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Monolith® NT.Automated

分子相互作用分析

Monolith® Instruments
for MicroScale Thermophoresis™

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分子相互作用分析



微量热泳动技术 MST

源于德国 NanoTemper®

微量热泳动技术能够简单、快速、精确地定量分析生物分子相互作用。它可以检测分子在微观温度梯度场中的运动，以及分子水化层、电荷和大小的变化。

MST 的技术优势：

快速得到实验结果

- 超智能软件：以引导模式进行实验设计和数据分析，10 分钟内获得 K_ds

测试方式优于传统技术

- 非常适合难以纯化和不稳定的样品

天然条件下的测量

- 无需样品固定，可用于所有缓冲液条件（细胞裂解液、血清等）

科研工作更高效

- 操作简便、无需纯化样品、无需维护

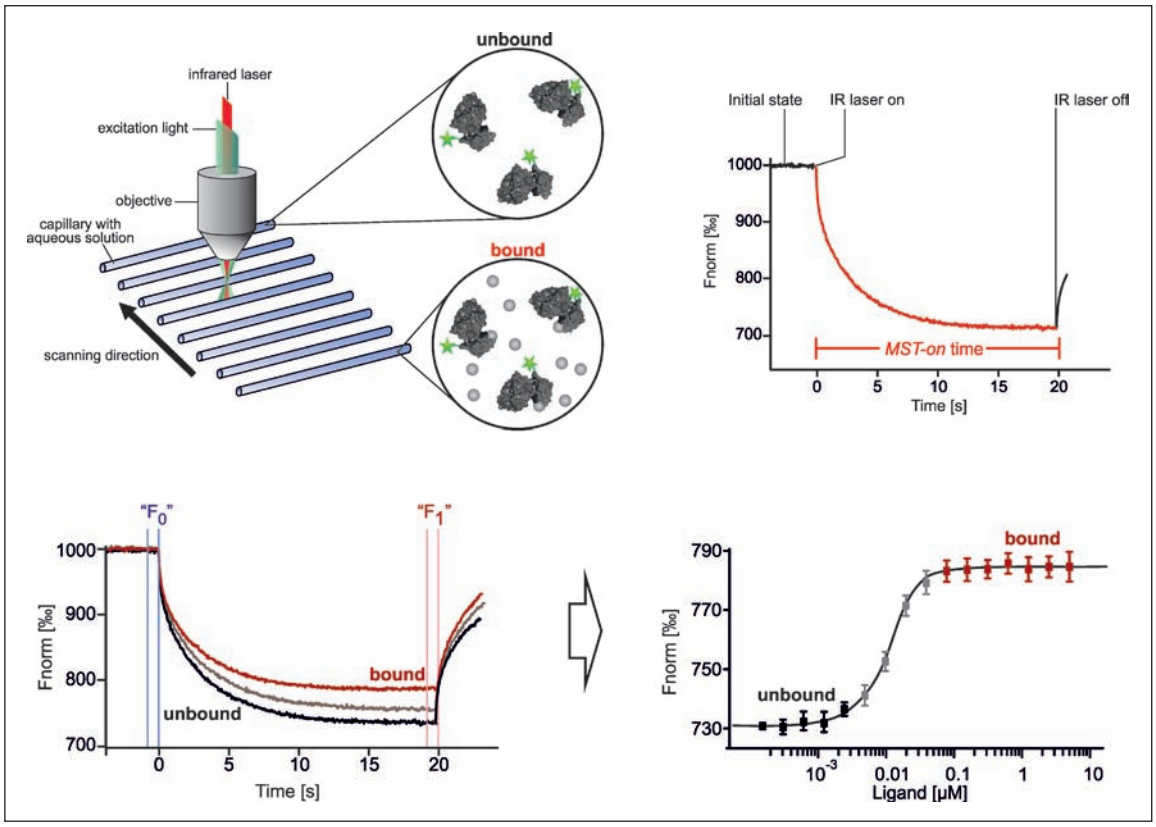
应用范围广泛

- 可以测定分子质量从离子至核糖体，结合亲和力 K_ds 从 pM 至 mM 范围内的所有分子

微量热泳动 MicroScale Thermophoresis™

微量热泳动 (MST) 是一种定量分析生物分子间相互作用的前沿技术。通过精确检测荧光变化，以及灵敏的热泳动现象，MST 提供了一种灵活、强大和快速测量分子间相互作用的方法。

