10/2014

Monolith NT.LabelFree

Product Information

Monolith Instruments
for MicroScale Thermophoresis

www.nanotemper-technologies.com

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Product Information

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
  –immobilization-free and label-free

Do your research efficiently:

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

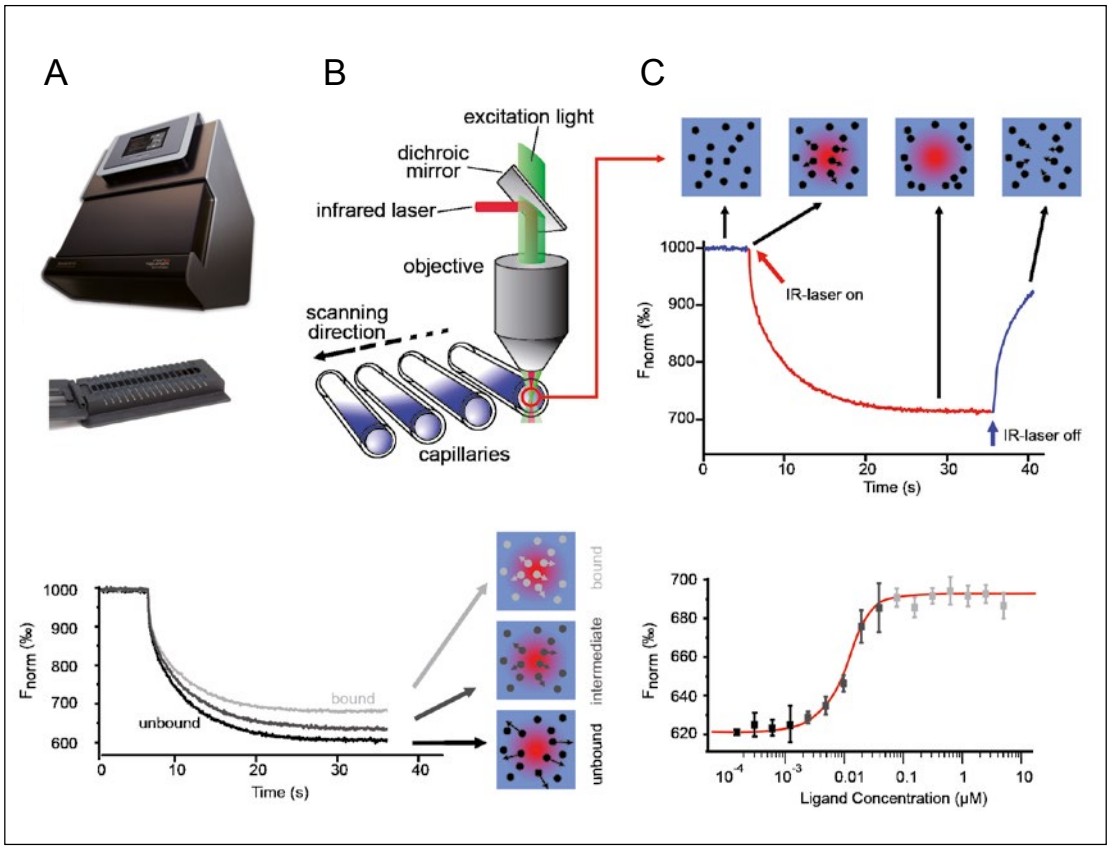
Work flexibly:

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

MicroScale Thermophoresis

A powerful technique

MicroScale Thermophoresis (MST) is a powerful technique to quantify biomolecular interactions. By combining the precision of fluorescence detection with the variability and sensitivity of thermophoresis, MST provides a flexible, robust and fast way to dissect molecular interactions. When performing a MST experiment, a mircoscopic temperature gradient is induced by an infrared laser and the directed movement of molecules is detected and quantified using intrinsic tryptophan fluorescence.

