

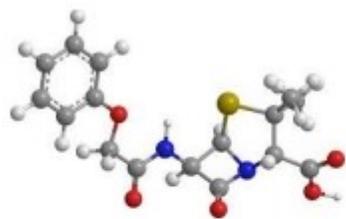
微量热泳动技术原理

(Microscale Thermophoresis, MST)

MST术语

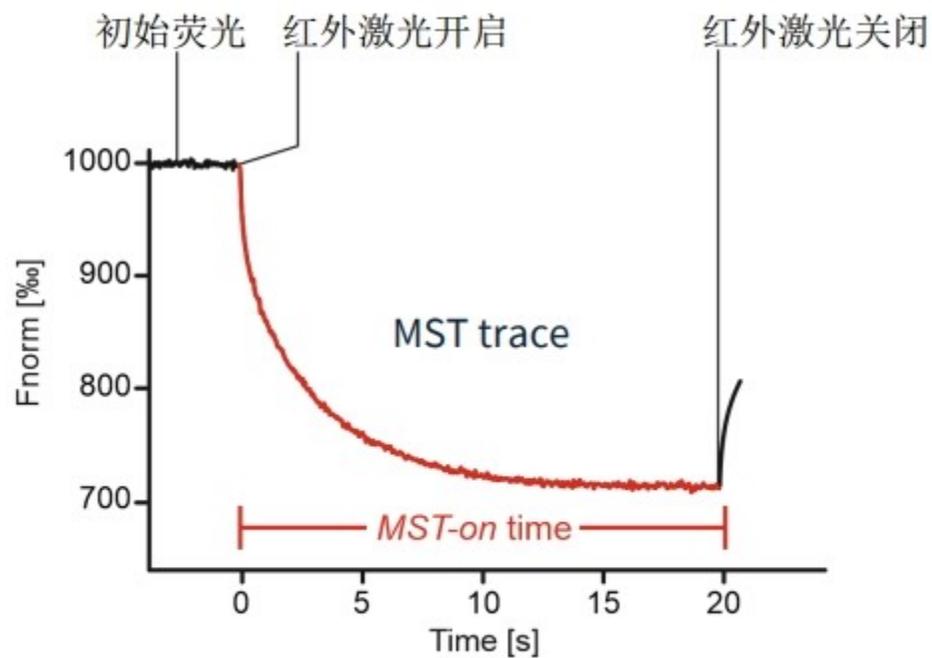
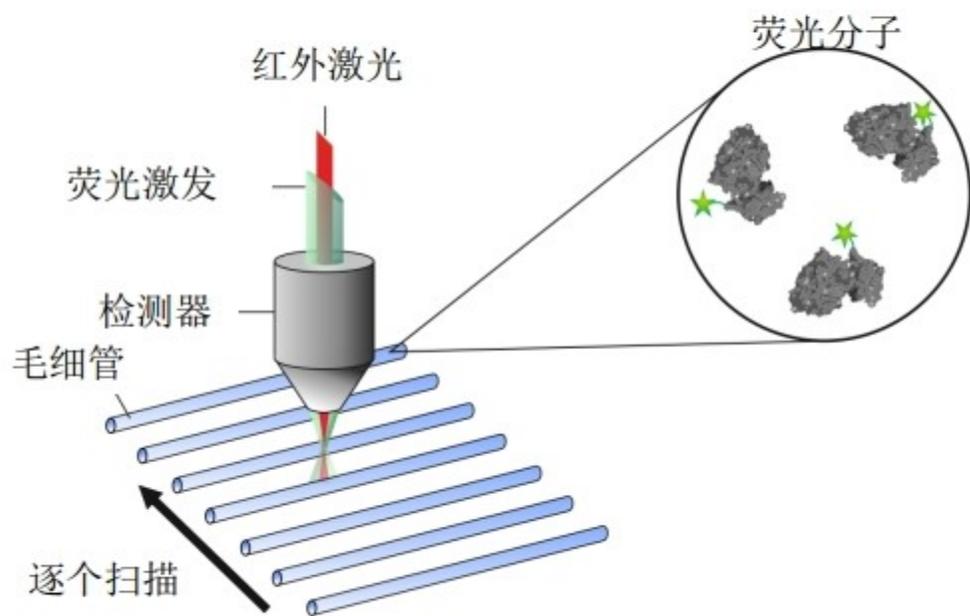


TARGET → 荧光分子



LIGAND → 不激发荧光分子,
配体

工作原理



MST 信号变化来源于热泳动和温度依赖的荧光强度变化

热泳动 (Thermophoresis)

- > 分子在温度梯度内的定向运动会改变荧光分子的**浓度**;
- > 荧光浓度变化的程度取决于分子的整体性质, 例如大小, 水化层和电荷

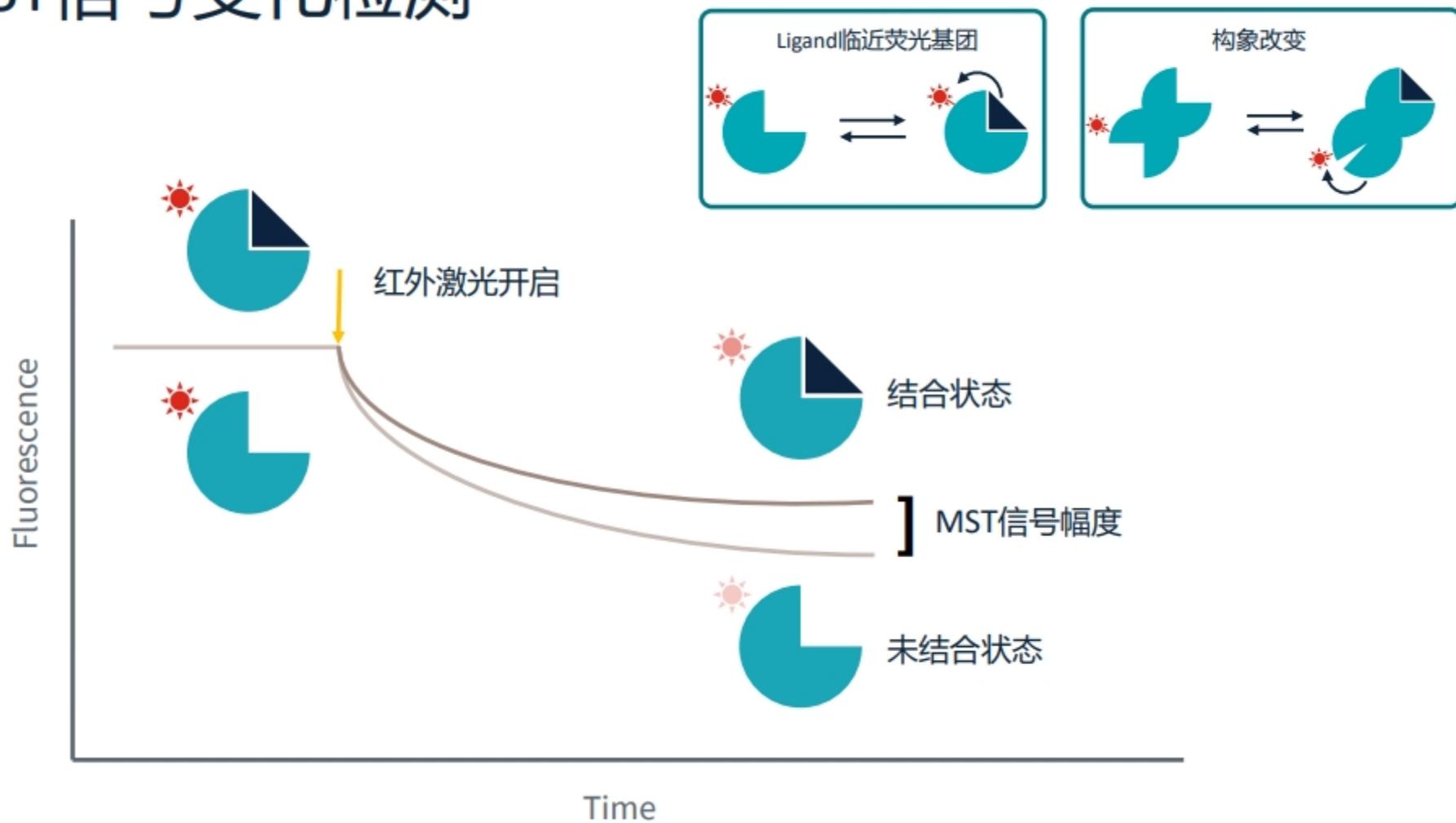


温度依赖的荧光强度变化 (TRIC)