运行 MST 实验时，红外激光瞬间加热生成一个微观温度梯度场，通过共价结合的荧光染料、荧光融合蛋白或蛋白自身色氨酸荧光来监测并定量分析分子的定向移动。



产品详情

在 Monolith 系列产品中，Monolith NT.Automated（全自动版）专门针对高通量应用而设计开发。

Monolith NT.Automated 涵盖了微量热泳动技术的所有优点，可同时容纳 96 个样品。高通量版可以与液体处理系统整合，建立起一套全自动的筛选平台。

样品自动装载到 24 支毛细管为一组的毛细管芯片 (24-Capillary Chips) 中进行分析检测。采用毛细管形式的优势在于 : 无样品固定、低样品消耗、亲和力的测定可在任意溶液中进行（如血清或细胞裂解液）。因此，可在接近天然环境中测量，并能为您节省大量的宝贵样品。

Monolith NT.Automated 具备强大的多功能性，可以整合任意两种不同的 Monolith 检测系统。因此，一套系统可以完美实现 Monolith NT.115 和 Monolith NT.LableFree（非标记型）的所有应用。

产品详情

Monolith NT. Automated 基础模块

目录号	仪器	检测器数量
G011	Monolith NT.Automated	2
G011R	Monolith NT.Automated 筛选系统	2

Monolith NT. Automated 检测器

目录号	检测器（最多可选 2 个）	亲和力范围 (K_d)	样品消耗量（每个 K_d）
F001	Nano-BLUE/GREEN	1 nM - mM	120 ng [1]
F002	Nano-BLUE/RED	1 nM - mM	120 ng [1]
F003	Nano-GREEN/RED	1 nM - mM	120 ng [1]
F004	Nano-RED	1 pM - mM	120 pg [2]
F005	LabelFree	10 nM - mM	1.2 µg [3]

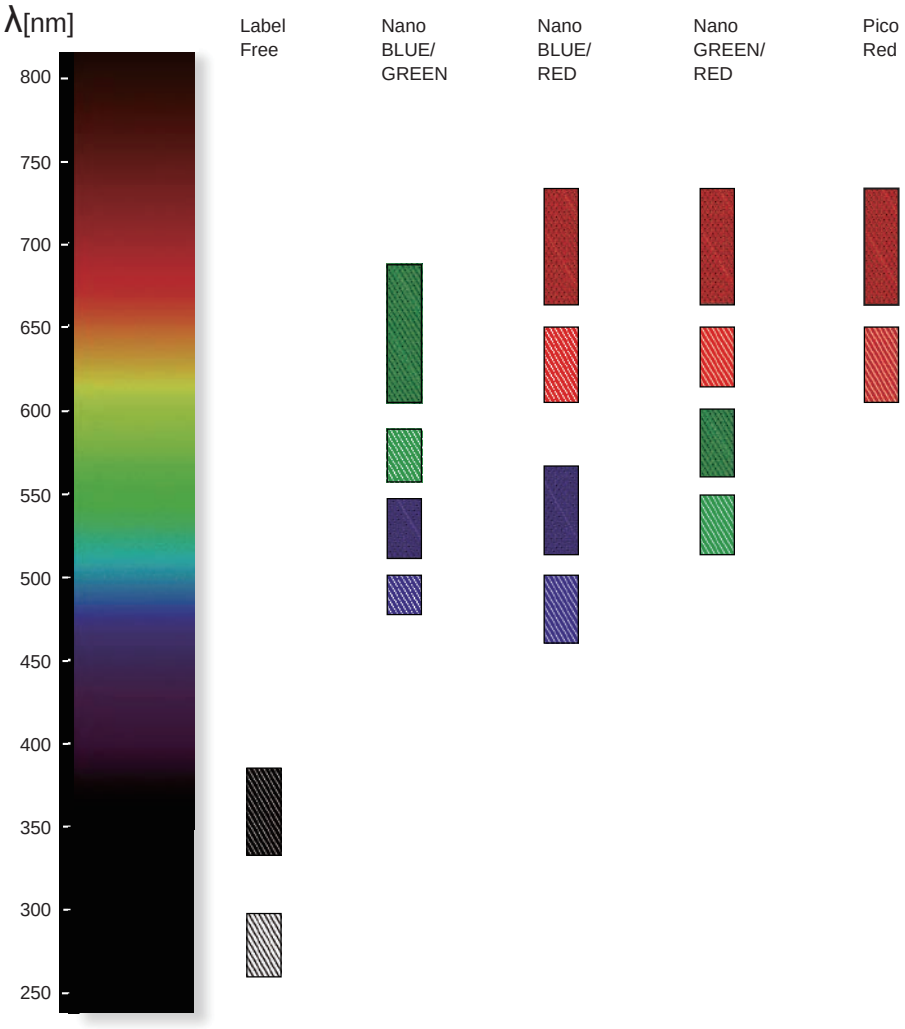
[1] 以 50 kDa，10 nM 荧光标记蛋白和 12 个数据点为例
[2] 以 50 kDa，10 pM 荧光标记蛋白和 12 个数据点为例
[3] 以 50 kDa，100 nM 有色氨酸自发荧光的蛋白和 12 个数据点为例

