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Monolith® NT.115 Series

分子相互作用分析

Monolith® Instruments
for MicroScale Thermophoresis™

www.nanotemper-technologies.com

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

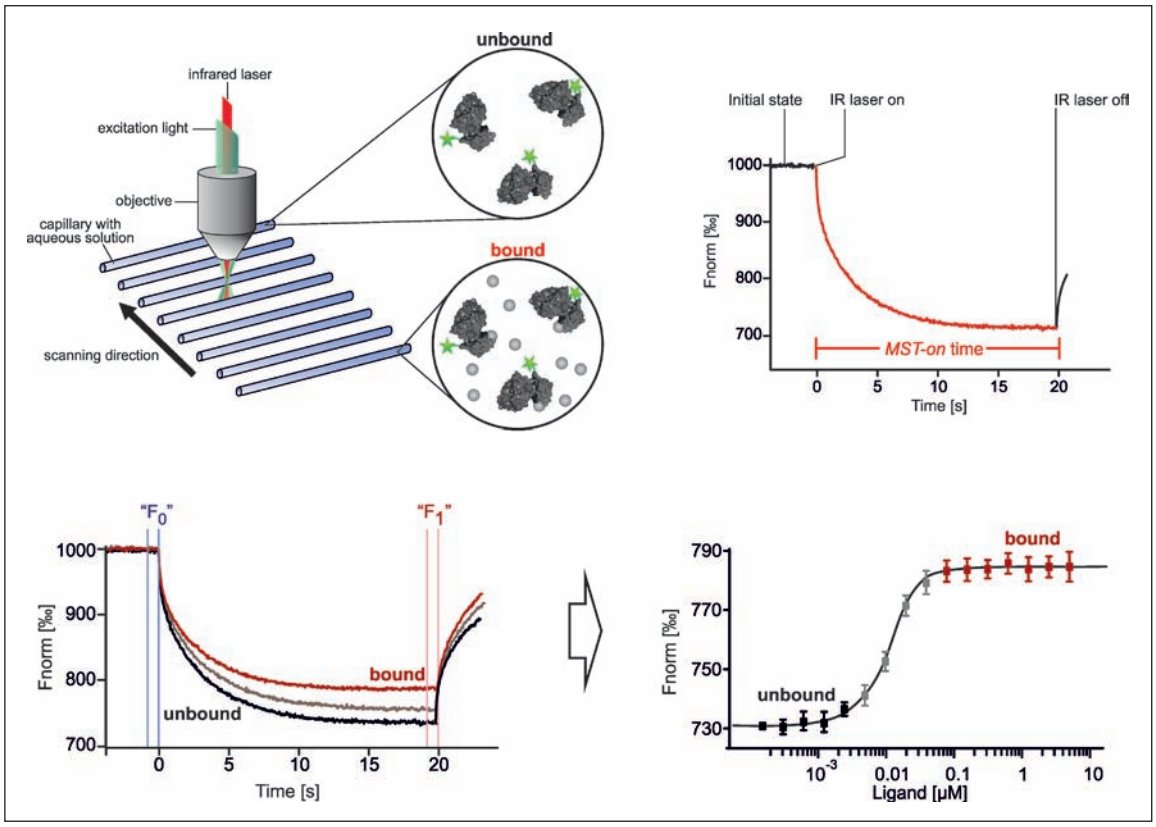
应用范围广泛

- 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。

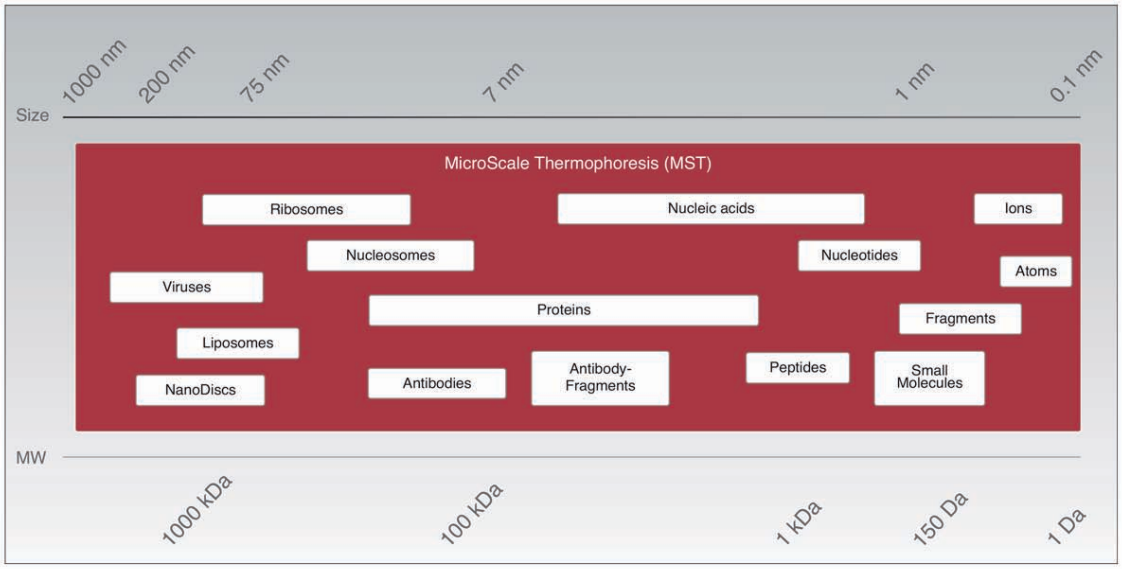


应用范围

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。