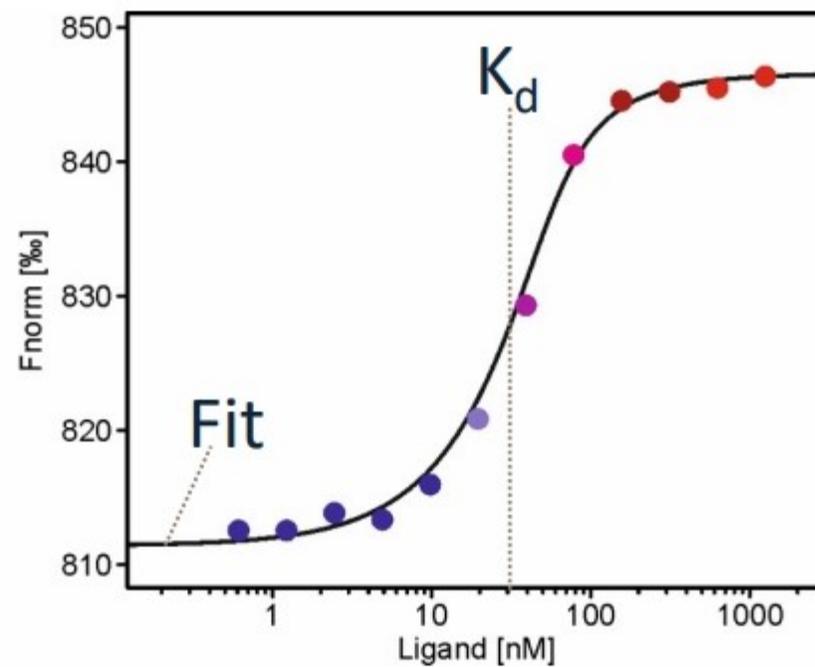
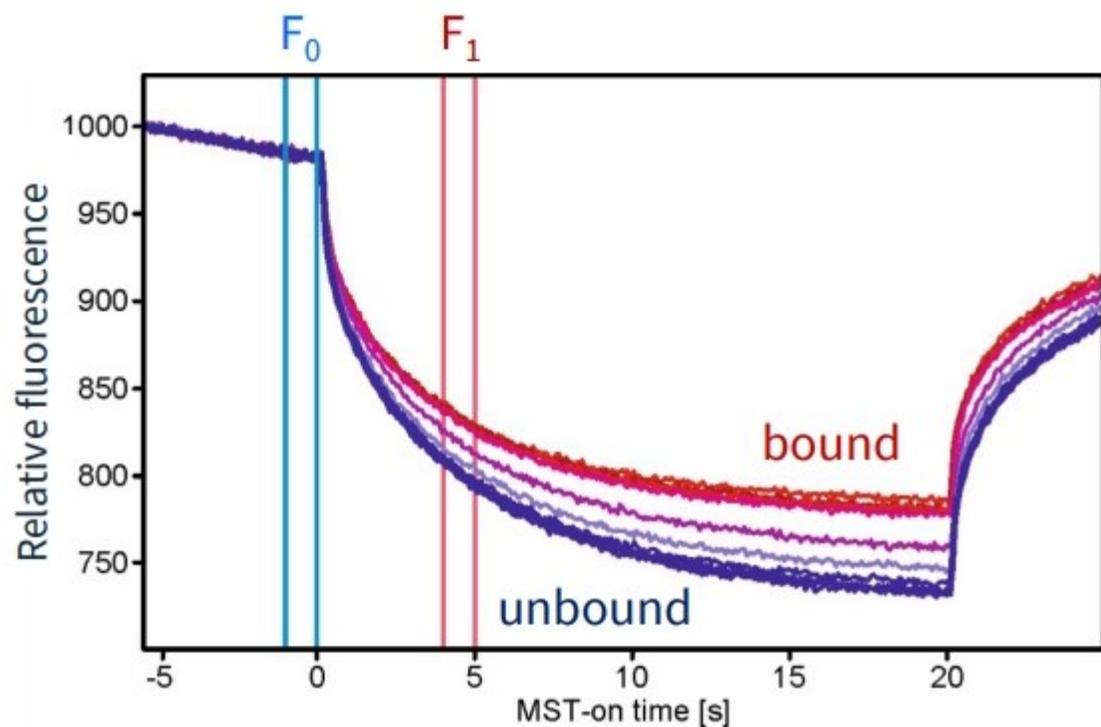
- > 荧光**强度**与温度有关
- > 荧光强度与荧光团的化学环境密切相关



基于MST信号变化检测

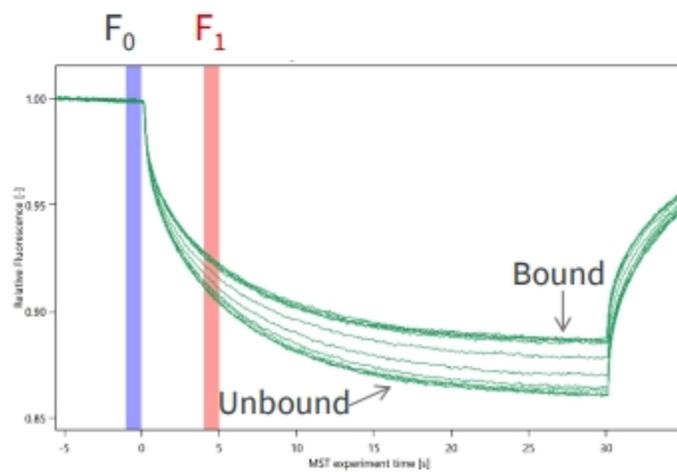


由MST结合曲线得出平衡解离常数 K_d

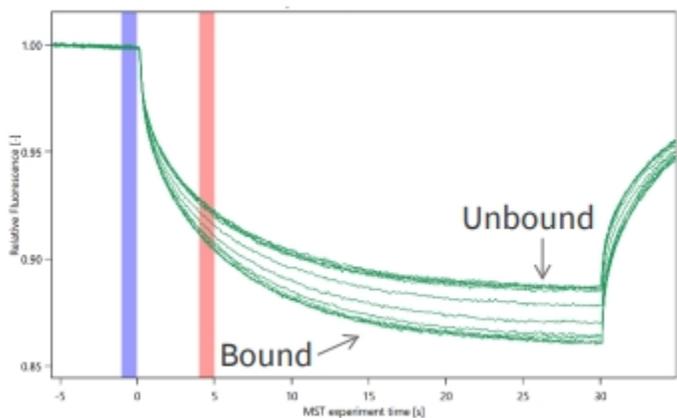


K_d 越小, 亲和力越强

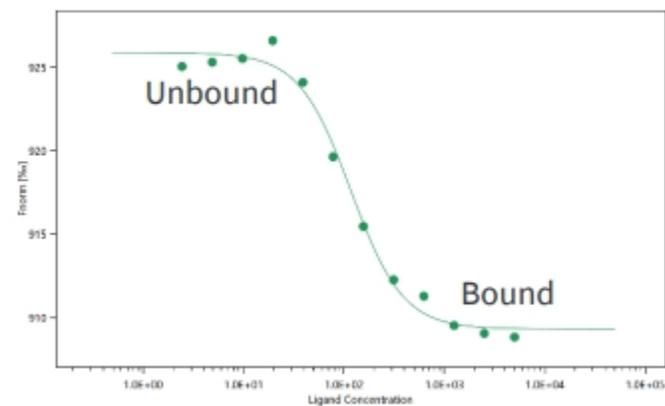
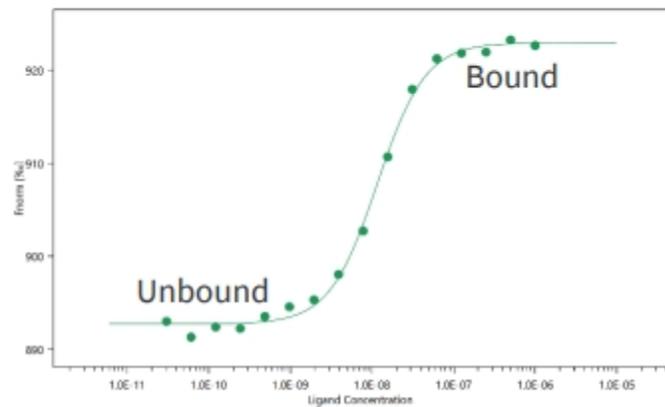
曲线方向和亲和力大小无关