技术参数

Monolith NT. 系列仪器	NT.Automated
样品数量	96
每台仪器的荧光通道	最多集成 2 个检测器
多路荧光	是
亲和力范围	这些参数取决于所选择的检测器：NT.Automated 仪器最多集成 2 个不同的检测器，可以从所有的 Monolith 检测系统中进行任意搭配（包括 Nano, Pico 和 LabelFree 检测器）。
是否需要标记	
荧光分子浓度	
应用范围	
是否需要色氨酸荧光	
复杂生物溶液中测量（血清、细胞裂解液）	
样品消耗量	<3 µl
分子量范围 (Da)	10^1 - 10^7
实验和分析时间	分钟
是否需要固定样品	否
温度控制	25 ℃（主动控制）
仪器维护	可选服务或性能诊断

检测器和光谱范围

Monolith NT.Automated 检测器的相应激发 / 检测波长如下图所示：



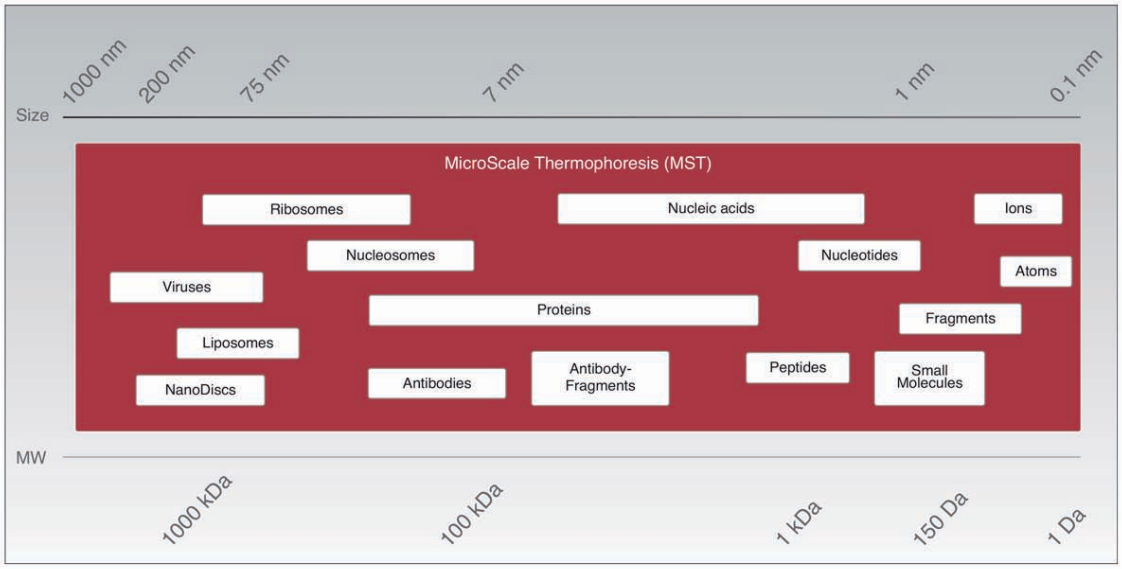
明暗阴影方块分别对应的是不同荧光检测器的激发 / 检测波长光谱。

应用范围

微量热泳动技术可以检测任何生物分子、化合物、纳米材料等分子间的相互作用，应用范围非常广泛，从离子、小分子、到高分子量的蛋白复合体。

热泳动现象，即分子在温度梯度场中的定向移动，不仅取决于目标分子的大小，也跟电荷和水化层相关。所以，即使相互作用的分子间并未出现明显地大小或分子量变化，MST 技术依然可以灵敏地检测到相互作用。

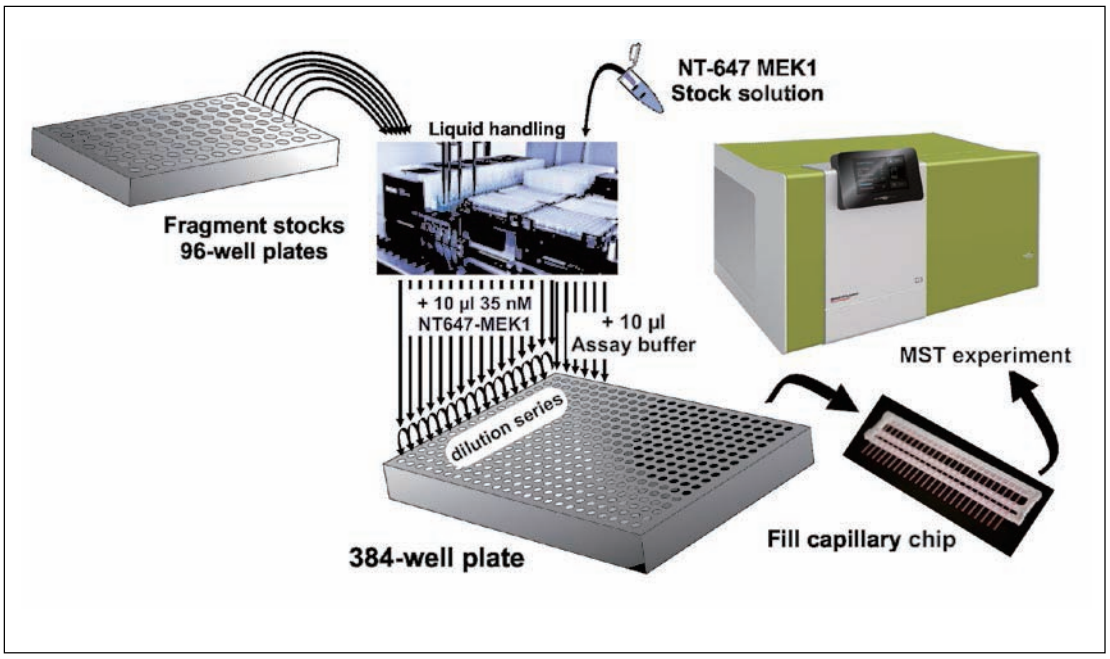
MST 可应用于任意缓冲液中操作，无需要表面固定。即使体积较大、不稳定的样品也同样适用，比如脂质体、纳米材料或者膜蛋白。



