10/2014

nano TEMPER technologies

Monolith NT.115 Series
Product Information

Monolith Instruments
for MicroScale Thermophoresis

www.nanotemper-technologies.com

MicroScale Thermophoresis

A technology by NanoTemper

MicroScale Thermophoresis is an easy, fast and precise way to quantify biomolecular interactions. It measures the motion of molecules along microscopic temperature gradients and detects changes in their hydration shell, charge or size.

Enjoy the benefits of MST:

Optimize assays quickly:

- Judge and improve sample quality immediately

Measure previously unmeasurable targets:

- Work with very small amounts and sensitive samples

Benefit from close-to-native conditions:

- Analyze in all buffers and bioliquids (cell lysate, serum) –immobilization-free

Do your research efficiently:

- Enjoy perfect ease-of-use, purification-free measurements and get rid of maintenance downtime

Work flexibly:

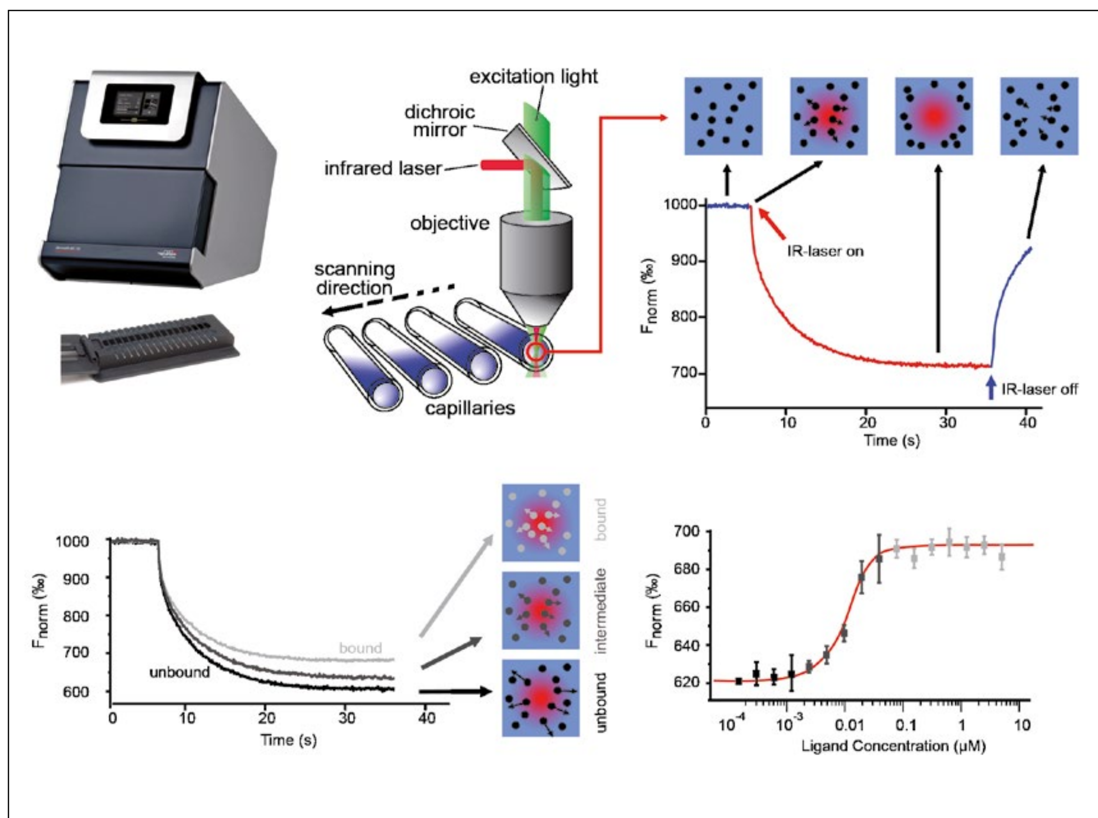
- K_ds for all molecular weights from ions to ribosomes and for pM to mM binding affinities

MicroScale Thermophoresis

A powerful technique

MicroScale Thermophoresis (MST) is a powerful technique for quantifying biomolecular interactions. By combining the precision of fluorescence detection with the flexibility and sensitivity of thermophoresis, MST provides a flexible, robust and fast way to measure molecular interactions.