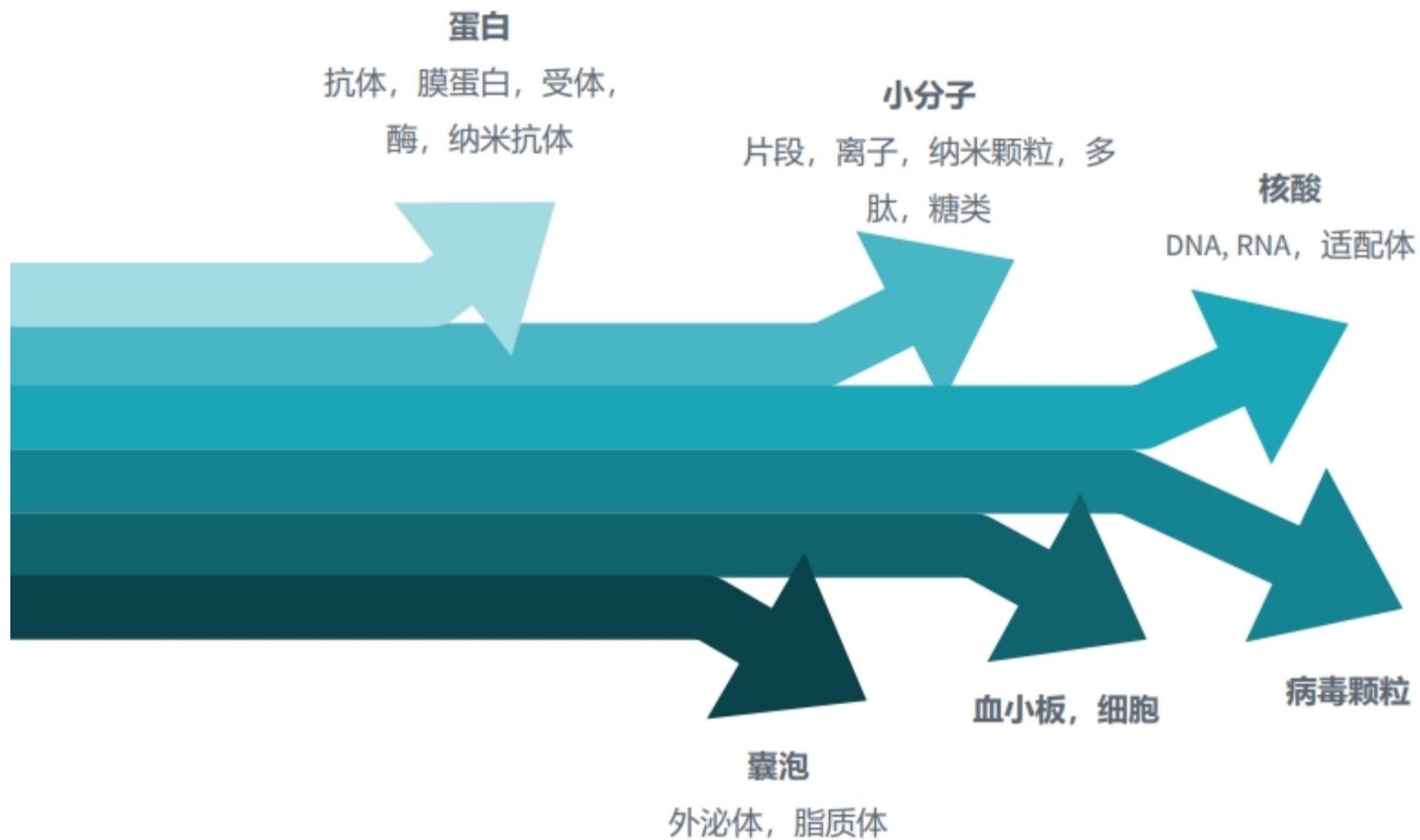
$$F_{\text{norm}} = F_1/F_0$$



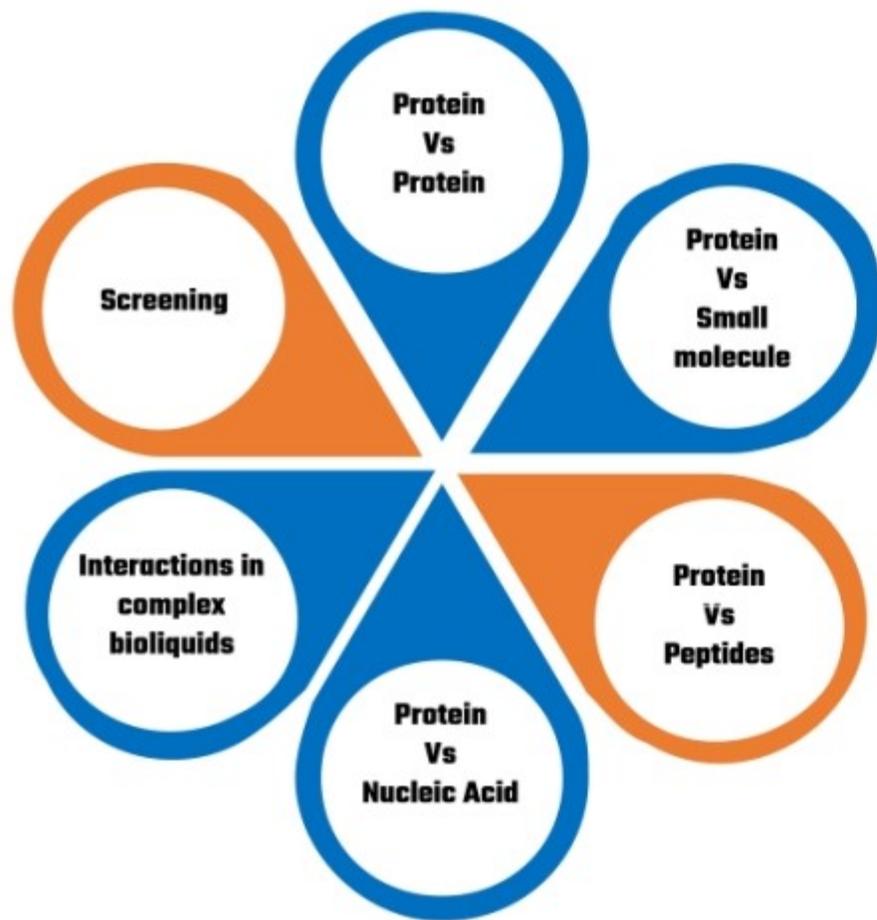
$$F_{\text{norm}} = F_1/F_0$$



检测样品类型丰富



MST技术应用领域



样品类型	研究方向
<ul style="list-style-type: none"> > 蛋白-蛋白 > 蛋白-小分子 > 蛋白-离子 > 抗原-抗体 > 蛋白-多肽 > 蛋白-脂类 > 蛋白-核酸 > 病毒颗粒 > 纳米颗粒 > 核酸适配体 > 细胞裂解液/血清 > 	<ul style="list-style-type: none"> > 基于靶标的药物筛选 > 蛋白结构验证 > 植物信号通路 > 神经退行性疾病 > 肿瘤抑制剂开发 > 中药活性成分 > 蛋白泛素化研究 > 竞争性结合实验 > 酶与底物的结合 > 农药残留检测 > 癌症标志物 >

Monolith技术的优势



MST实验设置与优化

MST 实验流程

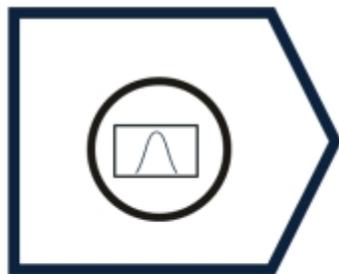
1. 蛋白质控



2. Target制备



3. Pretest



4. 亲和力检测



5. 实验优化

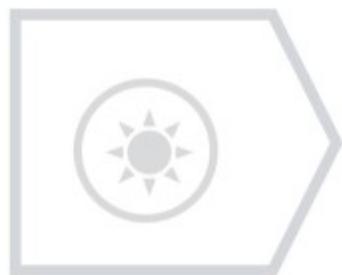


MST 实验流程

1. 蛋白质控



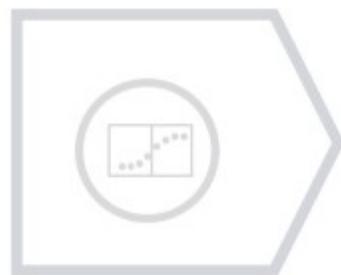
2. Target制备



3. Pretest



4. 亲和力检测



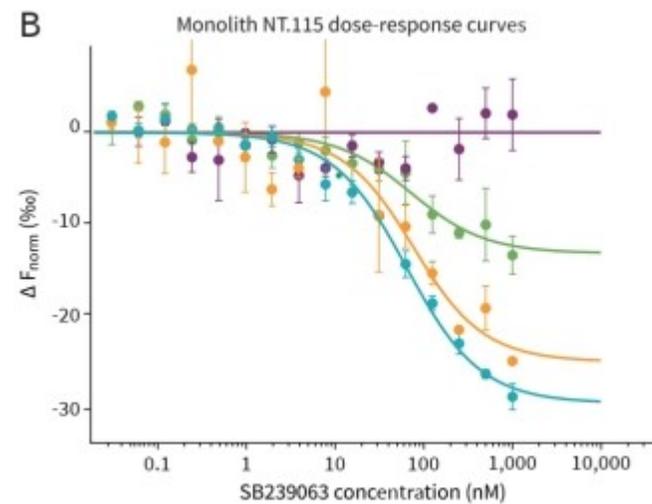
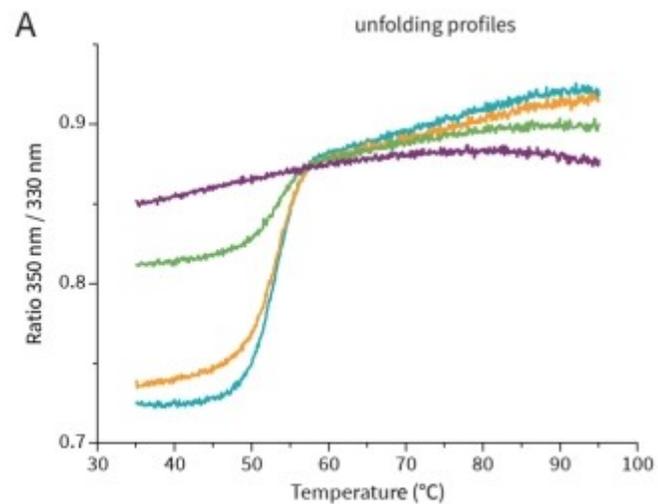
5. 实验优化