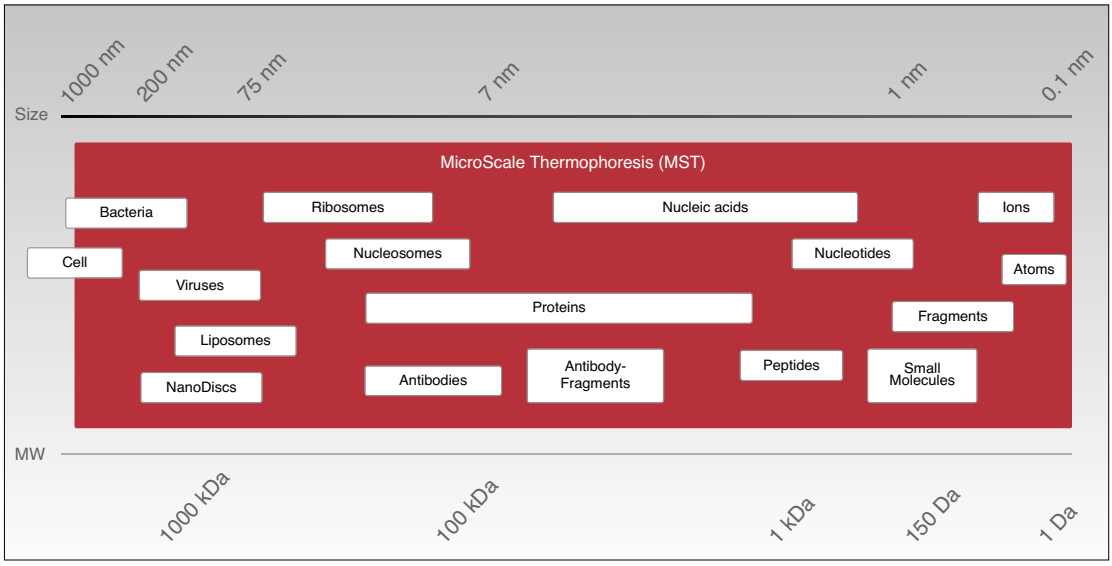


Discover the Application Range

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.



Product Details

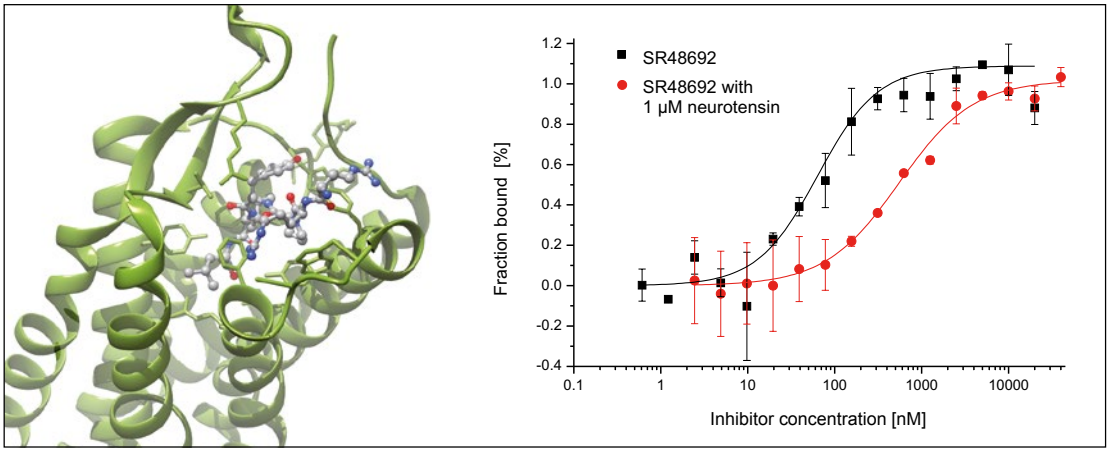
The Monolith NT.LabelFree measures MST via intrinsic tryptophan fluorescence [280 nm (ex) and 360 nm (em)], therefore providing a truly label-free reaction setup which is most beneficial for difficult samples such as membrane proteins. In addition, the experiment can be performed in any buffer. The NT.LabelFree is exceptionally sensitive for proteins binding to small molecules such as fragments, inhibitors or ions.

