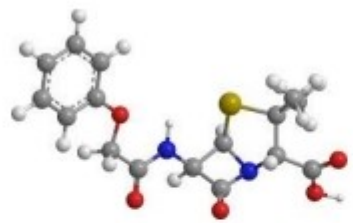
微量 热泳动技术原理
(Microscale Thermophoresis, MST)

NANOTEMPER

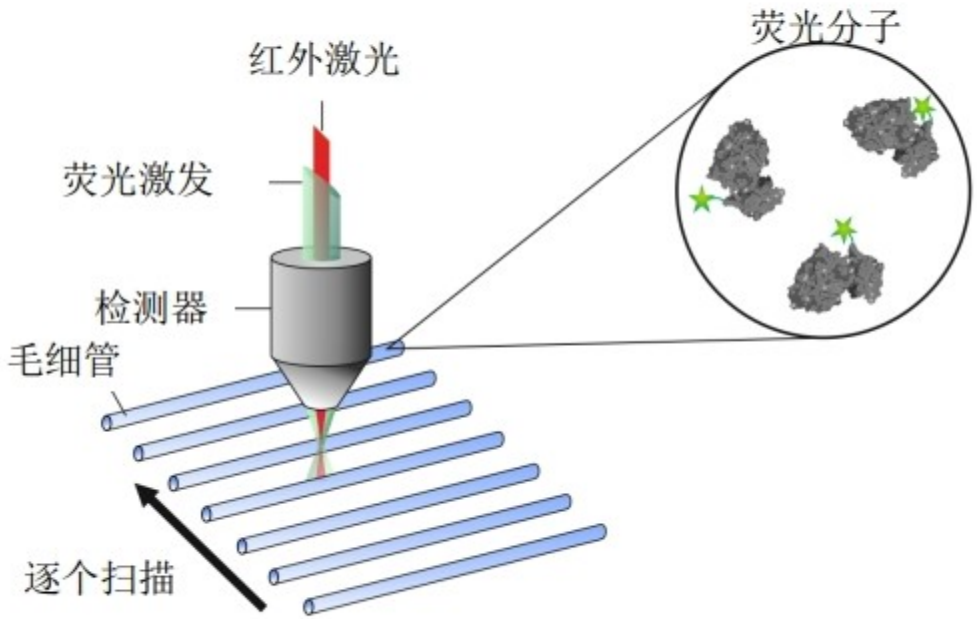
MST术语

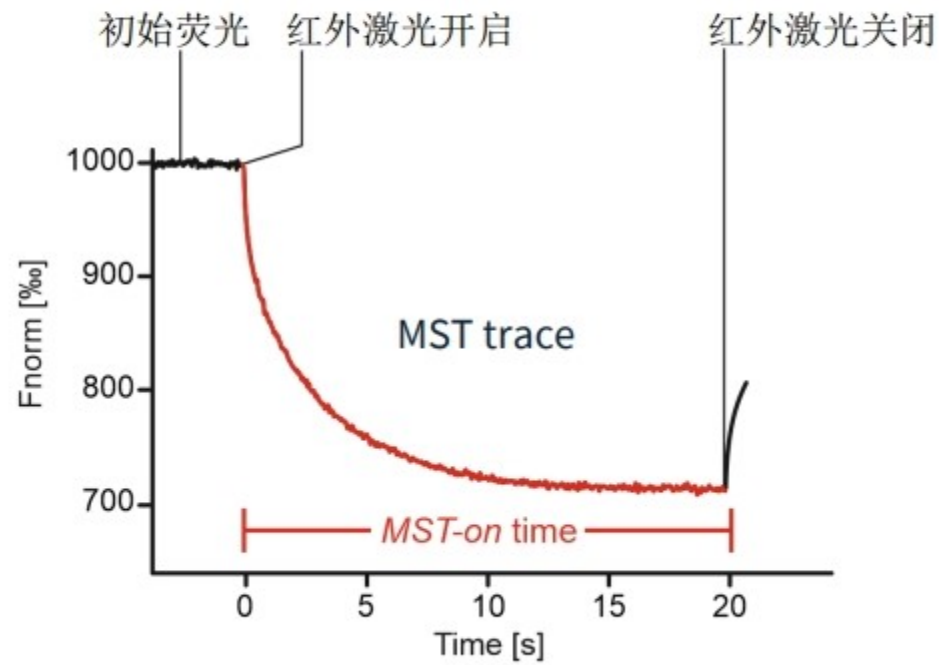
TARGET → 荧光分子



LIGAND → 不激发荧光分子，配体

工作原理


荧光分子
红外激光
荧光激发
检测器
毛细管
逐个扫描


初始荧光
红外激光开启
红外激光关闭
1000
900
800
700
Fnorm [‰]
MST trace
MST-on time
0
5
10
15
20
Time [s]