片段筛选：项目设计

片段库（MEK1 激酶）的全自动筛选项目案例研究

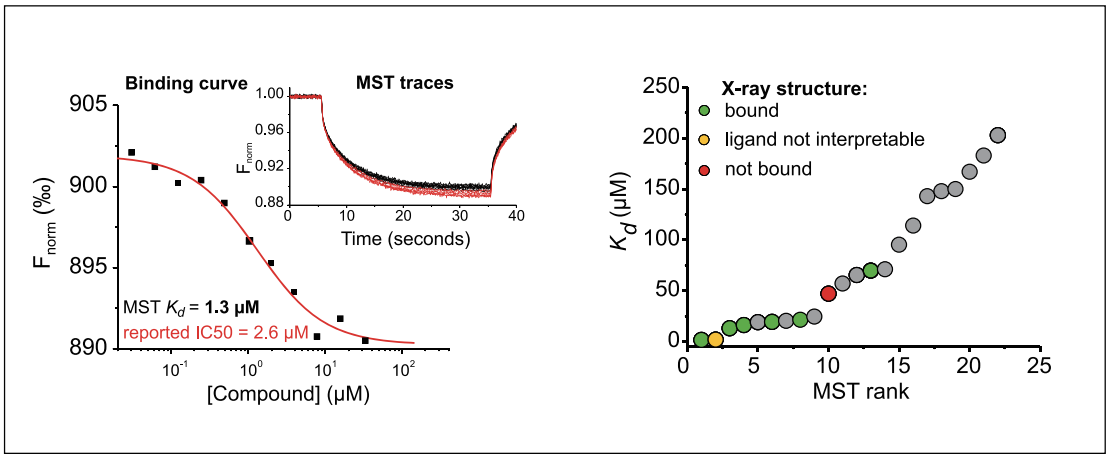
Monolith NT.Automated 可以实现快速的、低样品消耗的化合物或者片段库筛选。创新型的毛细管芯片模式能够简化实验操作，并且可以将 MST 实验集成到全自动化系统中。在此研究中，经由模拟计算获得的 193 个片段库，通过一系列正交实验，如 DSF（差示扫描荧光），SPR（表面等离子共振）和 MST，检测它们与药物靶标 MEK1 的相互作用。



	每个数据点	每个 K_d	总计 （193 个反应和 10 个阳性对照）
数据点个数	1	12	2436
测试时间	10 秒	120 秒	6 小时 26 分
蛋白用量	0.3 ng	3.1 ng	628 ng

片段筛选：实验设置与结果

为建立稳定的实验体系，缓冲液条件先经过一系列优化，以确保蛋白的活性以及稳定性，提高实验的重复性。首先，荧光标记了 NT-647 的 MEK1 靶标蛋白与其天然底物 ATP 进行结合反应分析，并作为后续实验的阳性对照。整个筛选项目中共计进行了 10 次 ATP 阳性对照，用于确定 MEK1 蛋白的功能性。这些对照实验的平均 K_d 值是 9.4 ± 0.8 µM, 证实了 MST 筛选方法的稳定性。此外，在对 193 个片段筛选前，先行测试了 MEK1 与已知结合的小分子的相互作用。