样品生物活性是下游实验成功的基础



— fresh — freeze-thaw cycles — 50% denatured — fully denatured



MST 实验流程

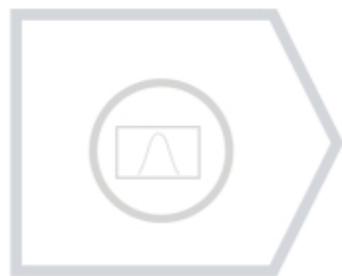
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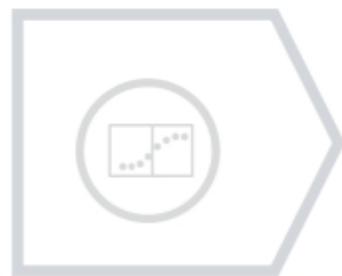
2. Target制备



3. Pretest



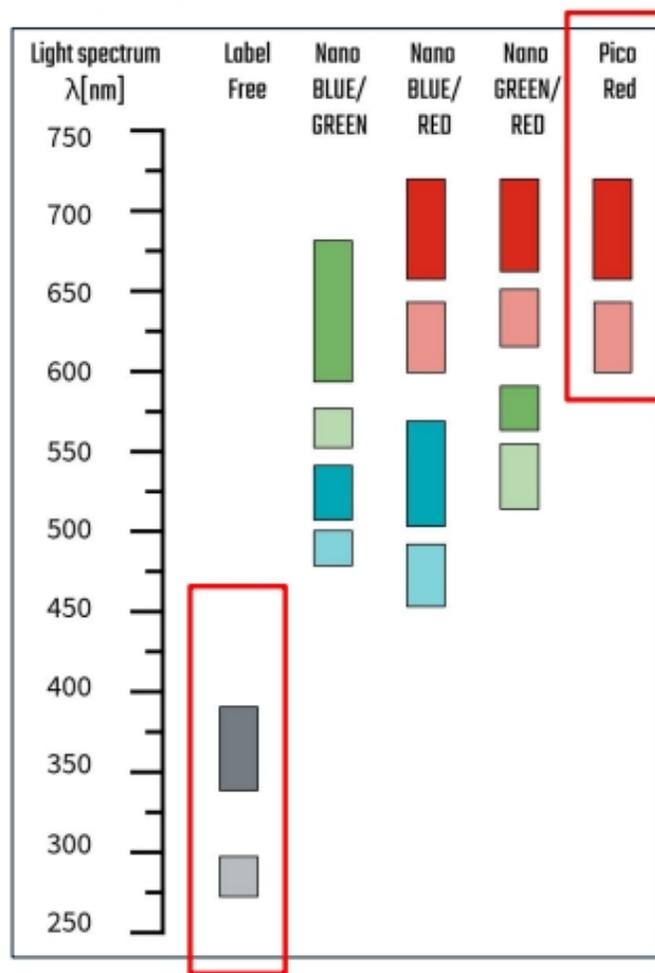
4. 亲和力检测



5. 实验优化

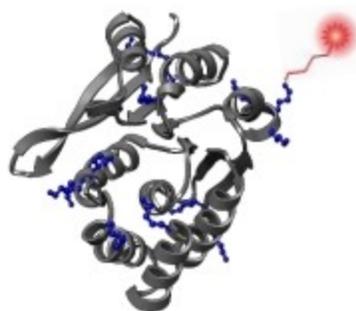


选择合适的通道 推荐使用红色通道

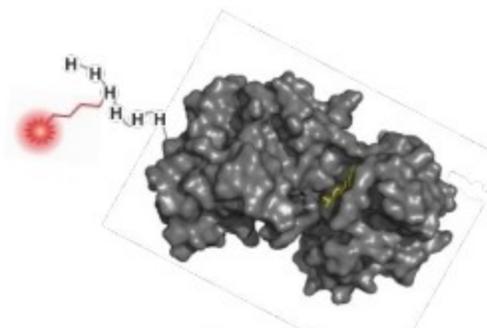


Fluorophore	Excitation [nm]		Emission [nm]
BCECF	480		525
GFP	488		507
NT-495 (BLUE)	493		521
Fluorescein (FITC)	495		519
Alexa488	495		519
YFP	514		527
Alexa532	530		555
TAMRA	546		579
Cy3	550		570
RFP	555		584
NT-547 (GREEN)	557		574
Alexa546	560		572
Cy5	649		670
NT-647 (RED)	650		670
Alexa647	652		668

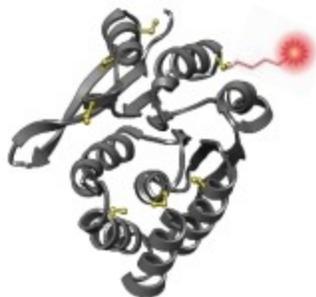
选择标记方式



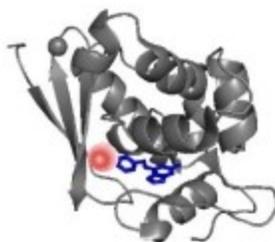
NHS kit: 通过赖氨酸侧链氨基偶联



Tris-NTA kit: His-tag
特异性标记

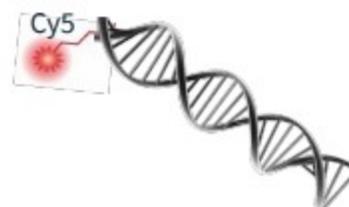


MALEIMIDE kit: 通过半胱氨酸偶联



Snap-tag kit: SNAP tag
特异性标记

NanoTemper
蛋白标记试剂盒
专为MST实验优化



直接合成荧光样品
(核酸、多肽...)



MST 实验流程

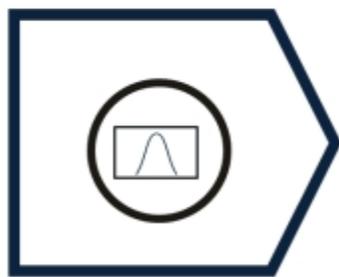
1. 蛋白质控



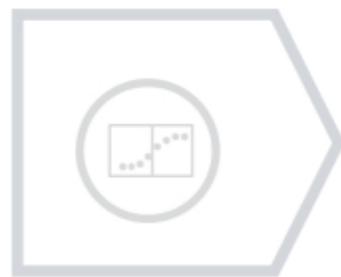
2. Target制备



3. Pretest



4. 亲和力检测



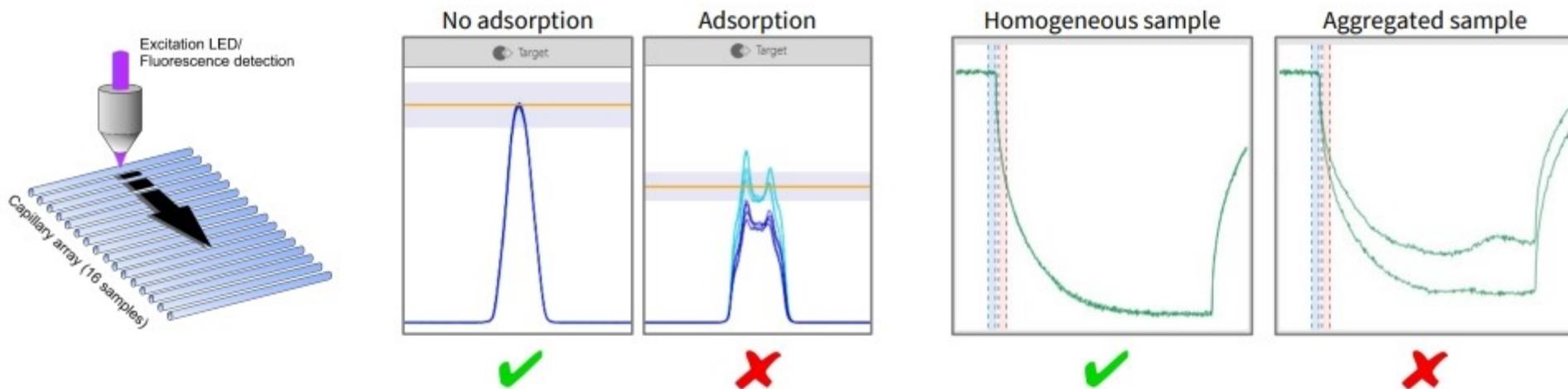
5. 实验优化



Pretest

- 确定产生足够荧光强度的Target浓度（应低于预估的Kd值）
- 检查Target是否与毛细管吸附
- 检测Target是否聚集

检测器	荧光强度
Pico Red	3000~20000



MST 实验流程

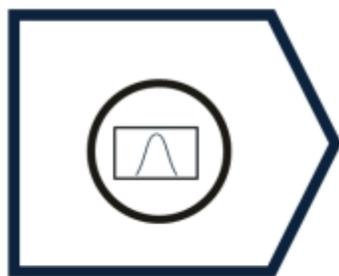
1. 蛋白质控



2. Target制备



3. Pretest



4. 亲和力检测

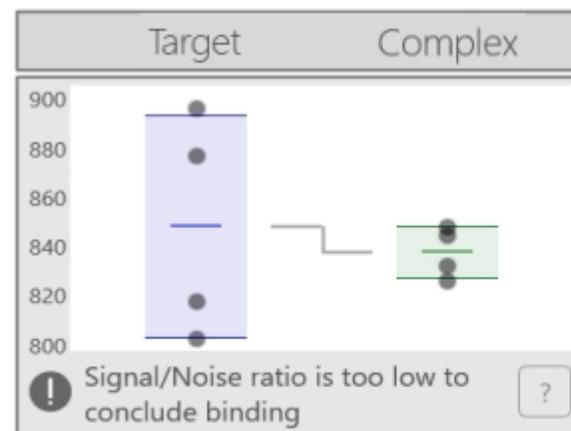
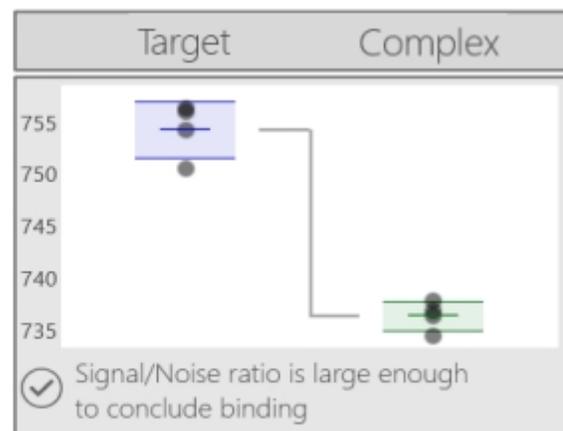
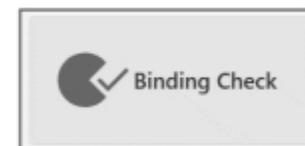