When performing a MST experiment, a microscopic temperature gradient is induced by an infrared laser, and the directed movement of molecules is detected and quantified using either covalently attached dyes, fluorescent fusion proteins, or intrinsic fluorescence.

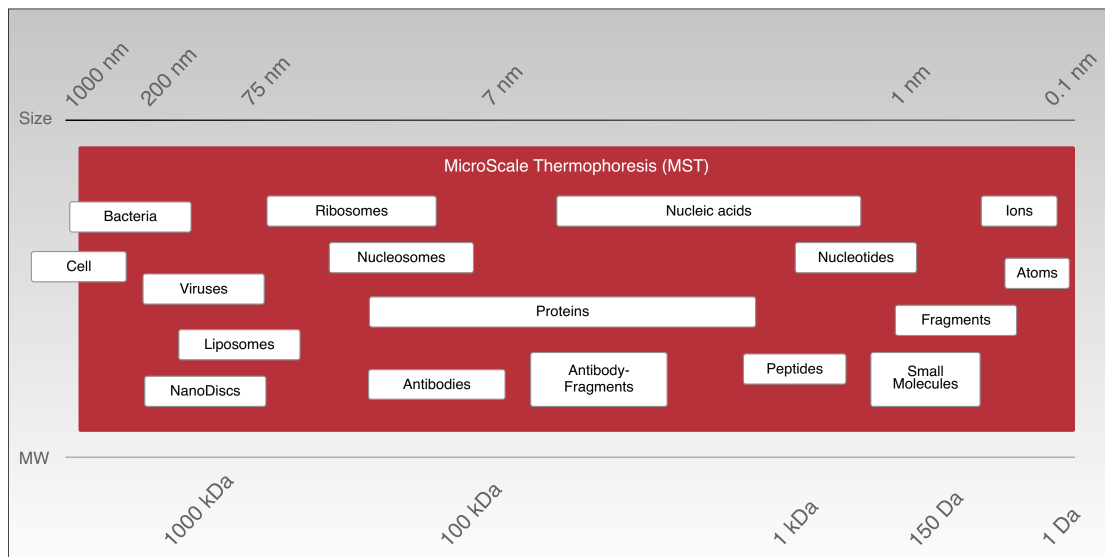


Discover the Application Range

MicroScale Thermophoresis detects interactions between any kind of biomolecules thus providing a large application range, from ions and small molecules to high molecular weight and multi-protein complexes.

Thermophoresis, the movement of molecules in temperature gradients, is not only dependent on the size, but also on the charge and the hydration shell of the molecule of interest. Therefore, binding events can be detected even without an increase in size or mass upon complex formation.

Since MST is performed free in solution without any surface immobilization, also bulky or sensitive molecule assemblies such as liposomes, nanodiscs or membrane proteins can be investigated.



Monolith NT.115 and NT.115 Pico

The NT.115 Series measures biomolecular interactions via detection of fluorescent dyes or fluorescent fusion proteins (such as GFP) providing the following benefits:

- Immobilization-free affinity determination from 1 pM to mM
- Broad application range
- Buffer independency: including serum or cell lysate
- Purification free: fluorescent fusion proteins

Cat #	Instrument	Channel 1	Channel 2	Affinity Range (K_d)	Sample Consumption (per K_d)
G006	NT.115 Pico	Pico – RED	-	1 pM to mM	120 pg [1]
G007	NT.115 Blue/Green	Nano – BLUE	Nano – GREEN	1 nM to mM	120 ng [2]
G008	NT.115 Blue/Red	Nano – BLUE	Nano – RED	1 nM to mM	120 ng [2]
G009	NT.115 Green/Red	Nano – GREEN	Nano – RED	1 nM to mM	120 ng [2]