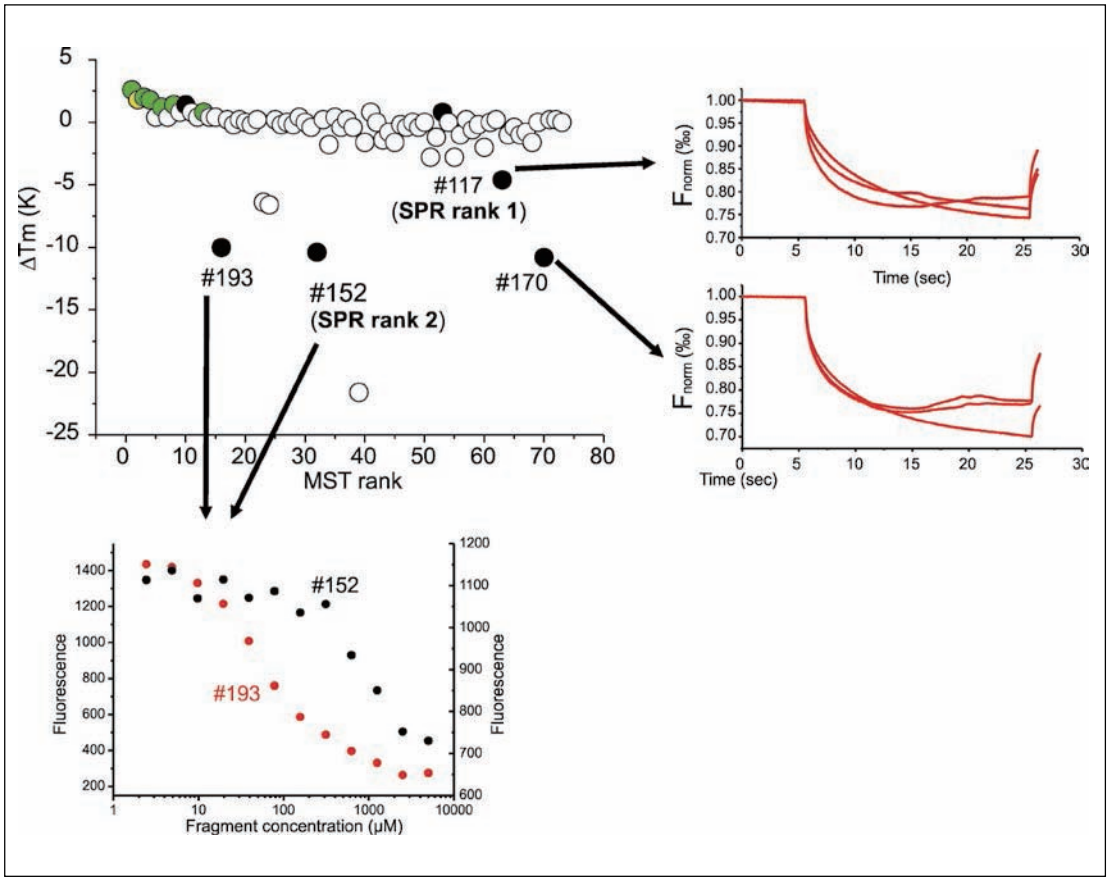
在 193 个片段的 MST 分析实验中，超过 70 个结合分子被证实其 K_d 值范围在 µM 到 mM 之间 , 16 个片段的 K_d 值在 100 µM 以下。尤为重要的是，8 个共结晶实验中有 7 个排在 MST 榜单的 Top15 中。

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 & medicinal chemistry letters 23:3620-3626
2. Breitsprecher D, et al., (2014) Case Study on Automated Screening Project of a Fragment Libirary against MEK1 kinase, Application Note NT021

片段筛选：其他优势

MST 排行结果与 DSF 的定性结果保持高度一致性。但 DSF 分析极依赖于配体结合后蛋白质变性温度的变化，因此只有少量片段可以被鉴定为 hits。而 MST 分析通过结合亲和力（K_ds）排行，从 193 个样品中确定出了 70 个 hits。

此外，MST 的原始信号图可直接鉴别出 SPR 筛选中的假阳性结果。高通量的 MST 分析是现代药物发现过程中一个完美的工具。



左上图显示了 DSF 筛选中变性温度变化（ΔT_m）与 MST 结果排序的相关性。负的 ΔT_m 代表片段会降低蛋白的稳定性。MST 可以基于多种现象来鉴定不稳定效应：蛋白聚集造成 “崎岖不平的踪迹”（请见片段 #117 和 #170），蛋白沉降导致荧光的损失（请见片段 #193 和 #152）。注意片段 #117 和 #152，在 SPR 筛选中被排序为 1 和 2。#193 最初在 MST 排序里被排在 #21，但是很容易被鉴定为假阳性结果。

用户评价

Dr. Markus Zeeb, Principal Scientist, Structural Research, Boehringer Ingelheim Pharma GmbH & Co. KG – Research Germany
德国勃林格殷格翰制药

"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 measusre 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
英国 UCB 优时比制药

"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."

Dr.Alexey Rak, Structural Biology & Biophysics, Sanofi R&D, France
法国赛诺菲集团

"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.

Dr. Timothy Sharpe, Head of the Biophysics Facility, Biozentrum, University of Basel, Switzerland
瑞士巴塞尔大学

“We have used our NanoTemper NT.115 MicroScale Thermophoresis (MST) instrument extensively in the last one and a half years to study many different types of interaction. 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 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.”

文献摘录

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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