5. 实验优化

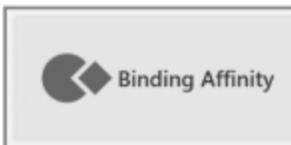


Binding Check

- 判断是否有结合



Binding Affinity



1. 正确填写Plan Your Experiment, 软件自动生成指引

Plan Your Experiment

Target: AptamerCy5 RED

Assay buffer: MST Buffer including 0.005% Tween-20

Use His-Tag Labeling:

Concentration of stock solution: 40 nM

Concentration in this assay: 20 nM

Ligand: AMP

Estimated Kd: optional μM

Concentration of stock solution: 50 mM

Ligand in organic solvent like DMSO:

Ligand buffer in this assay: 50.0%

Highest concentration in this assay: 25 mM

Capillary: Monolith Capillary

System settings: Excitation Power, MST-Power, Auto-detect 20%, Medium

Instructions

Below are detailed instructions on how to prepare the samples necessary for your experiment. For general information on binding affinity experiments, refer to the guidance on the right.

The minimum required stock solution and buffer volumes for this experiment are:

- 8.0 μl target stock solution (1 μM of Aptamer Cy5 RED)
- 30 μl ligand stock solution (50 mM of AMP)
- 202 μl MST Buffer including 0.05% Tween (assay buffer)
- 200 μl buffer of the ligand stock solution (ligand buffer)

1. Start by preparing the following intermediate samples:

- Dilution of target Aptamer Cy5 RED (1 μM) to 40 nM:
To obtain a 40 nM solution of Aptamer Cy5 RED, add 8.0 μl of stock solution to 192 μl of assay buffer.
- No predilution required. Use 30 μl of 50 mM AMP stock solution for the following steps.
- No predilution required. Use 200 μl of ligand buffer for the following steps.

2. Prepare a serial dilution of the diluted ligand using the diluted ligand buffer.

Remember to discard 10 μl of the lowest concentration sample in Tube 16 to get an equal volume of 10 μl in all samples.

Less info

i. Transfer 20 μl of 50 mM AMP into tube 1.

ii. Transfer 10 μl of diluted ligand buffer into tubes 2 to 16.

iii. Now transfer 10 μl of 50 mM AMP from Tube 1 to Tube 2 and mix carefully by pipetting up and down.

iv. Transfer 10 μl from Tube 2 Tube 3.

v. Continue the serial dilution up to the final step where you transfer 10 μl from Tube 15 to Tube 16.

vi. Discard 10 μl from tube 16 to get an equal volume of 10 μl

10 μl 10 μl 10 μl 10 μl 10 μl

1 2 3 4 ... 16 Waste

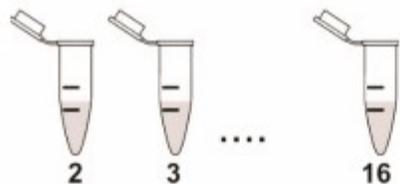
Back Print Instructions Start Measurement

Binding Affinity

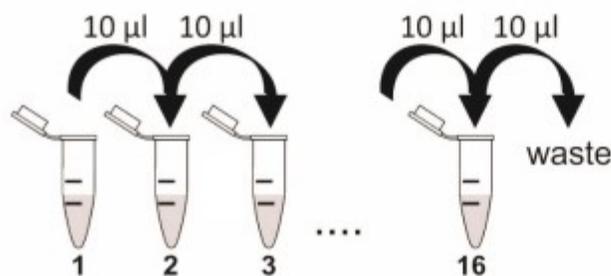
2. Ligand 稀释



准备20 μ l Ligand
(浓度为Kd的20~50倍)



向其他15个PCR管中各加入10 μ l Buffer



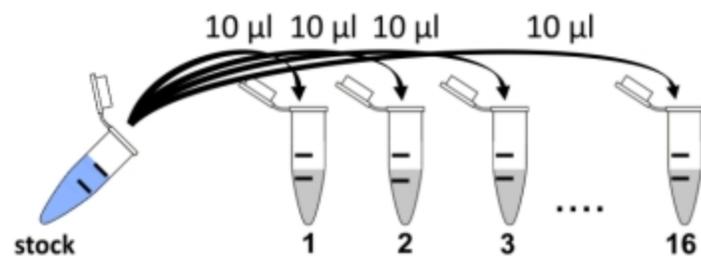
依次完成梯度稀释, 最后一管弃去10 μ l

Binding Affinity

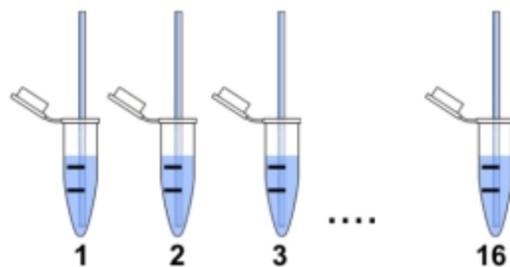
3. 加入Target



准备200 μ l Target
(浓度 $<K_d$, 通常20~100nM)



向所有PCR管中各加入10 μ l Target并混匀



使用毛细管吸取样品

样品处理的注意事项

- **荧光分子(target)的浓度**: 低于预估的 K_d 值, 一般20-100nM
- **配体(ligand)的最高浓度**: **预估 K_d 的20~50倍**
- **使用小管进行倍比稀释**: PCR strips, tubes
- **在倍比稀释过程中, 稀释缓冲液的成分不应发生变化**: e.g. DMSO
- **准确的移液至关重要**
- **用移液枪混合样品, 不要涡旋 (vortex)**
- **不要用手触摸毛细管的中间部分**

10 min 得到结果

Various interaction_20200916 - MO.Control v2.0.1*

NanoPedia Support

31 Binding Affinity

Plan Instructions Results Details

Change Monolith 2020 (TNG) Completed 09/16/2020

Hsp90 20nM ADP 4.5mM MST Tween 0.05% Premium 57% Nano - RED Medium

You can enter here some comments about this experiment

Session Overview

Close Save

10 Binding Check Aptamer binding check

14 Binding Check Aptamer binding check

16 Binding Check Aptamer binding check

17 Binding Check Aptamer test

19 Binding Check Aptamer test

20 Pretest Hsp90 std

22 Pretest Hsp90 premium

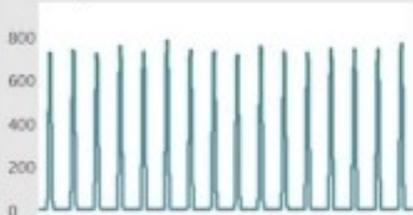
25 Binding Check Hsp90 ADP bad

26 Binding Check Hsp90-ADP bad

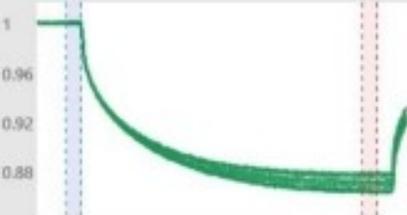
27 Binding Check

+ New Experiment

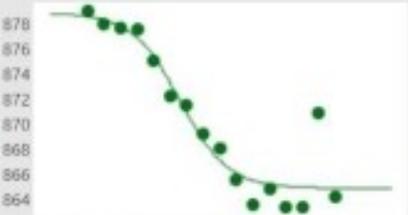
Capillary Scans



MST Traces



Dose Response



Fluorescence intensity

No fluorescence variation

No adsorption

No aggregates

No ligand induced photobleaching rate changes

Signal/Noise ratio is large enough to conclude binding

Review Review Review

Conclusion

Recommendations for improving the assay

Please review the quality checks on the details pages and change them if necessary in order to update the conclusion.

Based on all quality checks, the following should be addressed:

Binding detected, $K_d = 6.43 \mu\text{M}$

Recommendations for addressing this can be found to the right. Please consider them one at a time.