Technical Details

Monolith Instruments NT.Series	NT.LabelFree
Samples per run	16 samples
Fluorescence channels per instrument	1 (UV)
Fluorescence multiplexing	No
Affinity range	10 nM to mM
Labeling required	No
Concentration of fluorescent molecule	10^{-8} - 10^{-3} M
Sample consumption	1.2 µg [1]
Range of accessible interactions	■■■□□
Biophysical parameters	Affinity, Stoichiometry, Enthalpy, Enzyme Kinetics
Tryptophan fluorescence required	Yes
Measurements in complex bioliquids (serum, cell lysate)	No
Volume per measurement	< 4 µl
Molecular weight range (Da)	10^{1} - 10^{7}
Time for experiment & analysis	Minutes
Immobilization required	No
Temperature controlled	22 - 45 °C
Maintenance required	No

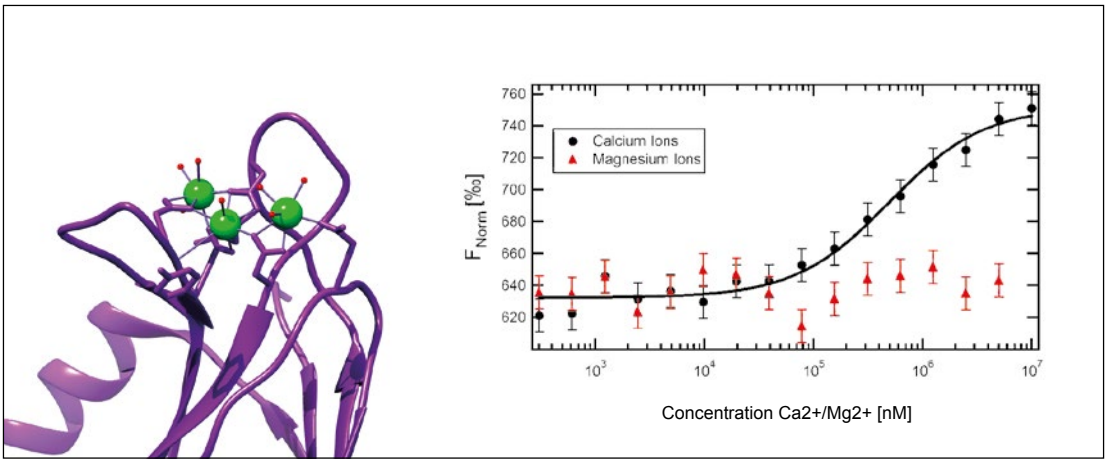
[1] calculated for a standard protein of 50 kDa, 12 data points per K_d and 100 nM tryptophan-containing protein.

Results



MST binding experiment of a GPCR Receptor binding to an inhibitor in the presence and absence of the natural ligand Neurotensin.