MST 信号变化来源于热泳动和温度依赖的荧光强度变化

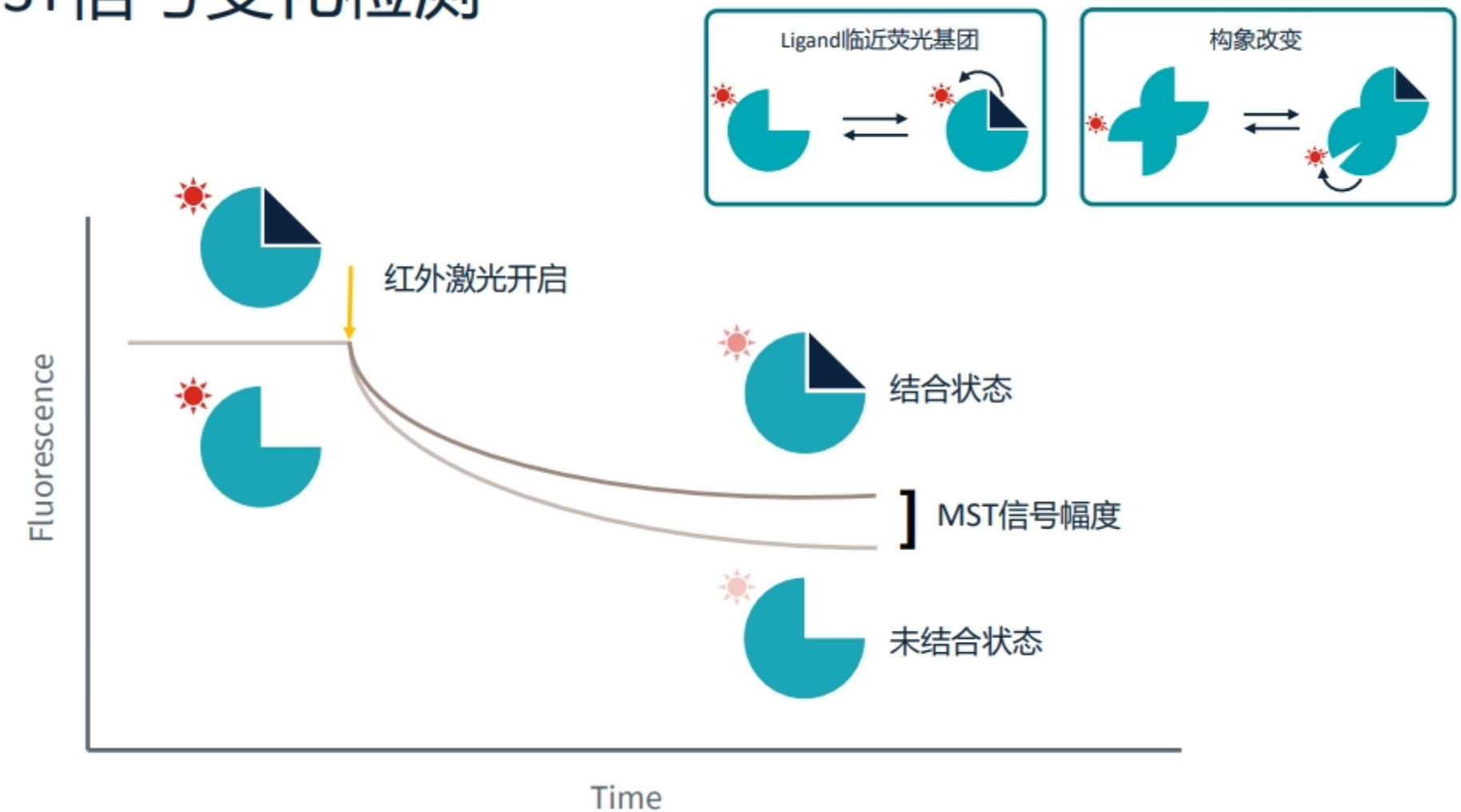
热泳动（Thermophoresis）

- 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

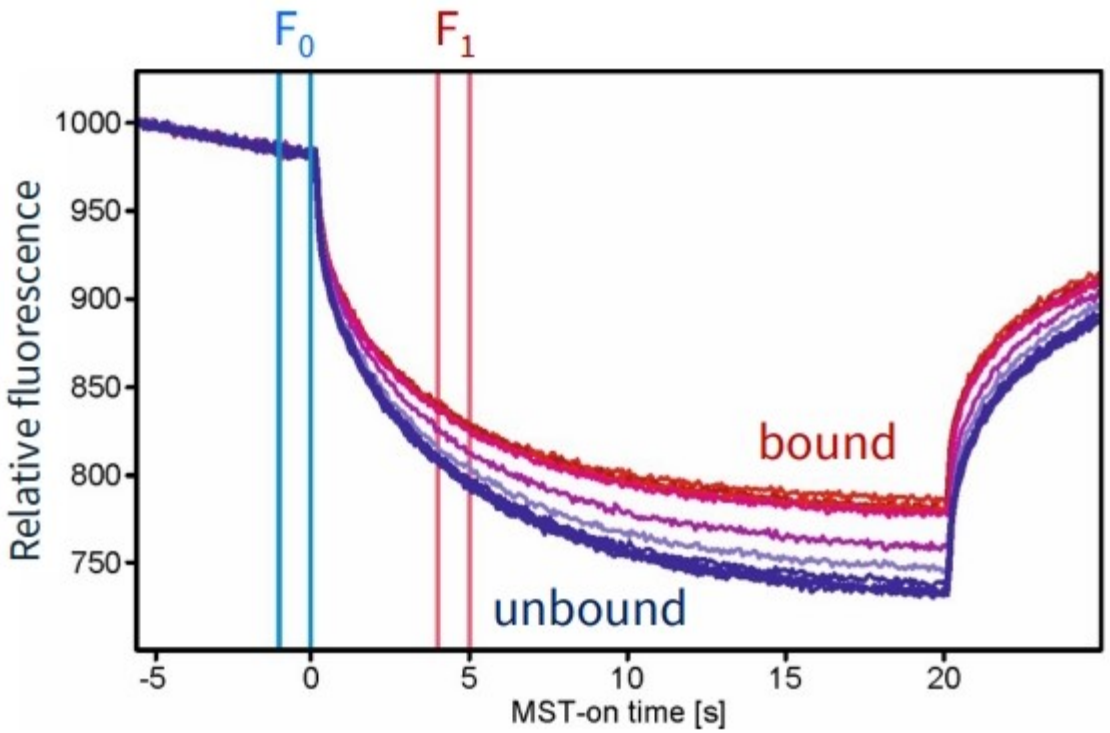
温度依赖的荧光强度变化（TRIC）

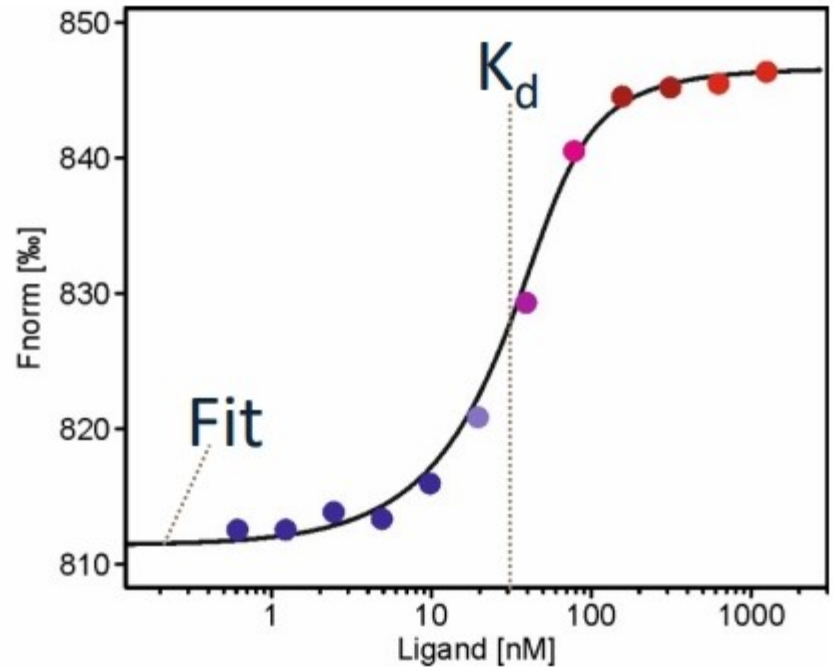
- 荧光**强度**与温度有关
- 荧光强度与荧光团的化学环境密切相关

基于MST信号变化检测



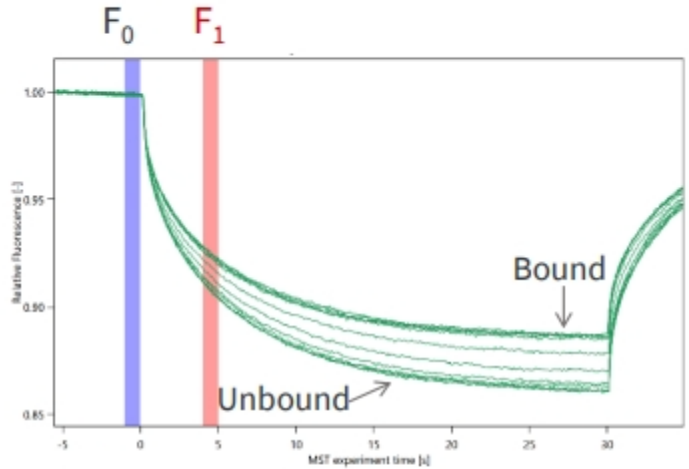
由MST结合曲线得出平衡解离常数Kd



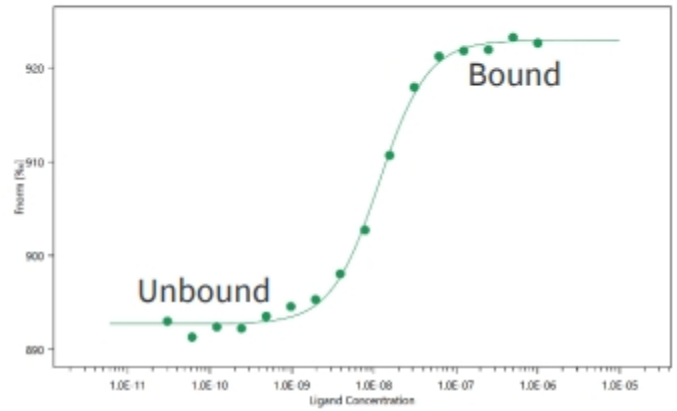


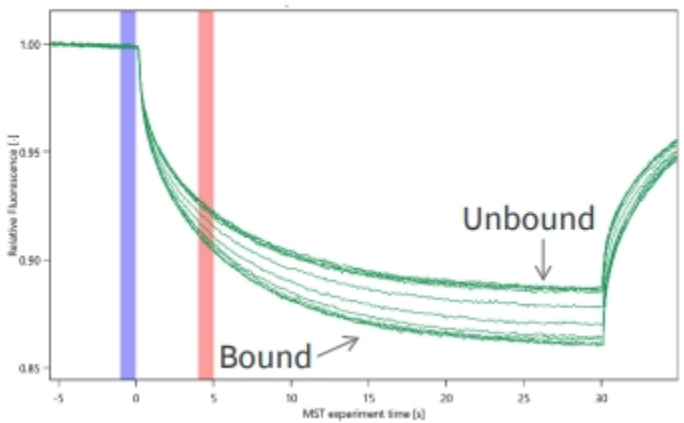
K_d越小，亲和力越强