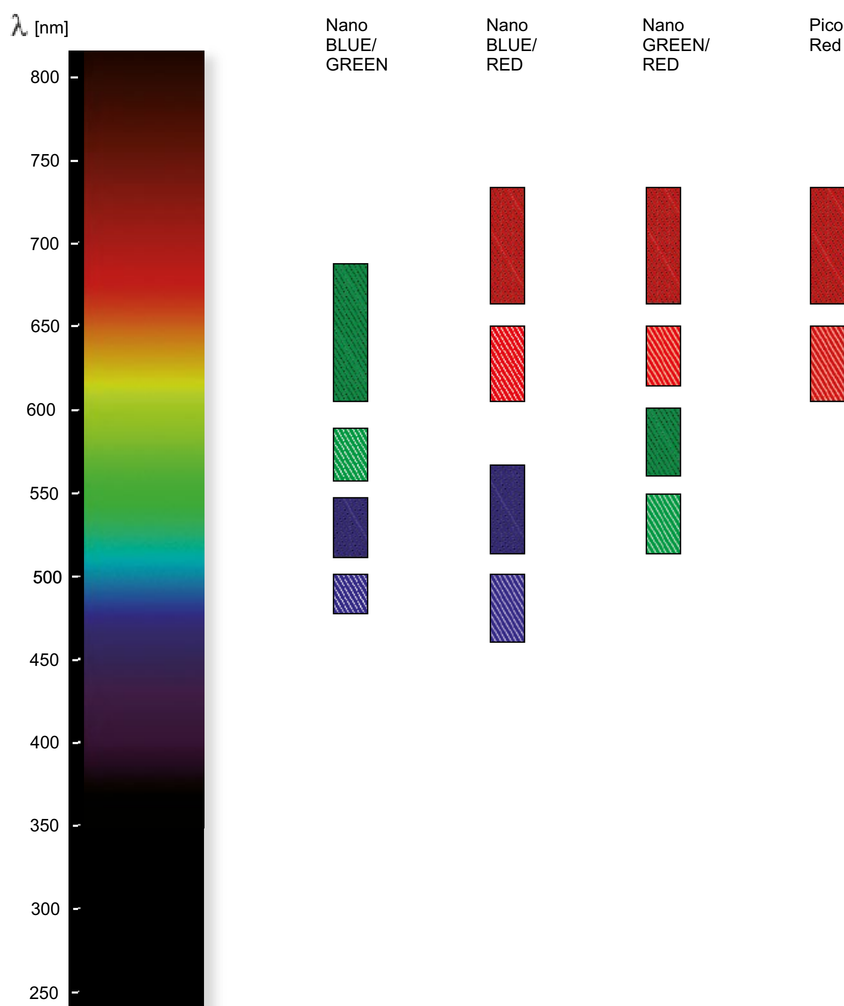
[1] calculated for a standard protein of 50 kDa, 12 data points per K_d and 10 pM fluorescently labeled protein.
[2] calculated for a standard protein of 50 kDa, 12 data points per K_d and 10 nM fluorescently labeled protein.

Technical Details

Monolith Instruments NT.115Series	NT.115	NT.115Pico
Samples per run	16 samples	16 samples
Fluorescence channels per instrument	2 (BLUE, RED or GREEN)	1 (RED)
Affinity range	1 nM to mM	1 pM to mM
Labeling required	Yes	Yes
Concentration of fluorescent molecule	10^{-9} - 10^{-3} M	10^{-11} - 10^{-3} M
Range of accessible interactions	■■■■■	■■■■■
Biophysical parameters	Affinity, Stoichiometry, Enthalpy, Enzyme Kinetics	Affinity, Stoichiometry, Enthalpy, Enzyme Kinetics
Tryptophan fluorescence required	No	No
Measurements in complex bioliquids (serum, cell lysate)	Yes	Yes
Volume per measurement	< 4 µl	< 4 µl
Molecular weight range (Da)	10^{1} - 10^{7}	10^{1} - 10^{7}
Time for experiment & analysis	Minutes	Minutes
Immobilization required	No	No
Temperature controlled	22 - 45 °C	22 - 45 °C
Maintenance required	No	No