If too few dose response points are in bound or unbound state, consider adjusting the ligand concentration range

Positive Negative Overridden

[How to work with quality checks...](#)

Back Create Report + Binding Affinity

MST 实验流程

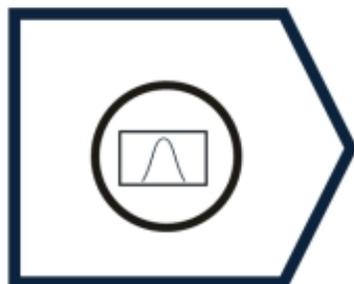
1. 蛋白质控



2. Target制备



3. Pretest



4. 亲和力检测

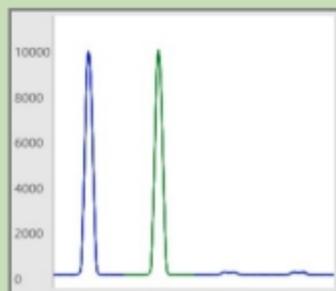


5. 实验优化

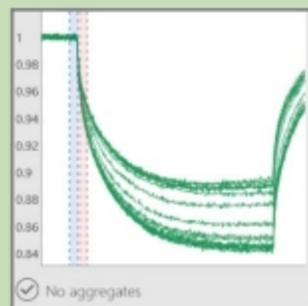


常见的样品问题类型

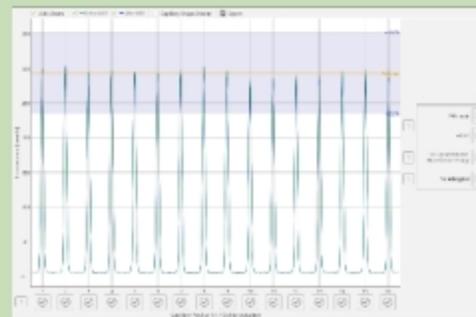
无吸附



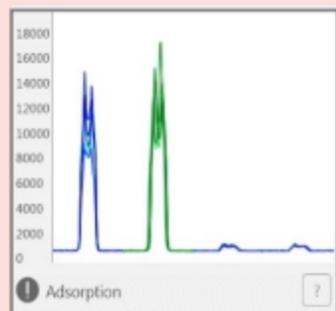
无聚集



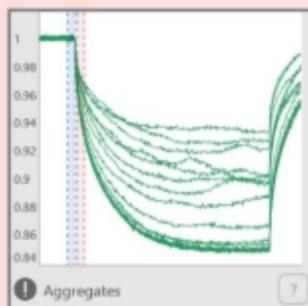
均一性好



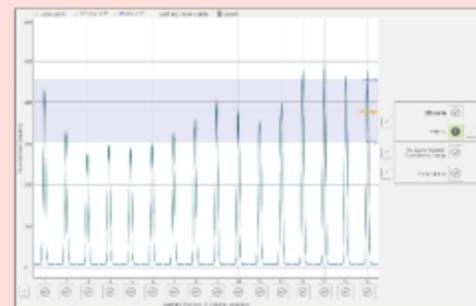
样品吸附



聚集



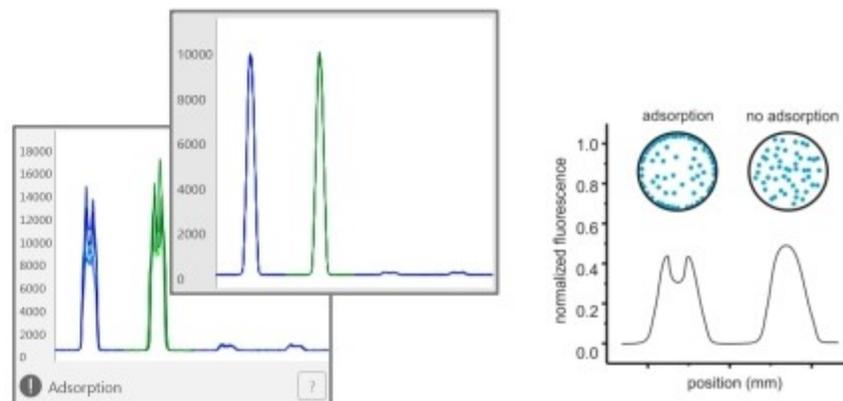
样品不均一



吸附和聚集

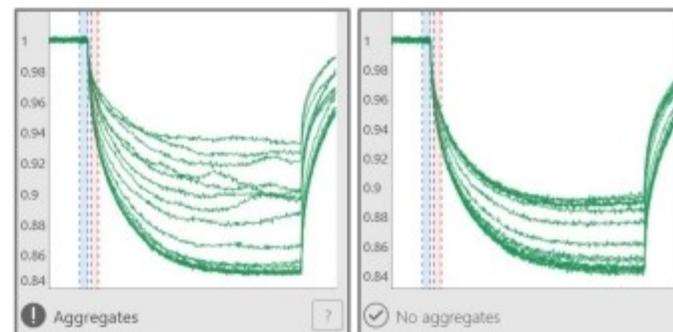
吸附

- 更换毛细管 (Standard → Premium)
- 添加去垢剂 (0.05% Tween 20, 0.1% Pluronic F-127)
- 调整缓冲液成分 (pH, salt concentration...)



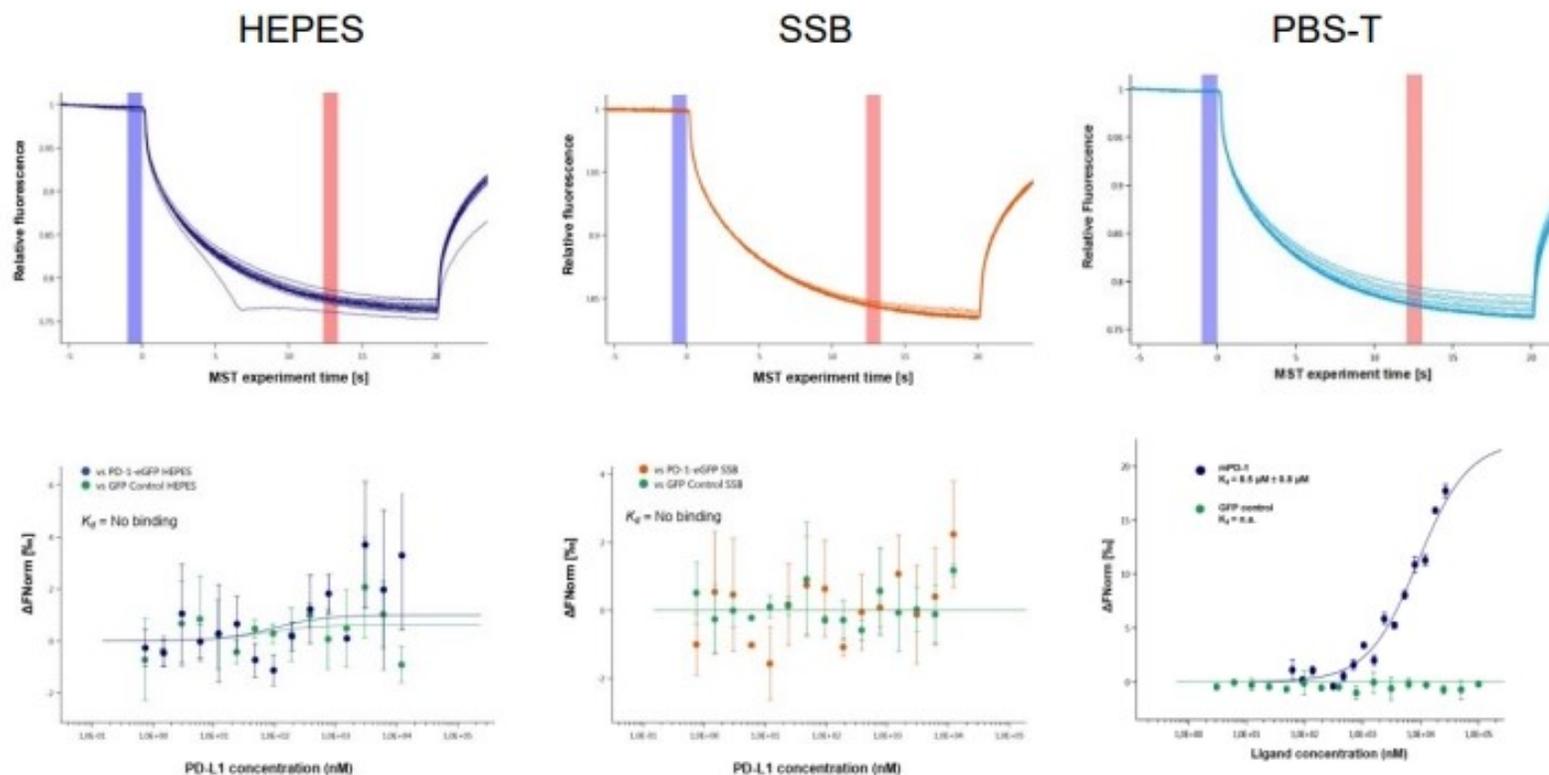
聚集

- 离心 (10 min >15.000 xg)
- 添加去垢剂 (0.05% Tween 20, 0.1% Pluronic F-127)
- 检查有机溶剂浓度 (DMSO...)
- 调整缓冲液成分 (pH, salt concentration...)



缓冲液优化

缓冲液对互作影响(盐浓度, pH值, 去垢剂和添加剂..)



Magnez, R., Thiroux, B., Taront, S., Segaula, Z., Quesnel, B., & Thuru, X. (2017). PD-1/PD-L1 binding studies using microscale thermophoresis. *Scientific Reports*, 7(1), 1–8.