曲线方向和亲和力大小无关

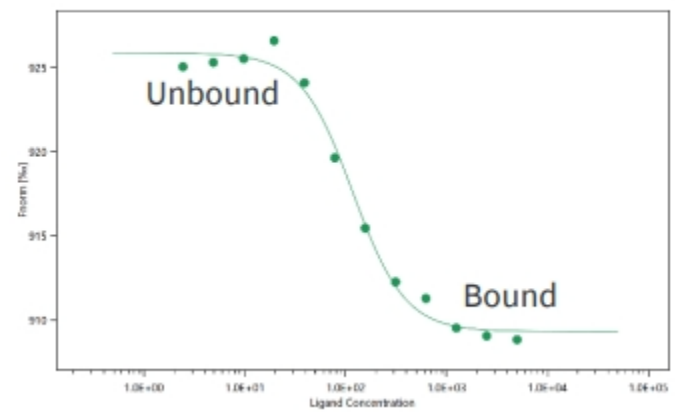


$F_{norm} = F_1/F_0$

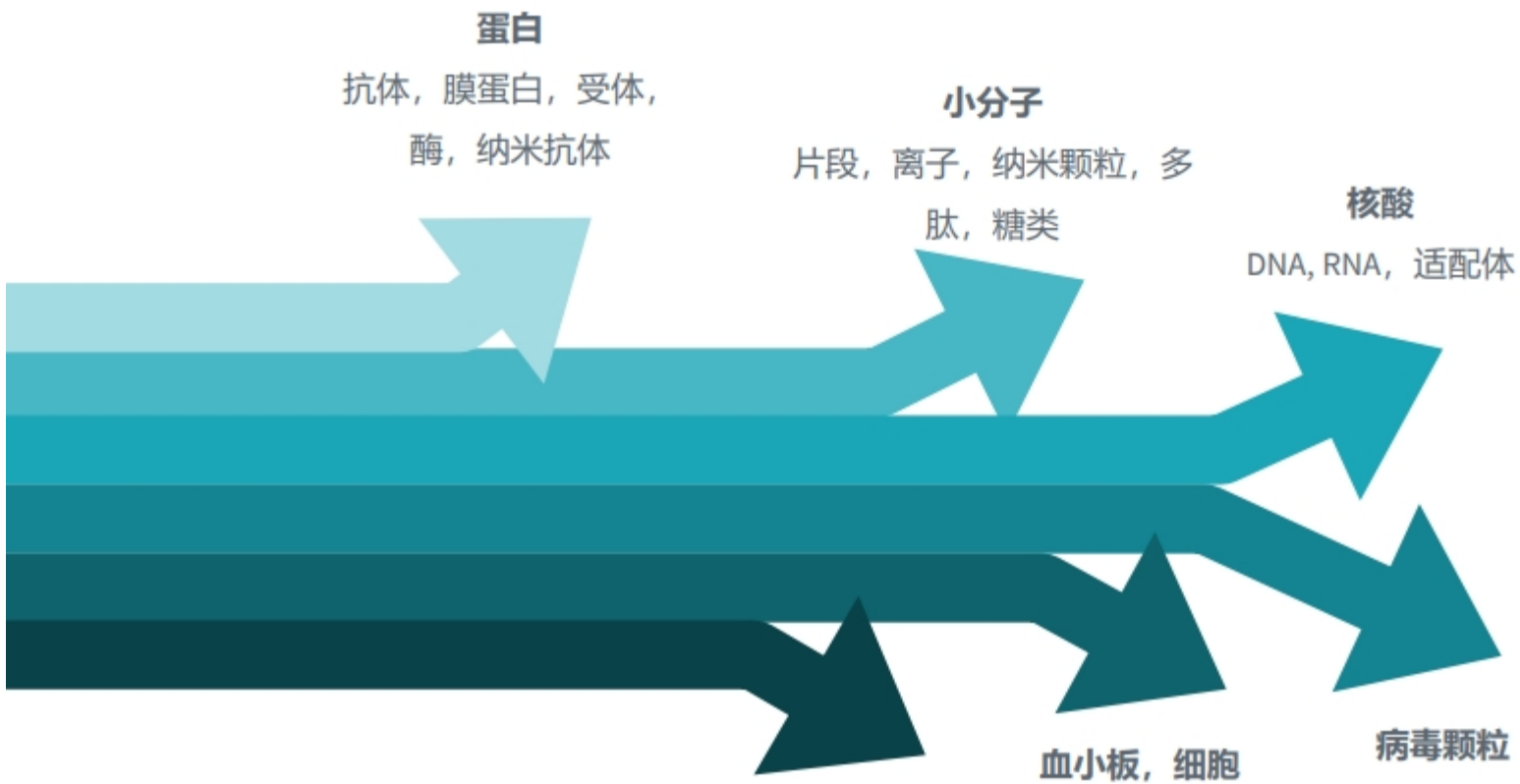




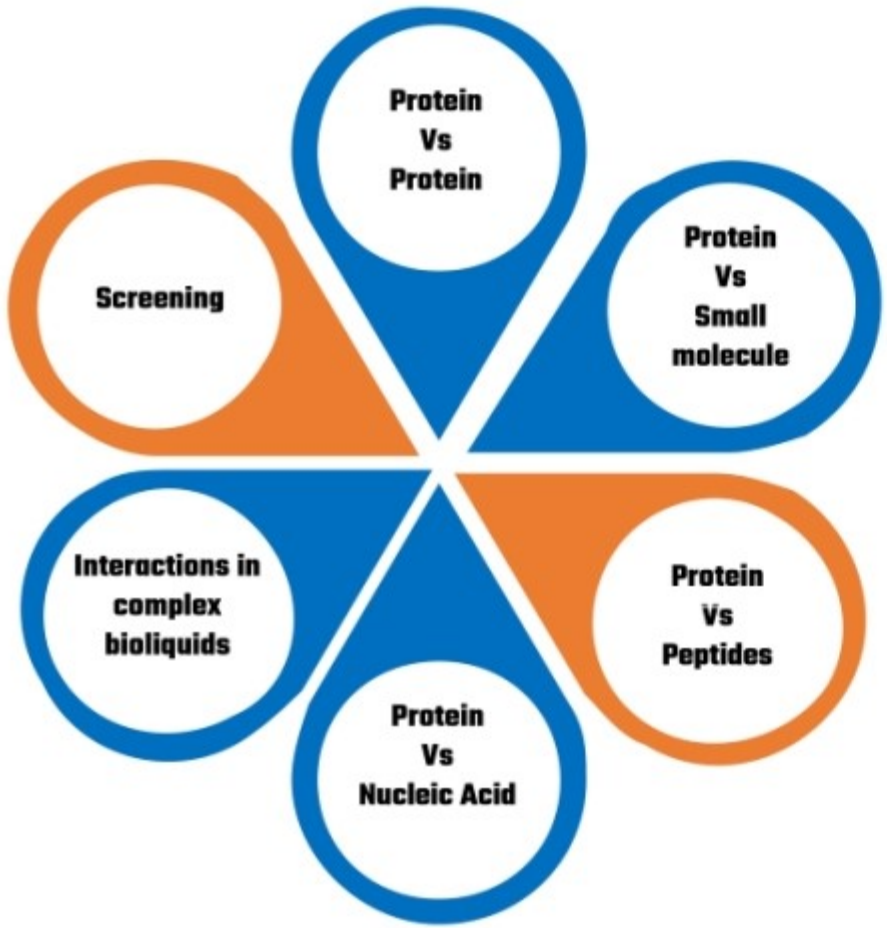
$F_{norm} = F_1/F_0$



检测样品类型丰富


蛋白
抗体，膜蛋白，受体，
酶，纳米抗体
小分子
片段，离子，纳米颗粒，多
肽，糖类
核酸
DNA, RNA，适配体
病毒颗粒
血小板，细胞
囊泡
外泌体，脂质体

MST技术应用领域



样品类型	研究方向
› 蛋白-蛋白	› 基于靶标的药物筛选
› 蛋白-小分子	› 蛋白结构验证
› 蛋白-离子	› 植物信号通路
› 抗原-抗体	› 神经退行性疾病
› 蛋白-多肽	› 肿瘤抑制剂开发
› 蛋白-脂类	› 中药活性成分
› 蛋白-核酸	› 蛋白泛素化研究
› 病毒颗粒	› 竞争性结合实验
› 纳米颗粒	› 酶与底物的结合
