10/2014

nano TEMPER technologies

Monolith NT.Automated

Product Information

Monolith Instruments
for MicroScale Thermophoresis

www.nanotemper-technologies.com

Monolith NT.Automated
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 and bioliquids (cell lysate, serum) –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

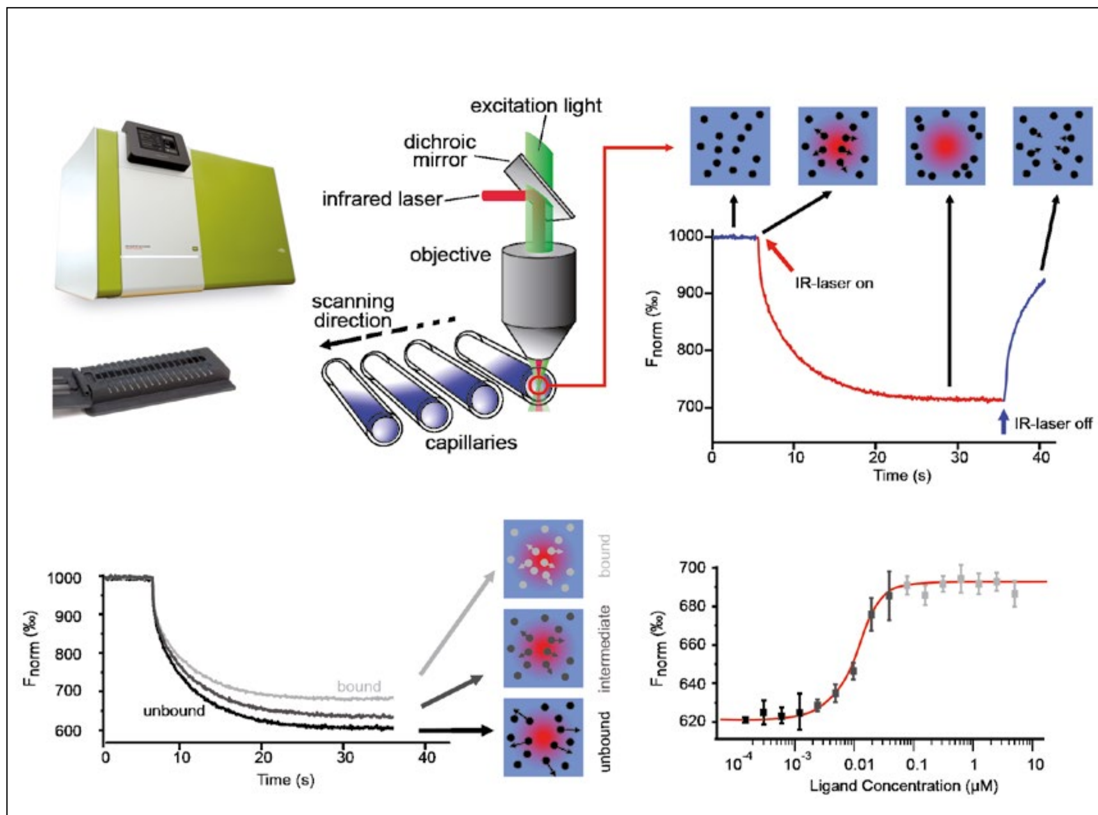
MicroScale Thermophoresis

A powerful technique

MicroScale Thermophoresis (MST) is a powerful technique to quantify biomolecular interactions. By combining the precision of fluorescence detection with the flexibility and sensitivity of thermophoresis, MST provides a flexible, robust and fast way to measure 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 either covalently attached fluorophores or intrinsic tryptophan fluorescence.

The applications range from small-molecule binding events to protein-protein interactions and interactions of multi-protein complexes.



Product Details

Within the Monolith Series, the Monolith NT.Automated is designed for high-throughput applications.

The Monolith NT.Automated comprises all benefits of the MicroScale Thermophoresis technology and is able to accommodate 96 samples at a time. Screening projects can be performed in an automated fashion by integrating the instrument into a liquid handling platform.