初始荧光

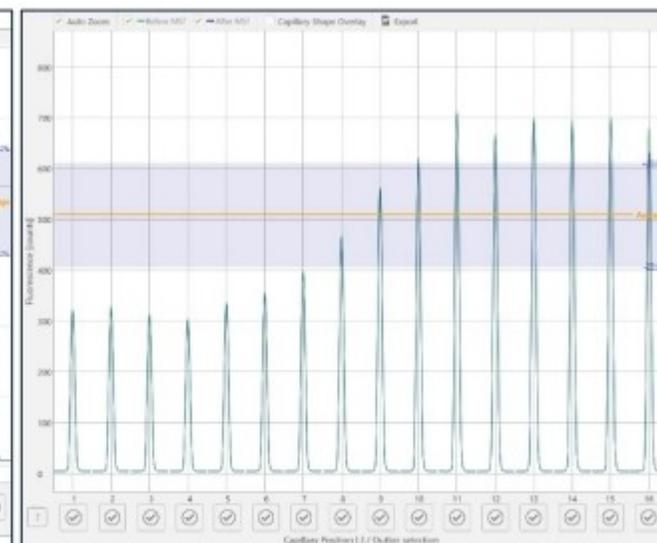
强度



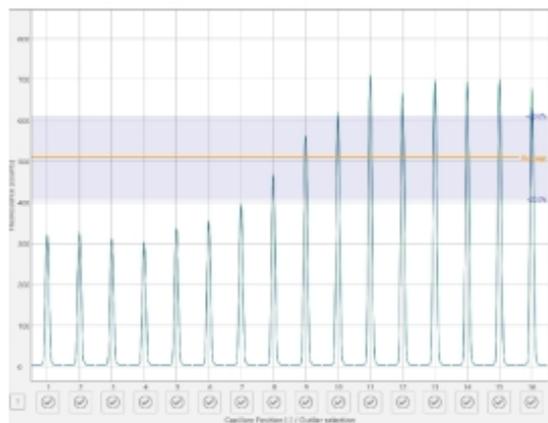
均一性



Ligand浓度依赖变化

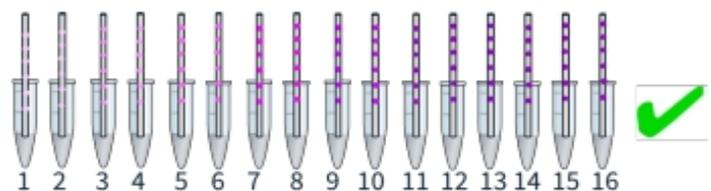


Ligand浓度依赖的初始荧光变化

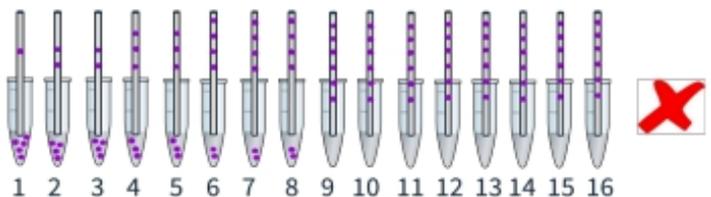


Specificity test
(SD-Test or ECP-Test)

> Ligand特异性结合引起荧光衰减或增强



> Ligand引起吸附或聚集产生的非特异性变化



In order to analyse only the
initial fluorescence of the signal:



优化反应条件

软件介绍

两个强大的软件帮助您进行MST实验



MO.Control

- 自动生成实验protocol
- 及时反馈问题并给出优化意见
- 检测完成后可立即查看Kd结果



MO.Affinity Analysis

- 数据合并/对比分析
- 导出图片/数据

MO.Control 软件

Session Overview

7 Binding Affinity

1 Plan 2 Instructions 3 Results 4 Details

Lysozyme NHS2nd gen 20 nM

NAG3 0.8 mM

PBS Pluronic 0.1%

Standard

Nano - RED, 61%

Medium

You can en

Alter data ?

更改plan

6 Binding Affinity

Lysozyme NAG3

7 Binding Affinity

20nM/0.8mM/pbs/0

8 Binding Affinity

Experiment 8

9 Binding Affinity

20nM/1uM/PBS/0min

Plan Your Experiment

Target

Lysozyme NHS2nd gen

Concentration of stock solution 4 μM

Concentration in this assay 20 nM

Ligand

NAG3

Estimated Kd optional μM

Concentration of stock solution 1.6 mM

Ligand in organic solvent like DMSO

Ligand buffer in this assay 50.0%

Highest concentration in this assay 0.8 mM

Assay buffer

PBS Pluronic 0.1%

Capillary Monolith Capillary

Excitation Nano - RED, 61%

MST-Power Medium

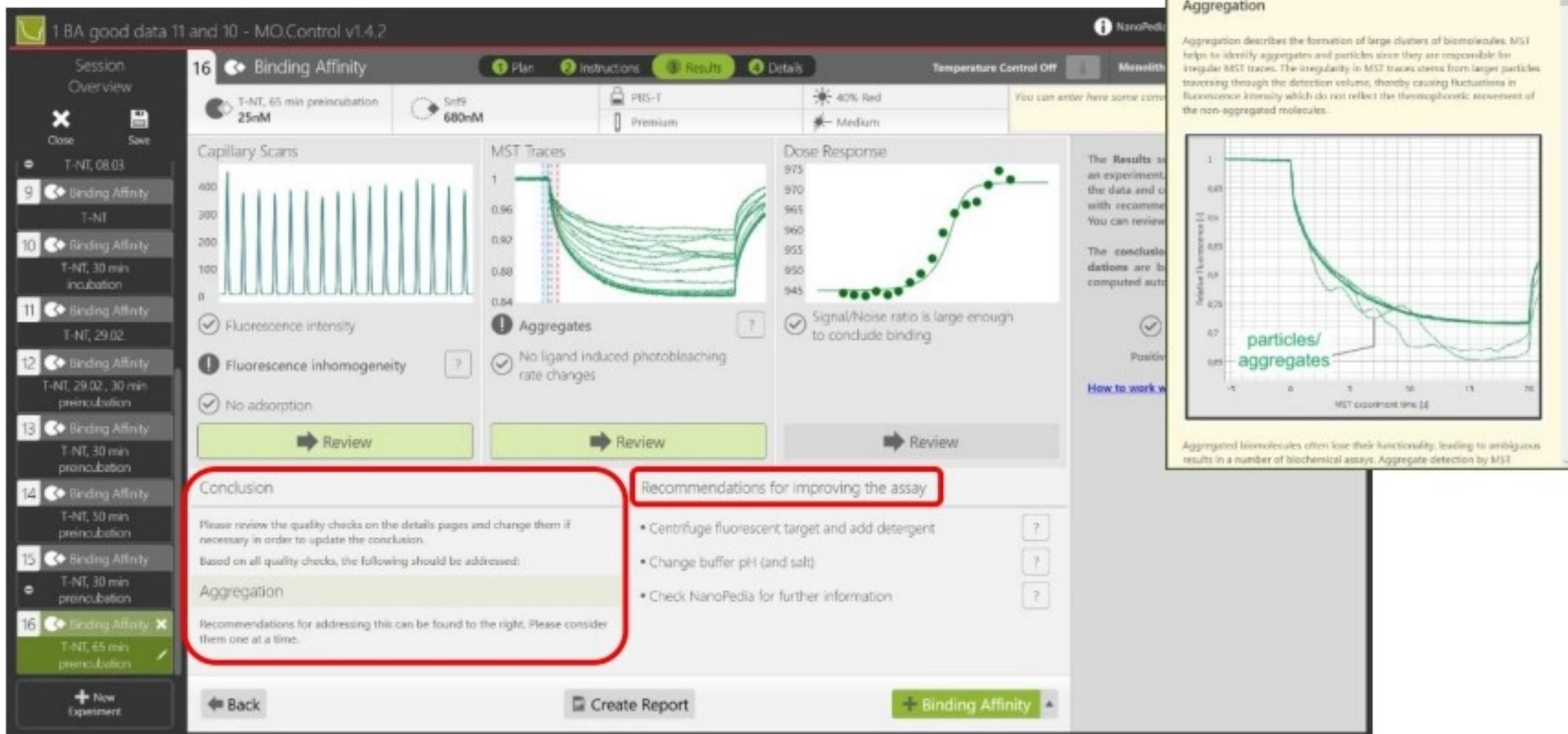
做好标注

Target浓度/ligand浓度/buffer/孵育时间

浓度填写准确

重复实验设置需一致

智能化的NanoPedia给您更清楚的结论和优化指南



MO. Affinity Analysis软件

The screenshot displays the MO. Affinity Analysis software interface. At the top, a navigation bar includes 'Home', 'Data Selection', 'Dose Response Fit', and 'Compare Results'. Below this, a secondary bar contains 'Add Raw Data', 'View MST Trace', 'Auto-Append', and 'Remove All Files'. The main workspace is divided into two columns. The left column shows a list of data runs with their respective MST traces. The right column shows a hierarchical view of 'Analysis-Set #1' containing the selected runs.