› 核酸适配体	› 农药残留检测
› 细胞裂解液/血清	› 癌症标志物
›	› .....

Monolith技术的优势

样品用量少（μL）
Do more with your precious target protein

测量范围广（Kd: pM to mM）
Measure broad range of affinities (from pM to mM) and develop assay faster

样品种类多
(人，动物，植物，微生物..)
Measure many sample types: mammalian, yeast and bacterial cultures, fungi, plant extracts, bioliquids

测量类型广 (蛋白-离子/代谢物)
Measure broad range of interaction, including protein-ion and protein: carbohydrate

不依赖配体 (大小，分子量)
Measure K_d independent of size and mass of binding partners

自然条件检测，无需处理
Measure in solution in close to native conditions. No immobilization required.

不挑缓冲液
Work with the buffer that keeps your protein stable

简单易用，无需维护
Don't halt or delay your project with daily maintenance and repairs

MST实验设置与优化

NANOTEMPER

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

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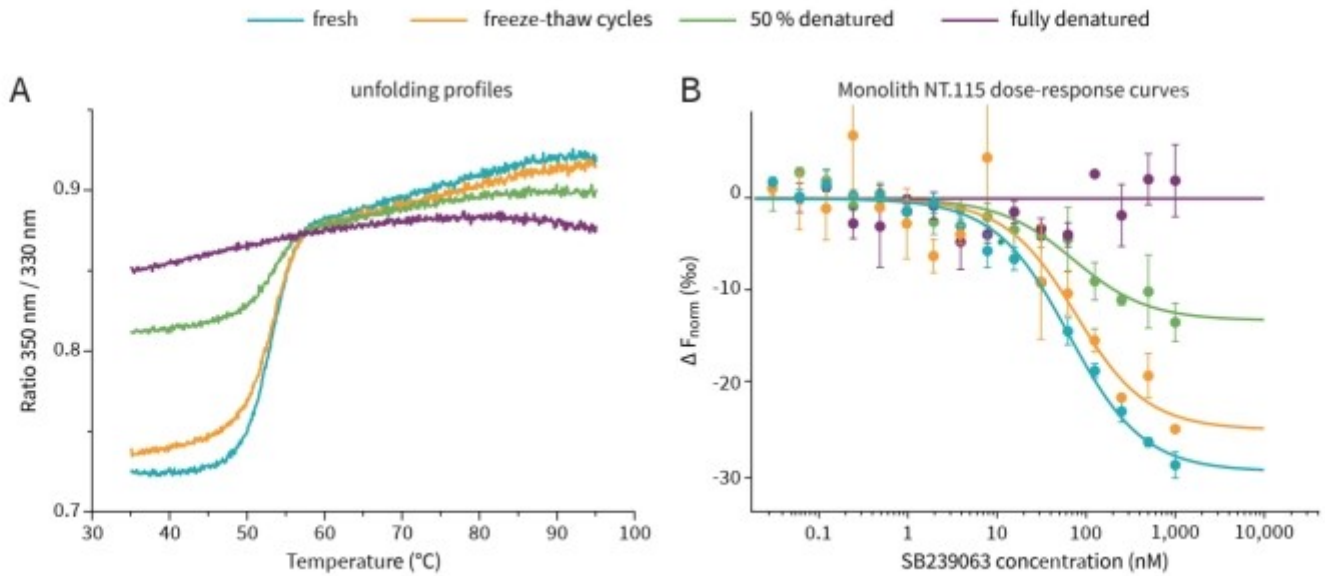
样品生物活性是下游实验成功的基础