Samples are loaded and analyzed in an automated fashion in 24-capillary chips. The capillary format ensures an immobilization-free, low volume reaction setup where binding affinities can be determined in any kind of buffer, even in serum and cell lysate. Thus, the instrument combines the benefits of close-to-native conditions with low volume and concentration requirements of your precious sample.

The Monolith NT.Automated provides a great versatility as this device can be equipped with up to two different Monolith detection systems. Therefore, high sensitivity fluorescence for high-affinity interactions and label-free applications can be combined in one instrument.

Product Details

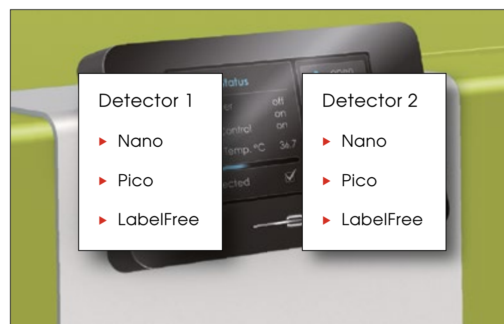
Monolith NT.Automated Base Modules

Cat #	Instrument	Detectors
G011	Monolith NT.Automated Base Module	2
G011R	Monolith NT.Automated Base Module Including liquid handling platform	2

Monolith NT.Automated Detectors

Cat #	Detector (choose up to 2)	Affinity Range (K_d)	Sample Consumption (per K_d) *
F001	Nano – BLUE/GREEN	1 nM to mM	120 ng [1]
F002	Nano – BLUE/RED	1 nM to mM	120 ng [1]
F003	Nano – GREEN/RED	1 nM to mM	120 ng [1]
F004	Pico – RED	1 pM to mM	120 pg [2]
F005	LabelFree	10 nM to mM	1.2 µg [3]

[1] calculated for a standard protein of 50 kDa, 12 data points per K_d and 10 nM fluorescently labeled protein.
[2] calculated for a standard protein of 50 kDa, 12 data points per K_d and 10 pM fluorescently labeled protein.
[3] calculated for a standard protein of 50 kDa, 12 data points per K_d and 100 nM fluorescently labeled protein.