Monolith NT.115 and NT.115 Pico

NT.115 系列通过检测荧光染料或者荧光融合蛋白（比如 GFP 蛋白）来测量生物分子间的相互作用，优点如下：

- 无需固定，亲和力测量范围从 1 pM 到 mM 级别
- 应用范围广泛
- 任意缓冲液：包括血清或者细胞裂解液
- 无需纯化：荧光融合蛋白

目录号	型号	通道 1	通道 2	亲和力范围 (K_d)	样品消耗量 (per K_d)
G006	NT.115Pico	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 – BLUE	Nano – RED	1 nM to mM	120 ng [2]

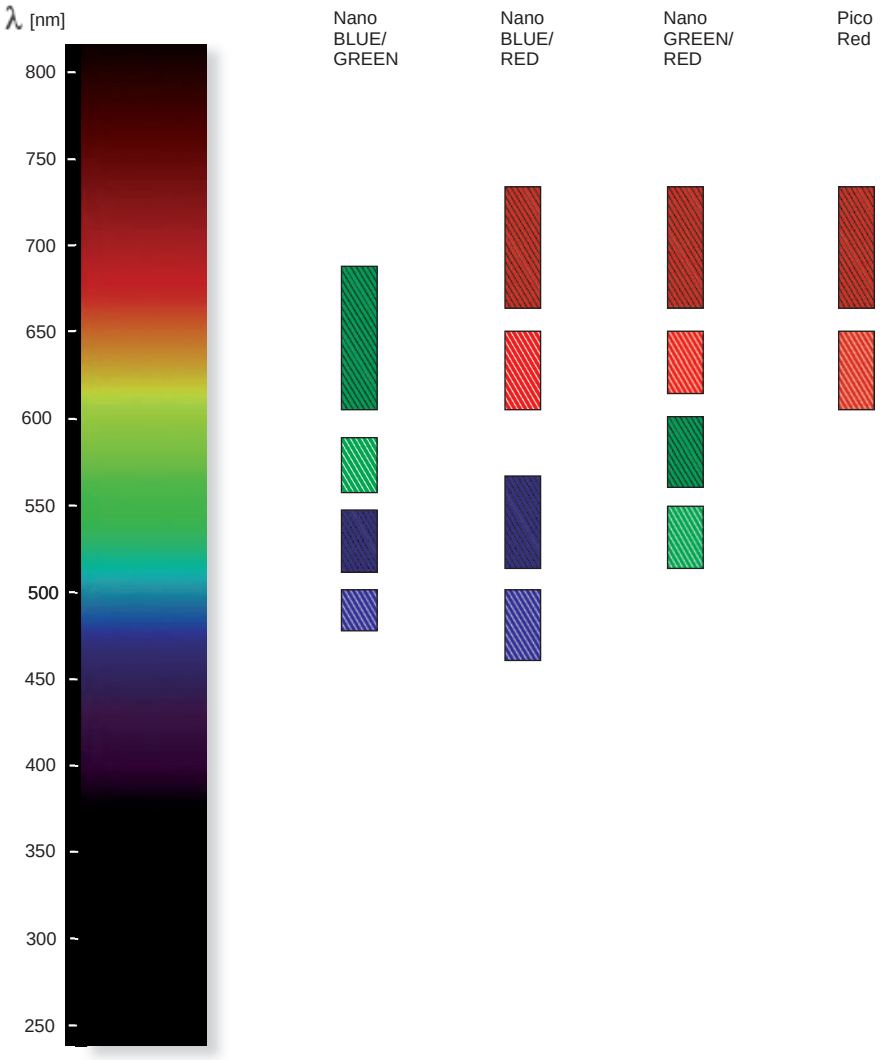
[1] 以50 kDa，10 pM荧光标记蛋白和12个数据点为例
[2] 以50 kDa，10 nM荧光标记蛋白和12个数据点为例