MST 实验流程

1. 蛋白质控
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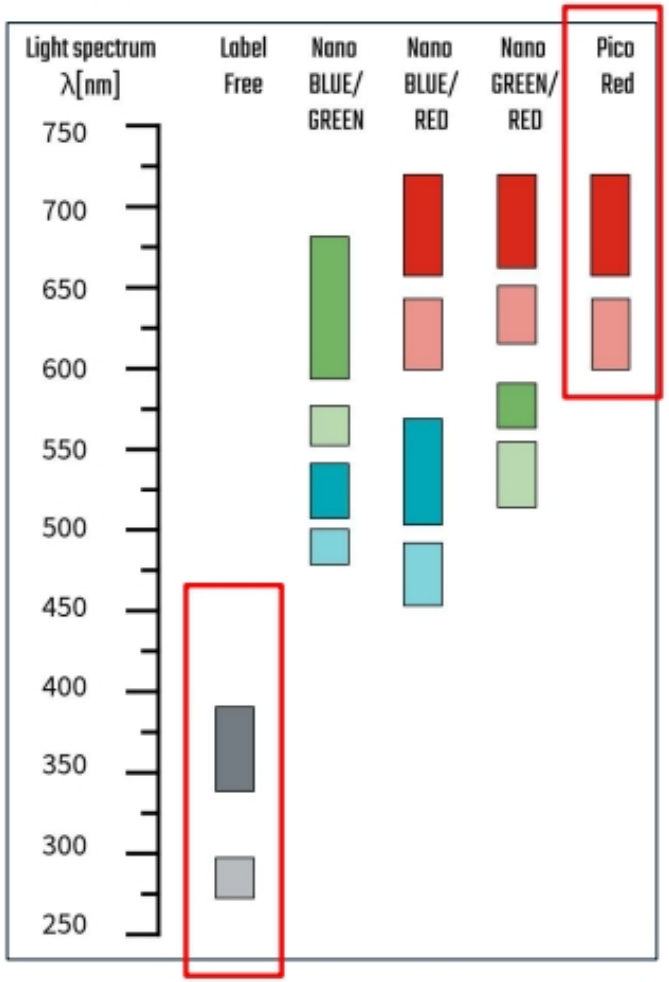
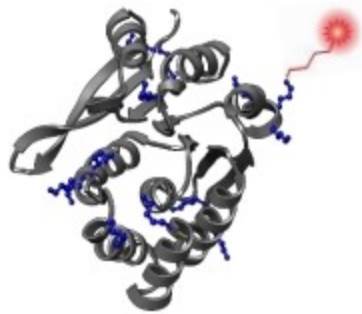
选择合适的通道 推荐使用红色通道

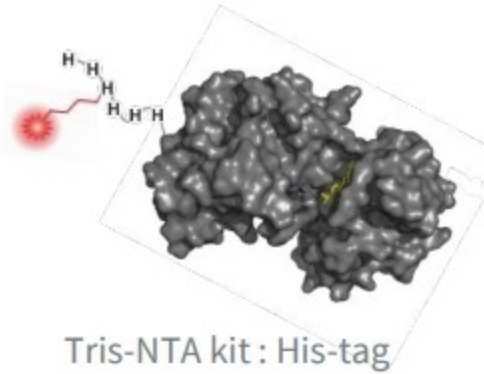


Fluorophore	Excitation [nm]		Emission [nm]
BCECF	480		525
GFP	488		507
NT-495 (BLUE)	493		521
Fluorescein (FITC)	495		519
Alexa488	495		519
YFP	514		527
Alexa532	530		555
TAMRA	546		579
Cy3	550		570
RFP	555		584
NT-547 (GREEN)	557		574
Alexa546	560		572
Cy5	649		670
NT-647 (RED)	650		670
Alexa647	652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

选择标记方式

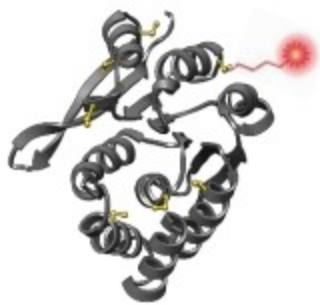


NHS kit：通过赖氨酸侧链氨基偶联

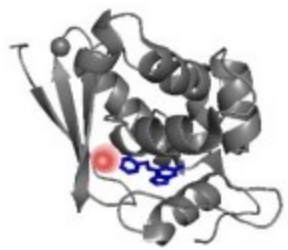


Tris-NTA kit : His-tag

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

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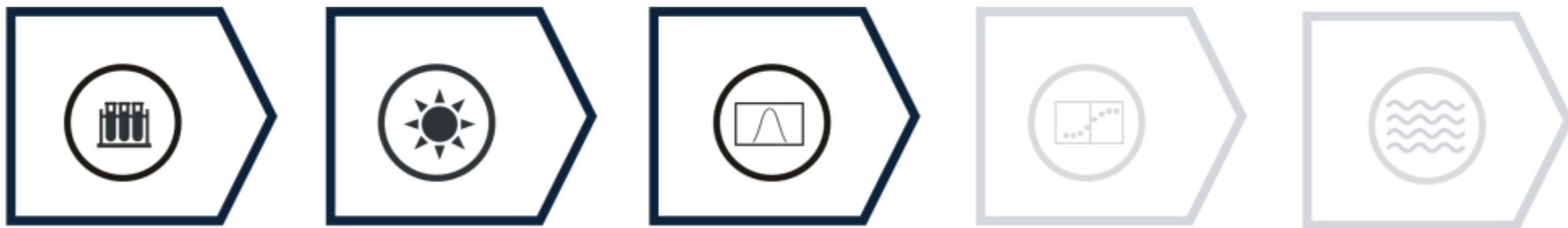
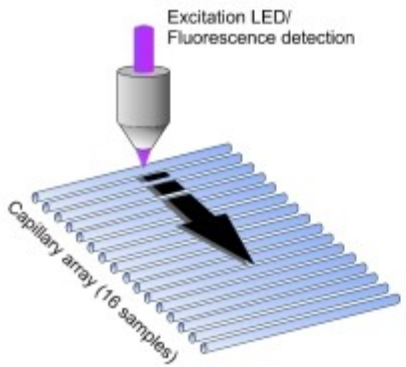
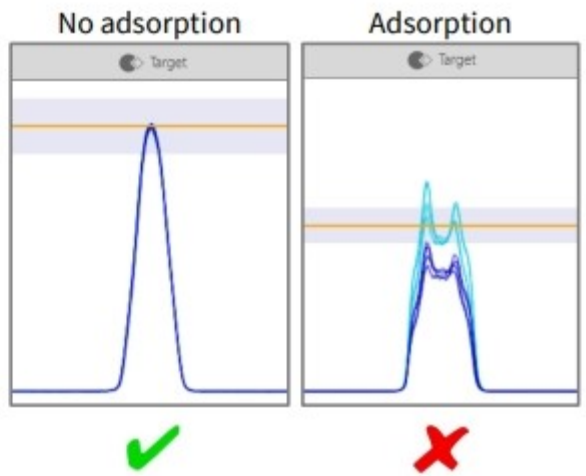