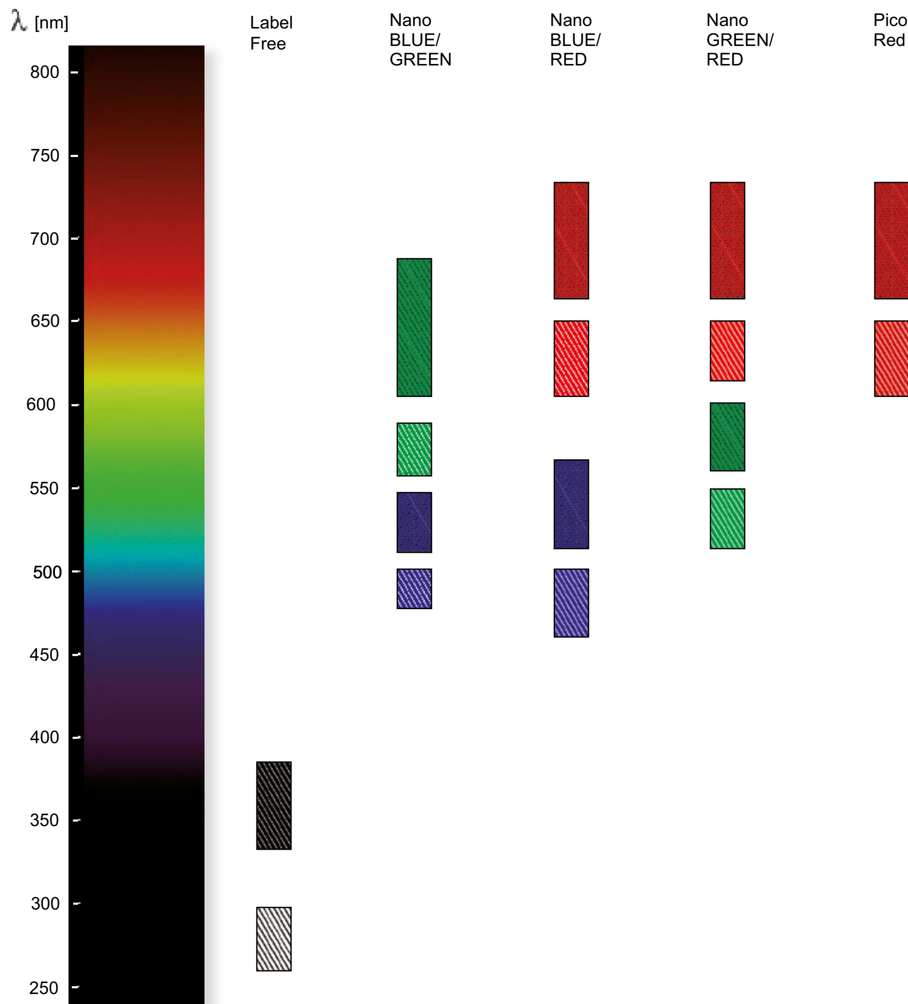


Technical Details

Monolith Instruments NT.Series	NT.Automated
Samples per run	96 samples
Fluorescence channels per instrument	Combination of up to 2 detectors
Fluorescence multiplexing	Yes
Affinity range	These parameters depend on the choice of the detectors: the NT. Automated can be equipped with a combination of two different detectors which can be freely chosen from all Monolith detection systems (Nano, Pico and LabelFree detectors)
Labeling required	
Concentration of fluorescent molecule	
Range of accessible interactions	
Tryptophan fluorescence required	
Measurements in complex bioliquids (serum, cell lysate)	
Volume per measurement	< 3 µl
Molecular weight range (Da)	10^1 - 10^7
Time for experiment & analysis	Minutes
Immobilization required	No
Temperature controlled	25°C (actively controlled)
Maintenance required	Optional service and performance diagnostic

Detectors and Spectra

The excitation/emission wavelength for the corresponding Monoltih NT.Automated detectors are illustrated below.



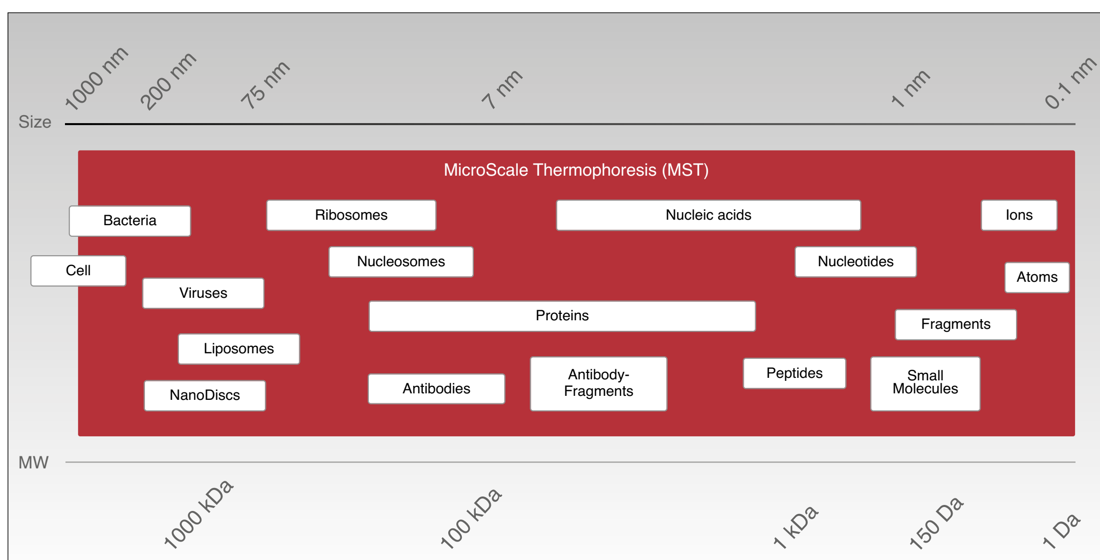
The light and dark shaded boxes correspond to the excitation and emission wavelength spectra of the different fluorescence detectors, respectively.

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.

