes as low as 15 microliters required for loading of capillaries.

Monolith NT.Automated—PicoRED Analysis

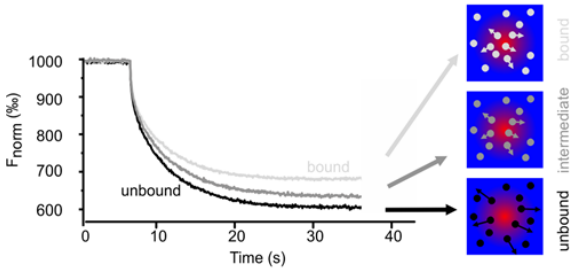
Theoretical Background

The Monolith uses a laser that shines on the sample to create temperature changes in the sample. This creates a temperature gradient that is the basis of Microscale Thermophoresis (MST). MST detects a temperature-induced change in the fluorescence of a target as a function of the concentration of the non-fluorescent ligand. This change in fluorescence is caused by both temperature related intensity change (TRIC) and thermophoresis.



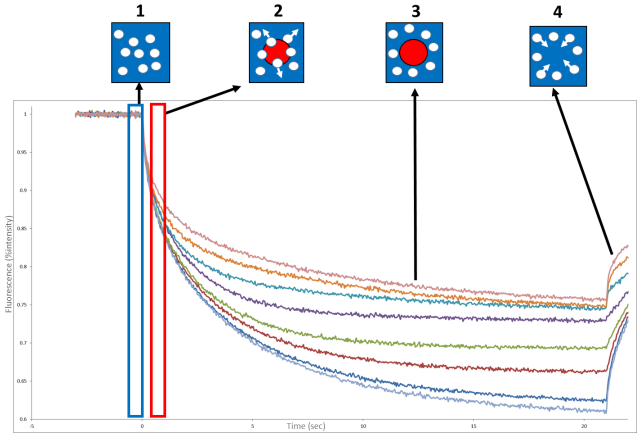
Any change of the fluorescent probe's chemical environment, such as a binding event with a partner, may alter the observed fluorescence when a temperature gradient is applied (known as TRIC).

A binding event between labelled protein and its partner will induce changes in the complex such that the free protein will diffuse differently from the complex and the rate at which the fluorescence signal decreases will be less for the complex than it will be for the free protein.



Overview of an MST experiment

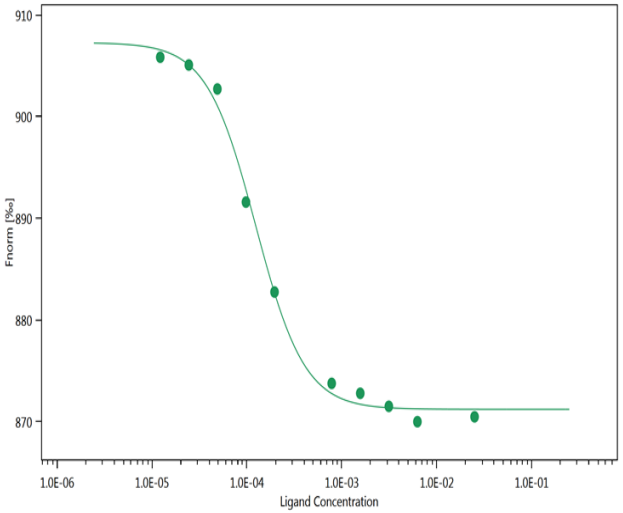
A typical MST experiment is comprised of 4 events. In the first event, the fluorescence within the capillary is measured to get an F_{cold} value (denoted by blue band). In the second event, the laser is turned on and the thermophoresis effect takes place, with samples diffusing out from where the laser is applied as a function of the temperature gradient. At a determined point, the fluorescence in the capillary within the laser's path is taken to get an F_{hot} value (denoted by red band).



In the third event, the fluorescence change plateaus as all of the sample has diffused out from the spot where the laser has been applied. In the fourth event, the laser is turned off and the sample can now diffuse back with the fluorescence increasing as a result. The normalized fluorescence $F_{norm}=F_{hot} / F_{cold}$ is then plotted as a function of ligand concentration and used to determine a k_d or EC_{50} value. One can alter the time points that define F_{hot} or F_{cold} in order to obtain more robust results with higher signal/noise ratios.

PicoRED detector validation

The PicoRed Control Kit (cat. #MO-C030) containing labelled AptamerCy5 RED and AMP were used to validate the PicoRED detector. Increasing amounts of AMP were titrated as a serial dilution then loaded into Premium Coated capillaries (cat. #MO-AK002) and read using a 20% laser power and medium MST power.



The resulting analysis gave an EC_{50} value of 120 µM for the interaction, comparing well to the reported value for the control kit.

Instrument specifications	
Excitation laser wavelength	1475 nm
Temperature control	25°C
Protein concentration	From pM to mM depending on detector selected
Read time per capillary	30 seconds