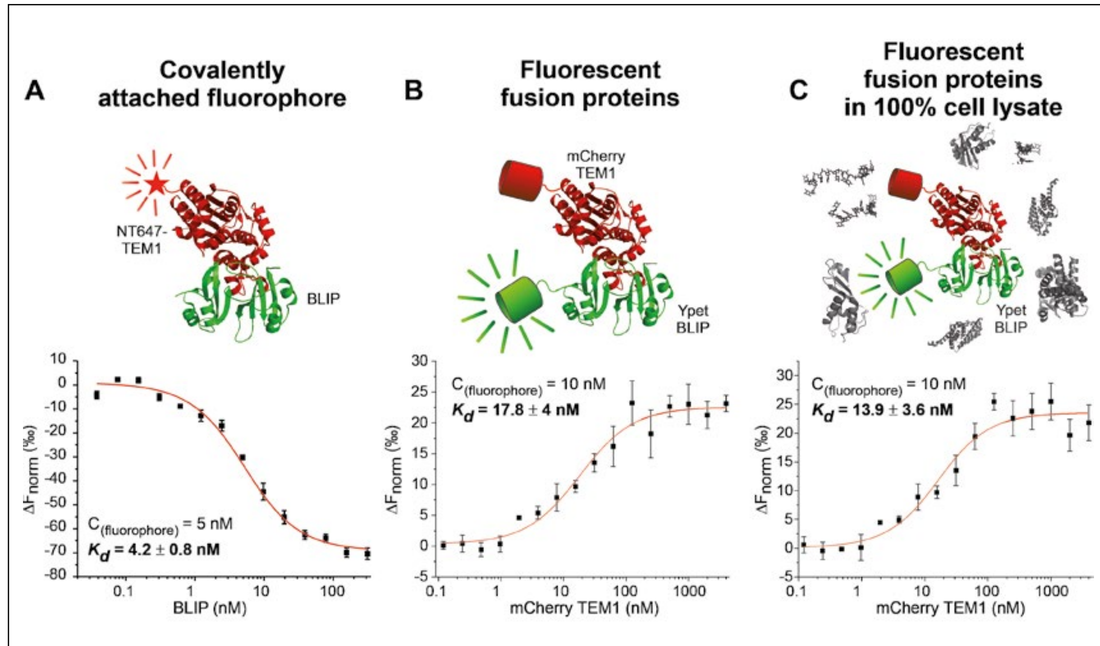
Detectors and Spectra

The excitation/emission wavelengths for the corresponding Monolith NT.115 Series detectors are illustrated below.



The light and dark shaded boxes correspond to the excitation and emission wavelength spectra, respectively, of the different fluorescence detectors.