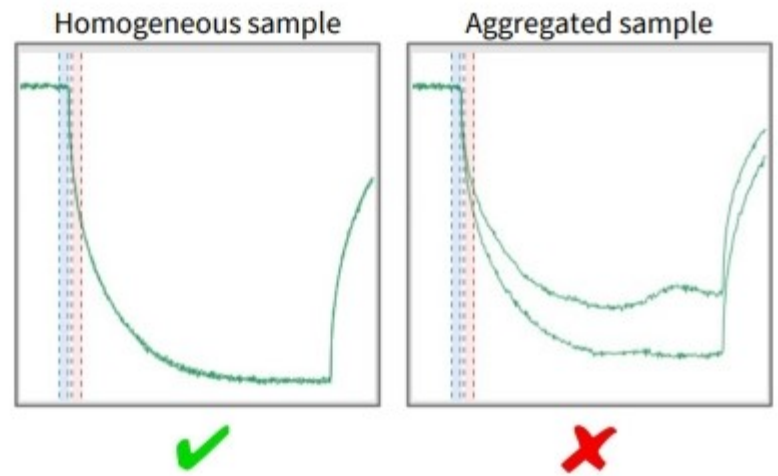
Pretest

检测器	荧光强度
Pico Red	3000~20000

- 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







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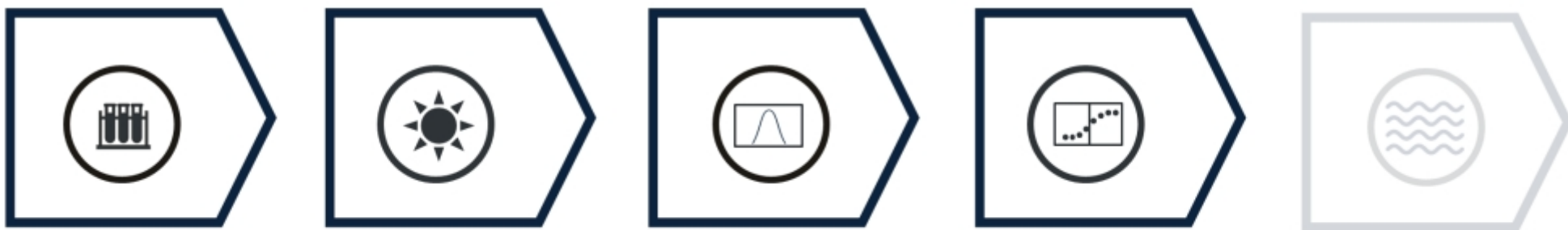
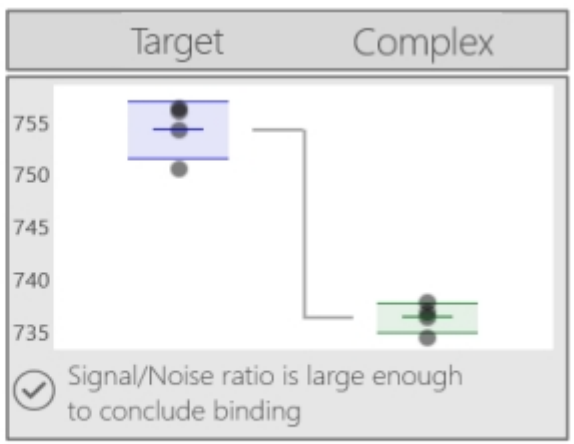
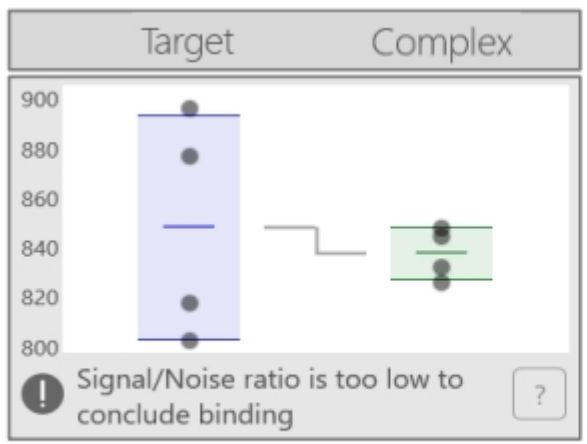