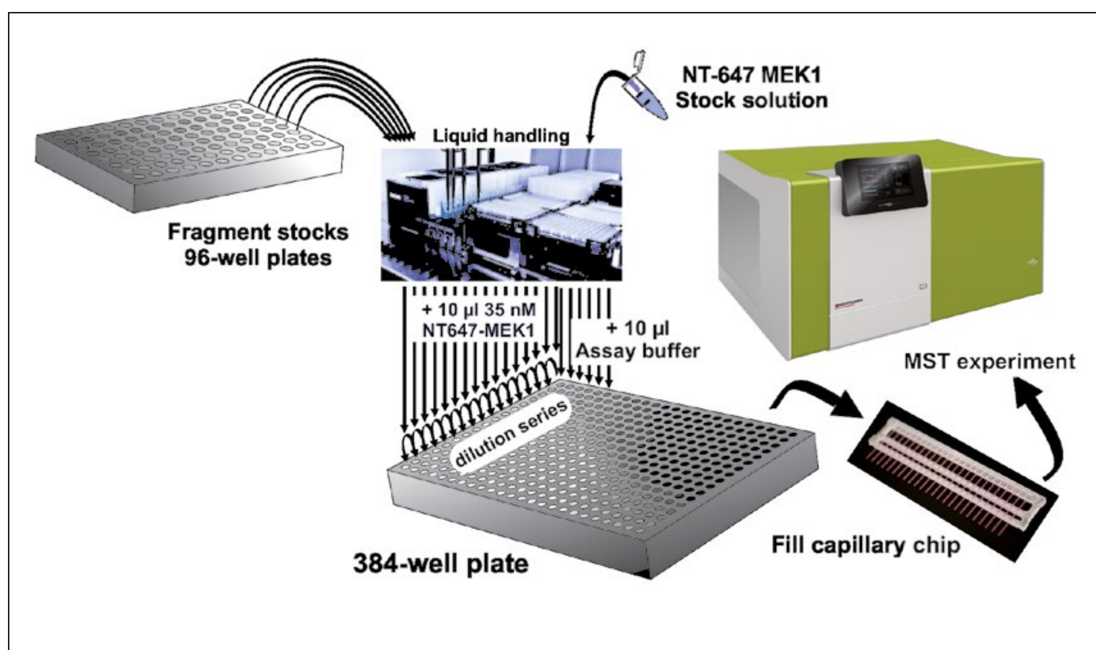
Since MST is performed free in solution without any surface immobilization, also bulky or sensitive molecule assemblies such as liposomes, nanodiscs or membrane proteins can be investigated.



Fragment Screening Project: Project Design

Case Study on Automated Screening Project of a Fragment Library against MEK1 kinase

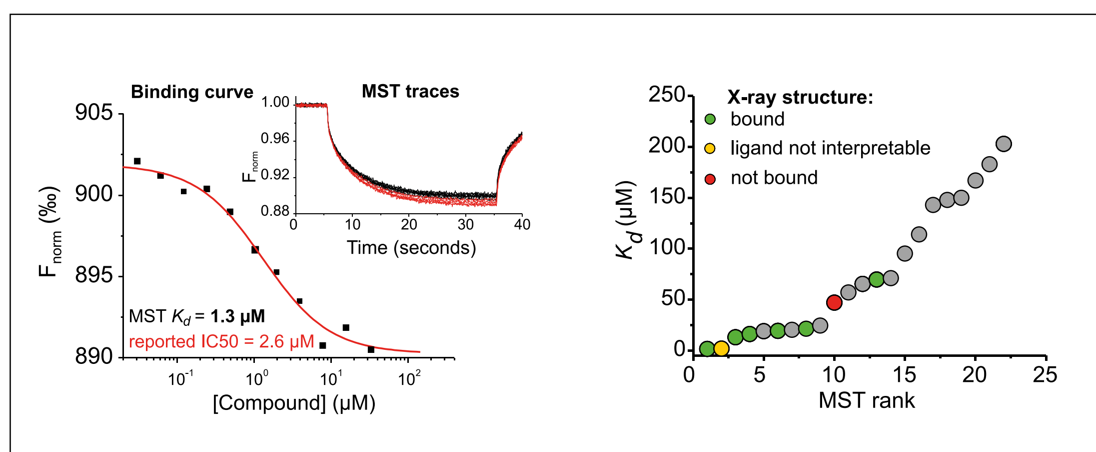
The Monolith NT.Automated allows for screening libraries of compounds or fragments in a timely manner with very low sample consumption. In addition, the innovative capillary chip format allows for easy handling and the integration of MST experiments into a fully automated setting. For this study, a library containing 193 *in silico* pre-selected fragments were screened for their interaction with the drug target MEK1 employing a set of orthogonal methods, DSF (differential scanning fluorimetry), SPR (surface plasmon resonance) and MST.