Results: Protein-Protein Interaction

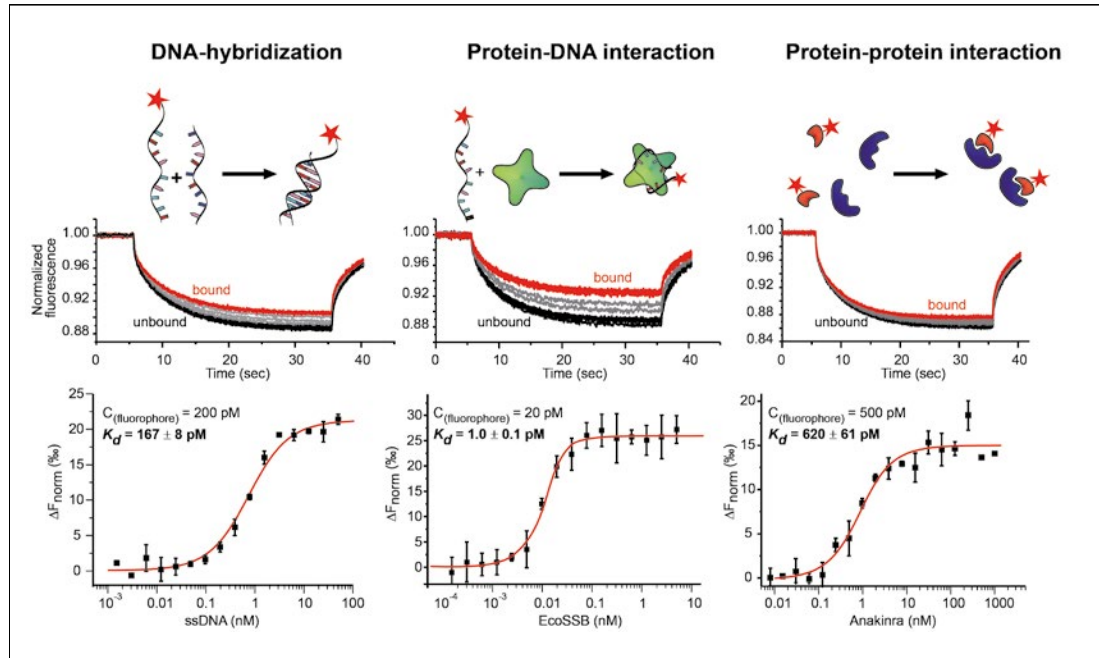


Thermophoretic analysis of a protein-protein interaction: this interaction has been investigated in a purified system using a covalently attached fluorophore (A) and using fluorescent fusion proteins in buffer (B) as well as in pure cell lysate (C).

Material was kindly provided by Prof. Gideon Schreiber, Weizmann Institute, Israel

Jerabek-Willemsen, M., André, T., Wanner, R., Roth, H. M., Duhr, S., Baaske, P., and Breitsprecher, D. (2014) MicroScale Thermophoresis: Interaction analysis and beyond. Journal of Molecular Structure

Results: High-Affinity Interactions



Detection of picomolar K_ds of different experimental systems using the Monolith NT.115^{Pico}.

Material was kindly provided by Dr. Ute Curth, Medical Unitersity Hanover, and Dr. Ahmed Besheer, Novartis, Basel.

Jerabek-Willemsen, M., André, T., Wanner, R., Roth, H. M., Duhr, S., Baaske, P., and Breitsprecher, D. (2014) MicroScale Thermophoresis: Interaction analysis and beyond. Journal of Molecular Structure

Customer Statements

Dr. Tim Sharpe, Biophysics Facility, Biozentrum, University of Basel, Switzerland

“We have used our Nanotemper NT.115 MicroScale Thermophoresis (MST) instrument extensively to study many different types of inteaction. […] Where we have been able to make comparisons, results from MST agree well with those from other established techniques (ITC, fluorescence intensity and anisotropy, SPR). However, we particularly appreciate two distinguishing aspects of MST. Firstly, MST can measure interpretable signal changes in circumstances where many other techniques struggle: i.e. small unlabelled molecules binding to large labeled molecules, where binding is tight (nM Kd) and one or both partners aggregate at micromolar concentrations. Secondly, one can often gain extra information about the interaction partners from MST titrations, particularly for systems that have changes in their conformational or oligomeric state upon binding. In several cases, multi-phasic titrations have inspired experiments with other techniques to characterize behaviours that can‘t be explained by the simplest binding models.”

Prof. Dr. Uffe Holmskov, Institute for Molecular Medicine, University of Southern Denmark, Odense, Denmark

“We are interested in elucidating interactions in close-to-native conditions: thus, we appreciate that the interactions are investigted free in solution and can be studied even in cell lysate. We are using MST for a number of different interactions, mainly to investigate protein-protein interactions but also to study protein-DNA interactions and protein binding to ions.”

Dr. Alexey Rak, Structural Biology & Biophysics, Sanofi R&D, France

“We routinely assess interaction affinity for both small molecule and biologics projects, with NanoTemper Technologies’ MicroScale Thermophoresis has proved a valuable tool for characterising small molecule-protein and protein-protein interactions, as well as for the study of protein aggregation concentration determination. There is very good agreement with other technologies such as Surface Plasmon Resonance (SPR) and Isothermal Titration Calorimetry (ITC), and we are particularly appreciative of this technology because of the extremely low protein consumption and relatively short time required for the assay setup.”