Left Column (Data Runs):

- Lysozyme NAG3 (#06)**: MST: Medium, Exc: 61% T=25°C, Target: Lysozyme NHS2nd gen=20 nM Ligand, 2020/09/16 17:02:57. The trace shows a binding curve starting at 1.0 and decreasing to approximately 0.75 over a concentration range of 0 to 20.
- 20nM/0.8mM/pbs/0 (Binding Affinity, #07)**: MST: Medium, Exc: 61% T=25°C, Target: Lysozyme NHS2nd gen=20 nM Ligand, 2020/09/16 17:25:07. The trace is similar to the first run.
- 20nM/0.8mM/pbs/0 (#07)**: MST: Medium, Exc: 61% T=25°C, Target: Lysozyme NHS2nd gen=20 nM Ligand, 2020/09/16 17:25:07. The trace is similar to the first run.

Right Column (Analysis-Set #1):

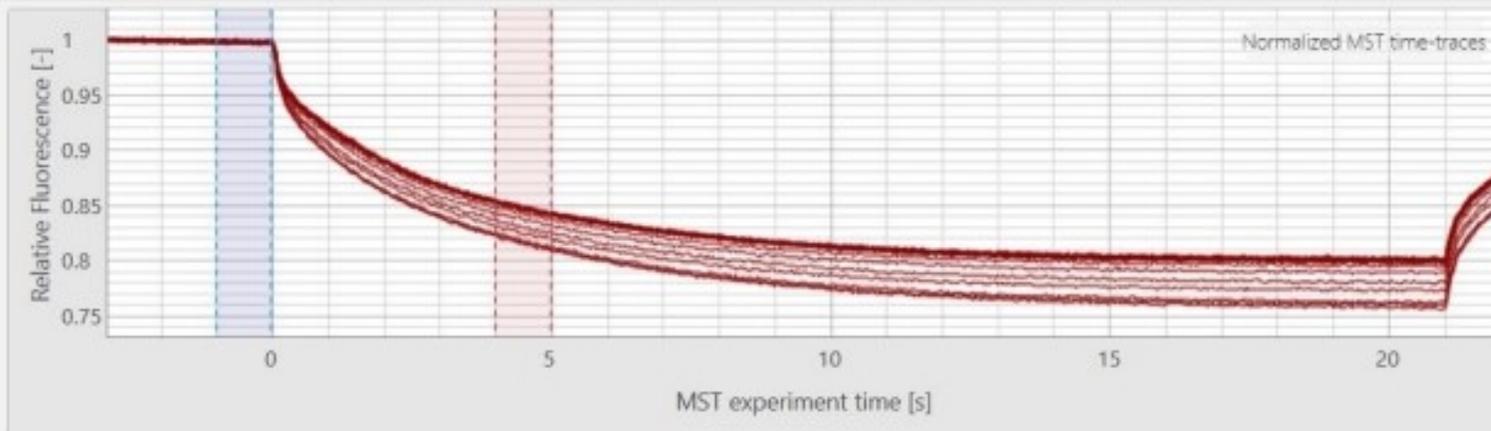
- Lysozyme NAG3 (#06)**: MST: Medium, Exc: 61% T=25°C, Lysozyme NAG3 (#06), 演示数据.moc2, 2020/09/16 17:02:57. This run is highlighted with a dashed green box.
- A prompt below the run says: "Drag Run here to create a new Merge-Set for this Run".

Annotations:

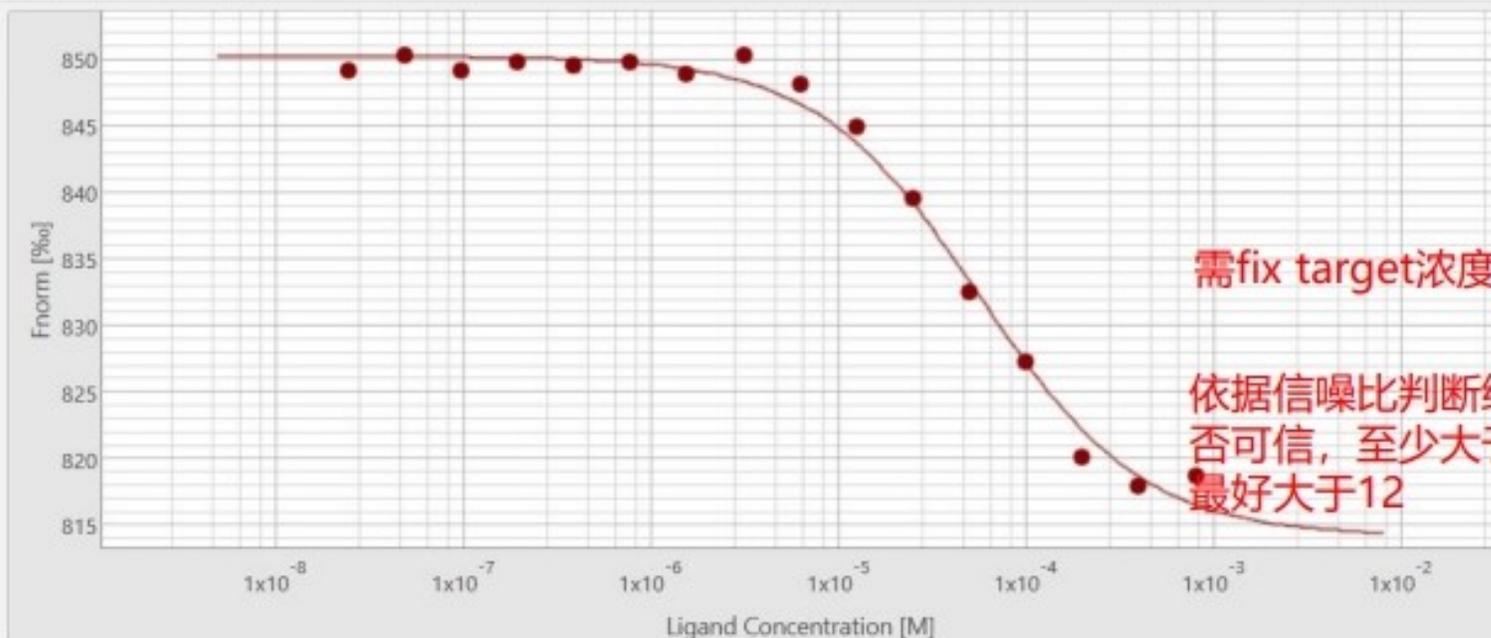
- Red arrows point from the text "合并数据" (Merge Data) to the dashed green box around the selected run in the Analysis-Set.
- Red arrows point from the text "对比数据" (Compare Data) to the same dashed green box.
- A red box highlights a small thumbnail of the Lysozyme NAG3 (#06) trace in the right column.
- A large grey arrow points from the thumbnail area down to the text: "Drag and drop the items you'd like to analyse into this area".

Zoom Extent Export

Generate Full Report



Zoom Extent Export



需fix target浓度

依据信噪比判断结果是否可信，至少大于5，最好大于12

Kd Model

Parameter	Result	Guess	Fix
Unbound	850.24	849	<input type="checkbox"/>
Bound	814.18	819	<input type="checkbox"/>
Kd [mM]	0.055936	0.0044	<input type="checkbox"/>

TargetConc [nM] 20 20

Response Amplitude: 36.0550

Kd Confidence [mM]: [0.0437 - 0.0715]

Standard Error: 1.2426

Reduced χ^2 : N/A

Signal to Noise: 31.2

Hill Model

选择信噪比最高的分析时间 (MST On Time), 可参考MO Control软件的选择

The screenshot displays the MO Affinity Analysis v3.0 software interface. The top navigation bar includes 'Home', 'Data Selection', 'Dose Response Fit', and 'Compare Results'. The main workspace is divided into three sections:

- Left Panel (Analysis-Set #1):** Lists analysis sets including 'MST spin 5min (#10)', 'MST spin 20min (#11)', and 'C030 (#13)'. A red box highlights a lightning bolt icon with the text '步骤一: 点击此符号' (Step 1: Click this symbol).
- Top Center Plot:** 'Normalized MST time-traces' showing 'Relative Fluorescence' vs 'MST experiment time [s]'. Vertical bars indicate time points at 1.00s, 0.00s, 0.00s, and 0.00s. A tooltip says 'Switch this Analysis-Set to Standard Mode'.
- Bottom Center Plot:** 'Fnorm [%]' vs 'Ligand Concentration [M]' on a semi-log scale, showing a fitted curve with data points.
- Right Panel (MST Evaluation Strategy):** Shows 'Manual' as the selected strategy. Under 'Custom On Time Presets', '1.5s On Time' is highlighted with a red box. Below it, '5s On Time' is highlighted with a red box and the text '步骤二: 手动选择 On Time' (Step 2: Manually select On Time).
- Bottom Right Panel (Kd Model):** A table of fit parameters:

Parameter	Result	Guess	Fix
Unbound	877.47	875	<input type="checkbox"/>
Bound	838.33	840	<input type="checkbox"/>
Kd [mM]	0.18556	0.14	<input type="checkbox"/>
TargetConc [nM]	20	20	<input checked="" type="checkbox"/>

Additional model statistics: Response Amplitude: 39.1374, Kd Confidence [mM]: [0.142 - 0.243], Standard Error: 1.7688, Reduced χ^2 : N/A, Signal to Noise: 23.8.



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折扣：

1	单笔订购1万9.5折
2	单笔订购5万9折
3	单笔订购10万9折，送套装(MO-X001)