技术参数

Monolith Instruments NT.115 系列	NT.115	NT.115^{Pico}
样品数量	16	16
荧光通道	2 （蓝、红或绿）	1 （红）
亲和力范围	1 nM to mM	1 pM to mM
是否需要标记	是	是
荧光分子浓度	10^{-9} - 10^{-3} M	10^{-11} - 10^{-3} M
应用范围	■ ■ ■ ■ ■	■ ■ ■ ■ ■
生物物理学参数	亲和力、化学计量学、热力学、酶动力学	亲和力、化学计量学、热力学、酶动力学
是否需要色氨酸荧光	否	否
复杂生物溶液中测量 （血清、细胞裂解液）	是	是
样品消耗量	< 4 µl	< 4 µl
分子量范围 (Da)	10^{1} - 10^{7}	10^{1} - 10^{7}
测试和分析时间	分钟	分钟
是否需要固定样品	否	否
温度控制	22 - 45 °C	22 - 45 °C
仪器维护	否	否

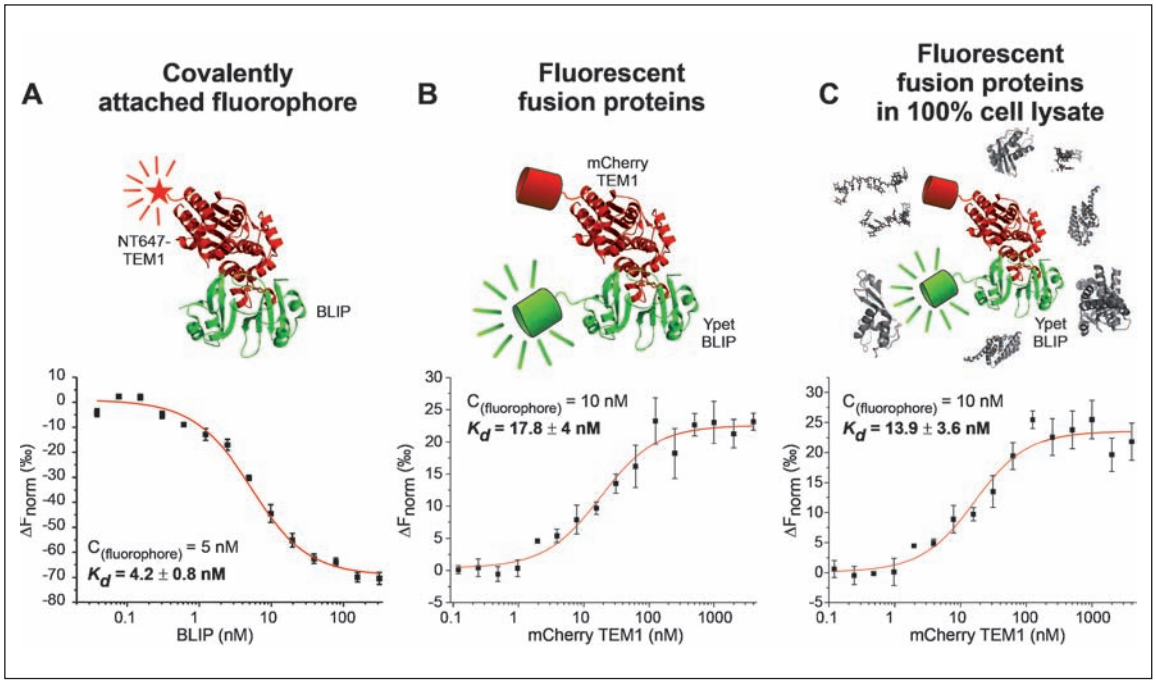
检测器和光谱范围

Monolith NT.115 系列检测器的相应激发 / 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。