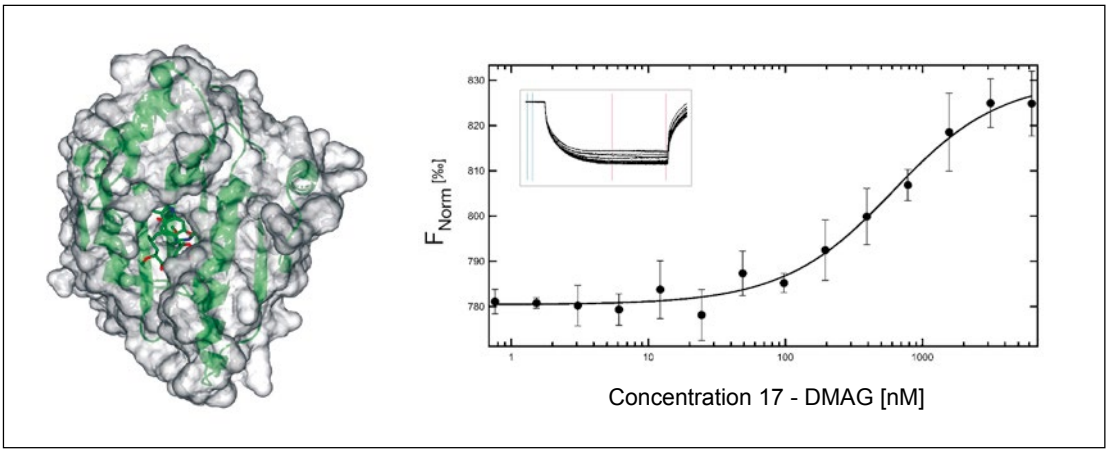
Prof. Anthony Watts, University of Oxford, Biochemistry, UK
Seidel et al, Angewandte Chemie, 2012



MST binding experiment of Synaptotagmin, a calcium sensor, to divalent cations.

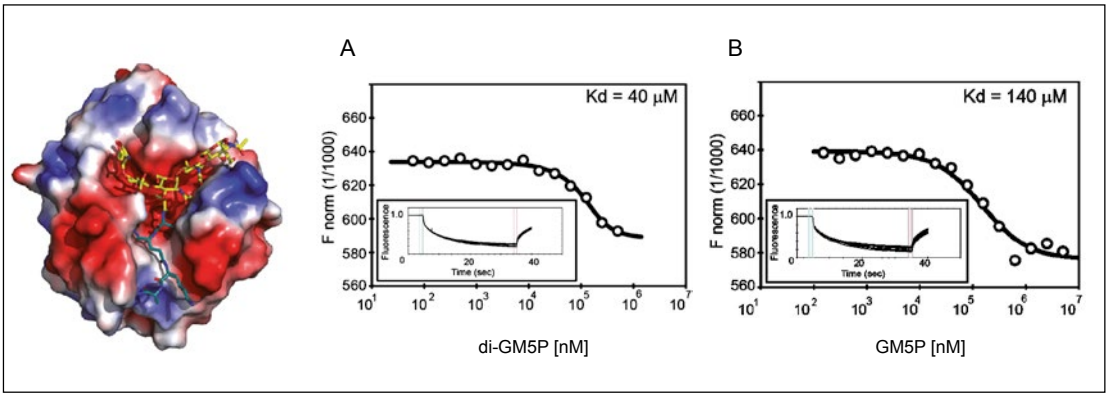
Karsten Meyenberg[1] & Geert van den Bogaart [2,]
[1] Institut für Organische und Biomolekulare Chemie, University Göttingen, Germany
[2] Max Planck Institute for Biophysical Chemistry, Göttingen, Germany

Results



MST binding experiment of HSP90 binding to the geldanamycin derivative 17-DMAG.

Stephen McLaughlin, MRC Laboratory for Molecular Biology, Cambridge, UK



Interaction of Lytic Amidase LytA with two different muropeptides

Mellroth, P., Sandalova, T., Kikhney, A., Vilaplana, F., Hesek, D., Lee, M., Mobashery, S., Normark, S., Svergun, D., Henriques-Normark, B., and Achour, A. (2014) Structural and Functional Insights into Peptidoglycan Access for the Lytic Amidase LytA of Streptococcus pneumoniae. MBio 5(1); doi:10.1128/mBio.01120-13.

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Customer Statements

Dr. Markus Zeeb, Boehringer Ingelheim Pharma GmbH & Co. KG – Research Germany

“One mission of the Structural Research group within the Lead Identification and Optimization department is to quantitatively validate and characterize interactions of small molecules as well as new biological entities with protein targets. We employ various traditional biophysical methods such as SPR, Thermal Shift, ITC, NMR and X-ray crystallography. Most recently we included MicroScale Thermophoresis (MST) in our standard project support workflow and extended its application from affinity determinations to fragment screening approaches.