	Per datapoint	Per K_d	Total (193 interactions + 10 positive controls)
# Datapoints	/	12	2436
Measurement time	10 sec	120 sec	6 h 26 min
Protein used	0.3 ng	3.1 ng	628 ng

Fragment Screening Project: Assay Setup and Results

To establish the assay, the buffer conditions were optimized to ensure reproducable protein activity and stability. First, the interaction of NT-647 fluorescently labeled MEK1 with its natural substrate ATP was analyzed, which later served as a positive control. A total of 10 ATP control experiments were performed throughout the screening to ensure MEK1 functionality. The average dissociation constant K_d of these control experiments was 9.4 +- 0.8 µM, corroborating the robustness of the MST screening approach.
In addition, the interaction of MEK1 with a pre-characterized test compound was determined prior to the screening of the 193 fragments.

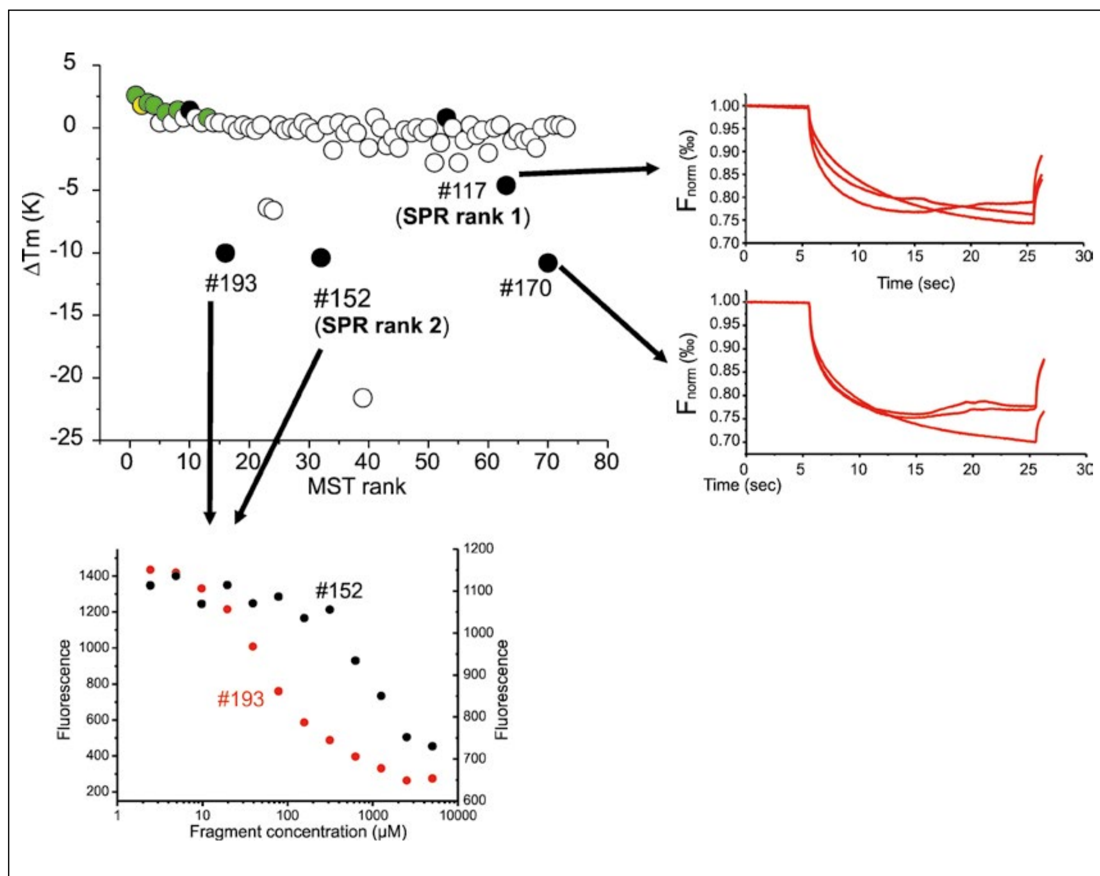


Among the193 fragments analyzed with MST in this study, > 70 binders were identified with K_ds ranging from the low µM to low mM range. 16 fragments displayed K_ds below 100 µM. Importantly, 7 out of 8 co-crystal validated hits were among the top-fifteen fragments from the MST-ranking.