蛋白—蛋白相互作用



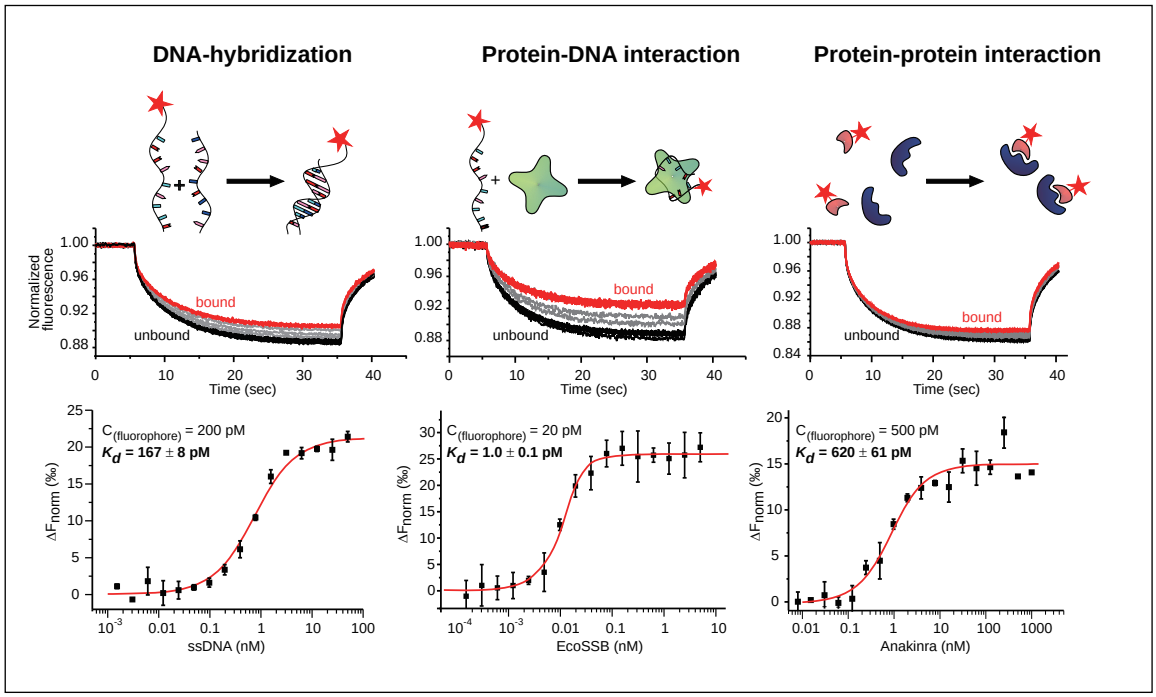
蛋白 - 蛋白相互作用的热泳动分析

相互作用分别在三种条件下测量：在纯化系统中，使用共价标记的荧光素 (A)；在缓冲液中使用荧光融合蛋白 (B)；在细胞裂解液中使用荧光融合蛋白 (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

pM 亲和力相互作用



不同实验体系下，使用 Monolith NT.115^{Pico} 测量 pM 级别的亲和力实验

DNA - DNA 相互作用（左）：用 MST 测量单链 DNA 与 Cy5 标记互补链的相互作用，该单链 DNA 包含 16 个碱基。对 MST 信号变化做曲线拟合，获得的 K_d 值为 167 ± 8 pM。

蛋白 - DNA 相互作用（中）：用 MST 分析单链结合蛋白 (EcoSSB) 与 Cy5 标记的低聚核苷酸 $(dT)_{70}$ 的相互作用，其中 EcoSSB 做梯度稀释，Cy5 标记的 DNA 浓度保持 20 pM 不变。对 MST 信号变化做曲线拟合，获得的 K_d 值为 1 ± 0.1 pM。

蛋白 - 蛋白相互作用（右）：实验中，NT647 标记的 Interleukin-1 受体的可溶性片段保持浓度不变，Anakinra 做梯度稀释。对 MST 信号变化做曲线拟合，获得的 K_d 值为 620 ± 61 pM。

Material was kindly provided by Dr. Ute Curth, Medial University 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

用户评价

Dr. Stephan Uebel, Max-Planck-Institute of Biochemistry, Martinsried, Germany
德国马普生化所

“For the Biochemistry Core Facility at the Max-Planck-Institute for Biochemistry it is of great importance to provide our users with instruments which are easy to use while offering a high information content.

Therefore, the Nanotemper Monolith NT.115 with its low sample consumption and maintenance-free design is a perfect addition to our instrument park. The straight forward handling of the instrument allows even first time user to access binding affinities usually within a day. Method development is often quick, certainly compared to other techniques, but the method puts a high demand on sample homogeneity and in particular on monodispersity.

As interactions are characterized free in solution, MST has been successful where SPR failed due to the limitations of a surface based approach.”

Prof.Dr. Uffe Holmskov, Institute for Molecular Medicine, University of Southern Denmark, Odense, Denmark
南丹麦大学

“we are interested in elucidating interactions in close-to-native conditions: thus, we appreciate that the interactions are investigated 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
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.

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