MST is a versatile and valuable tool which we quickly adapted in our repertoire of methods. The impressive advantages of MST, namely the low sample consumption, the broad application range, and swift assay development make it a unique biophysical method. The measurement in free solution without the need of surface coupling saves time and avoids a potential source for false positive or negative results. Our Structural Research group now also added the label-free version of the Monolith to our MST instrumentation portfolio, which gives us the possibility to choose to measure with high selectivity and sensitivity (NT.115). The labelfree version (NT.LabelFree) allows us to measure without any additional sample modification depending on the need of the particular assay. In some cases, LabelFree MST allowed us to perform assays with otherwise “very ill” behaved proteins which were not amenable to any other biophysical technique. Generally, we find very good consistency between quantitative MST measurements and results stemming from other biophysical methods.“

Dr. Timothy Sharpe, Head of the Biophysics Facility, Biozentrum, University of Basel, Switzerland

“We have used our Nanotemper MicroScale Thermophoresis (MST) instrument extensively in the last one and a half years to study many different types of interaction: e.g. protein-small molecule interactions (biological ligands and compounds from medicinal chemistry), protein-metal ion interactions, [...]. MST has become one of the most frequently used techniques in the facility, and has produced a great deal of useful data. 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).”

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 being the most recent addition to the pool of instruments we use to carry out these measurements. It 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 new technology because of the extremely low protein consumption and relatively short time required for the assay setup. NanoTemper customer support has been a key factor in enabling us to familiarise ourselves with the new technology. We would like to deploy increasing numbers of applications based on MST technologies and continue to interact with NanoTemper Technologies Company, a dynamic, scientifically driven company."

Dr. Nicolas Basse, UCB-Celltech, UK

"As part of our drug discovery projects we use Microscale Thermophoresis (MST) as an orthogonal method to measure the binding affinity of compounds to their protein target and apply this fragment hit ID through to lead optimisation. MST complements our biophysical platform and has correlated well with other more established technologies. Because it uses small amounts of protein, MST has proved to be particularly useful to look at molecular interaction involving proteins that are difficult to express or purify. MST requires a relatively short time to setup new assays and is a powerful technique for buffer optimisation. Using the NT.115 MST instrument we have successfully measured small molecule-protein and protein-protein interactions in complex media. Finally, LabelFree MST is one of the few true label-free/immobilisation-free instruments capable of measuring molecular interactions. We have appreciated the professionalism and support from NanoTemper Technologies."

Selected Publications

1. 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

2. Mellroth, P., Sandalova, T., Kikhney, A., Vilaplana, F., Hesek, D., Lee, M., Mobashery, S., Normark, S., Svergun, D., Henriques-Normark, B., and Achour, A. (2014) Structural and Functional Insights into Peptidoglycan Access for the Lytic Amidase LytA of Streptococcus pneumoniae. MBio

3. 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

4. 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

5. Parent, A., Pietschner, M., and Rak, A. (2011) Interactions of small fragment–like molecules with a model protein - Carbonic anhydraze II. Application Note NT010

6. McLaughlin, S. H. (2011) Binding of the geldanamycin derivative 17-DMAG to Hsp90 measured with fluorescence label and label-free. Application Note NT001

7. Morlot, C., Bayle, L., Jacq, M., Fleurie, A., Tourcier, G., Galisson, F., Vernet, T., Grangeasse, C., and Di Guilmi, A. M. (2013) Interaction of Penicillin-Binding Protein 2x and Ser/Thr protein kinase StkP, two key players in Streptococcus pneumoniae R6 morphogenesis. Molecular microbiology, n/a-n/a

8. Meyenberg, K., and Van den Bogaart, G. (2011) Binding of Calcium Ions to Synaptotagmin measured with fluorescence label and label-free. Application Note NT006

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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