1. Amaning K, Rak A, et al., (2013) The use of virtual screening and differential scanning fluorimetry for the rapid identification of fragments active against MEK1. Bioorganic & me dicinal chemistry letters 23: 3620-3626
2. Breitsprecher D, et al., (2014) Case Study on Automated Screening Project of a Fragment Library against MEK1 kinase, Applicaton Note NT021

Fragment Screening Project: Additional Benefits

The MST ranking showed a very strong correlation with a qualitative DSF screening. Since DSF, however, requires a change in melting temperature of the protein upon ligand binding, only few fragments could be identified as hits whereas MST could rank 70 out of 193 fragments according to their binding affinity.
In addition, false-positive hits from SPR screening could be identified directly from the MST time traces. Therefore, high-throughput MST analysis is a perfect asset in modern drug discovery processes.



The upper left panel displays a plot of the change in melting temperature (T_m) from the DSF screen against the MST rank. Negative T_m values correspond to protein destabilization due to fragment effects. MST can identify destabilizing effects based on multiple phenomena: Protein aggregation results in “bumpy traces” (see fragment #117 and #170), and protein precipitation leads to a loss of fluorescence (see fragment #193 and #152). Note that fragments #117 and #152 were ranked as 1 and 2 in the SPR screen. #193 was initially ranked as #21 in the MST rank but could be easily identified as a false positive.

Customer Statements

Dr. Markus Zeeb, Principal Scientist, Structural Research, 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. Nicolas Basse, Department of Structural Biology, 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.”

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. 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: protein-small molecule interactions (biological ligands and compounds from medicinal chemistry), small molecule competition assays, protein-metal ion interactions, protein-protein interactions, antibody-antigen interactions and protein oligomerisation.

MST offers high sensitivity with low material consumption, robust and low maintenance instruments. [...] 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). 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 labelled molecules, where binding is tight (nM K_d) 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.

The Facility‘s staff and users have benefitted greatly from the excellent customer support provided by Nanotemper. Advice is always practical, relevant and often delivered in-person. Nanotemper‘s Applications Scientists are professional, knowledgeable and display an impressive commitment to the success of new users‘ experiments.“

Selected Publications

1. Ascher, D. B., Wielens, J., Nero, T. L., Doughty, L., Morton, C. J., and Parker, M. W. (2014) Potent hepatitis C inhibitors bind directly to NS5A and reduce its affinity for RNA. Sci. Rep. 4

2. 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

3. Liebner, R., Mathaes, R., Meyer, M., Hey, T., Winter, G., and Besheer, A. (2014) Protein HESylation for half-life extension: Synthesis, characterization and pharmacokinetics of HESylated anakinra. European Journal of Pharmaceutics and Biopharmaceutics

4. Barandun, L. J., Immekus, F., Kohler, P. C., Ritschel, T., Heine, A., Orlando, P., Klebe, G., and Diederich, F. (2013) High-affinity inhibitors of Zymomonas mobilis tRNA-guanine transglycosylase through convergent optimization. Acta Crystallographica Section D 69, 1798-1807

5. 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

6. 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

7. Ramakrishnan, M., Alves De Melo, F., Kinsey, B. M., Ladbury, J. E., Kosten, T. R., and Orson, F. M. (2012) Probing cocaine-antibody interactions in buffer and human serum. PloS one 7, e40518

8. Lippok, S., Seidel, S. A., Duhr, S., Uhland, K., Holthoff, H. P., Jenne, D., and Braun, D. (2012) Direct detection of antibody concentration and affinity in human serum using microscale thermophoresis. Analytical chemistry 84, 3523-3530

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

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