Selected Publications

1. Schulze, R. J., Komar, J., Botte, M., Allen, W. J., Whitehouse, S., Gold, V. A. M., Lycklama a Nijeholt, J. A., Huard, K., Berger, I., Schaffitzel, C., and Collinson, I. (2014) Membrane protein insertion and proton-motive-force-dependent secretion through the bacterial holotranslocon SecYEG–SecDF–YajC–YidC. Proceedings of the National Academy of Sciences 111, 4844-4849

2. Parker, J. L., and Newstead, S. (2014) Molecular basis of nitrate uptake by the plant nitrate transporter NRT1.1. Nature 507, 68-72

3. Jerabek-Willemsen, M., André, T., Wanner, R., Roth, H. M., Duhr, S., Baaske, P., and Breitsprecher, D. (2014) MicroScale Thermophoresis: Interaction analysis and beyond. Journal of Molecular Structure

4. Gaffarogullari, E. C., Krause, A., Balbo, J., Herten, D.-P., and Jäschke, A. (2013) Microscale thermophoresis provides insights into mechanism and thermodynamics of ribozyme catalysis. RNA biology 10, 0--1

5. Khavrutskii, L., Yeh, J., Timofeeva, O., Tarasov, S. G., Pritt, S., Stefanisko, K., and Tarasova, N. (2013) Protein Purification-free Method of Binding Affinity Determination by Microscale Thermophoresis. Journal of Visualized Experiments, e50541

6. Ems-McClung, Stephanie C., Hainline, Sarah G., Devare, J., Zong, H., Cai, S., Carnes, Stephanie K., Shaw, Sidney L., and Walczak, Claire E. (2013) Aurora B Inhibits MCAK Activity through a Phosphoconformational Switch that Reduces Microtubule Association. Current Biology

7. Taft, M. H., Behrmann, E., Munske-Weidemann, L.-C., Thiel, C., Raunser, S., and Manstein, D. J. (2013) Functional Characterization of Human Myosin-18A and its Interaction with F-actin and GOLPH3. Journal of Biological Chemistry

8. Schulz, S., Doller, A., Pendini, N. R., Wilce, J. A., Pfeilschifter, J., and Eberhardt, W. (2013) Domain-specific phosphomimetic mutation allows dissection of different protein kinase C (PKC) isotype-triggered activities of the RNA-binding protein HuR. Cellular Signalling

9. Alexander, C. G., Jürgens, M. C., Shepherd, D. A., Freund, S. M. V., Ashcroft, A. E., and Ferguson, N. (2013) Thermodynamic origins of protein folding, allostery, and capsid formation in the human hepatitis B virus core protein. Proceedings of the National Academy of Sciences

10. 10. Seidel, S. A., Wienken, C. J., Geissler, S., Jerabek-Willemsen, M., Duhr, S., Reiter, A., Trauner, D., Braun, D., and Baaske, P. (2012) Label-free microscale thermophoresis discriminates sites and affinity of protein-ligand binding. Angew Chem Int Ed Engl 51, 10656-10659

11. Xiong, X., Coombs, P. J., Martin, S. R., Liu, J., Xiao, H., McCauley, J. W., Locher, K., Walker, P. A., Collins, P. J., Kawaoka, Y., Skehel, J. J., and Gamblin, S. J. (2013) Receptor binding by a ferret-transmissible H5 avian influenza virus. Nature 497, 392-396

12. Lin, C. C., Melo, F. A., Ghosh, R., Suen, K. M., Stagg, L. J., Kirkpatrick, J., Arold, S. T., Ahmed, Z., and Ladbury, J. E. (2012) Inhibition of basal FGF receptor signaling by dimeric Grb2. Cell 149, 1514-1524

13. Seidel, S. A., Dijkman, P. M., Lea, W. A., van den Bogaart, G., Jerabek-Willemsen, M., Lazic, A., Joseph, J. S., Srinivasan, P., Baaske, P., Simeonov, A., Katritch, I., Melo, F. A., Ladbury, J. E., Schreiber, G., Watts, A., Braun, D., and Duhr, S. (2013) Microscale thermophoresis quantifies biomolecular interactions under previously challenging conditions. Methods 59, 301-315

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Europe / International

NanoTemper Technologies GmbH
Flößergasse 4
81369 München
Germany

Phone +49 (0)89 4522895 0
Fax +49 (0)89 4522895 60

info@nanotemper-technologies.com

USA / Canada

NanoTemper Technologies, Inc.
395 Oyster Point Blvd. Suite 135
South San Francisco,
CA 94080, USA

Phone + 1 650 763 1658
Fax + 1 650 350 4390

info@nanotemper-technologies.com