Binding Check

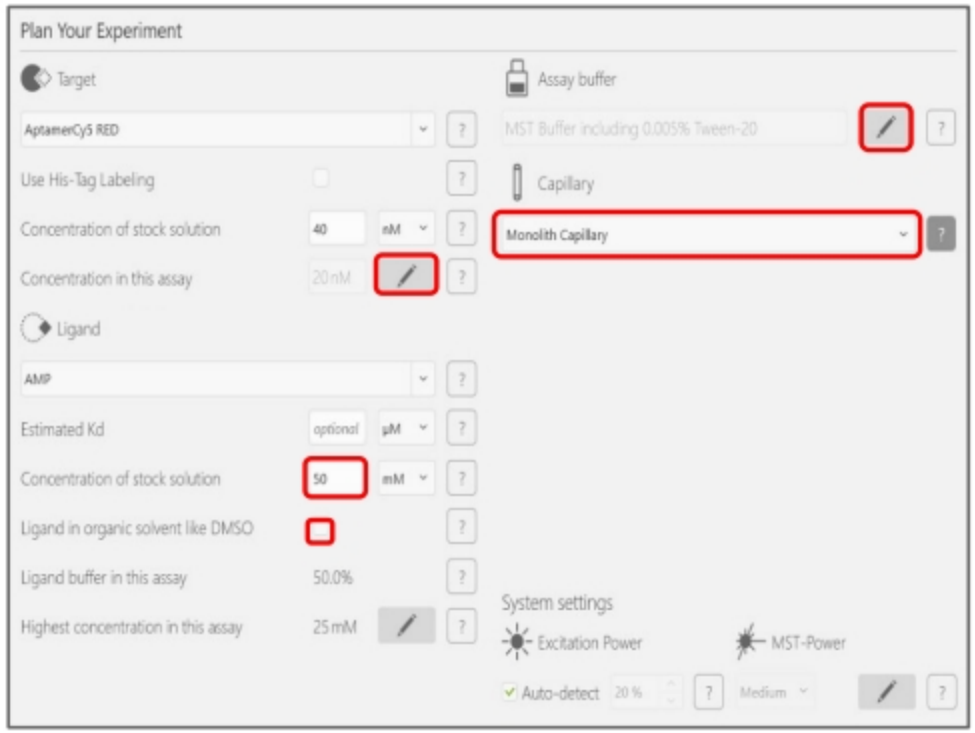
- 判断是否有结合

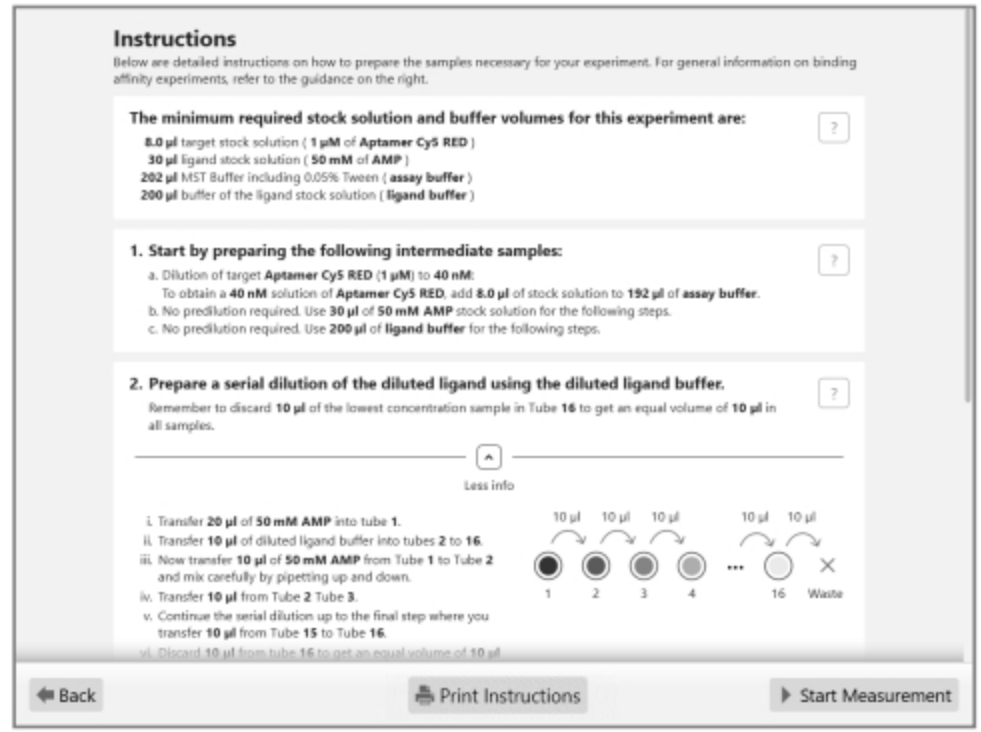




Binding Affinity

1.正确填写Plan Your Experiment，软件自动生成指引



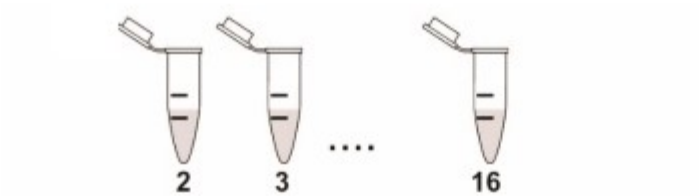


Binding Affinity

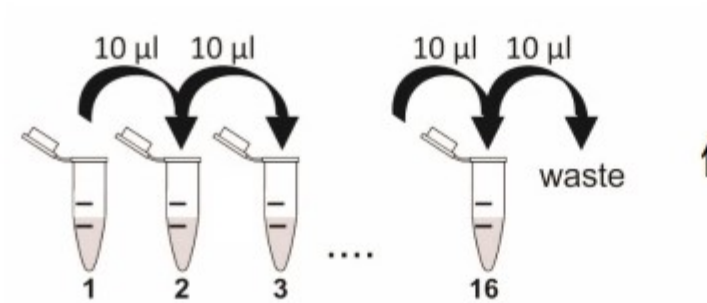
2. Ligand稀释

准备20μl Ligand

(浓度为Kd的20~50倍)



向其他15个PCR管中各加入10μl Buffer



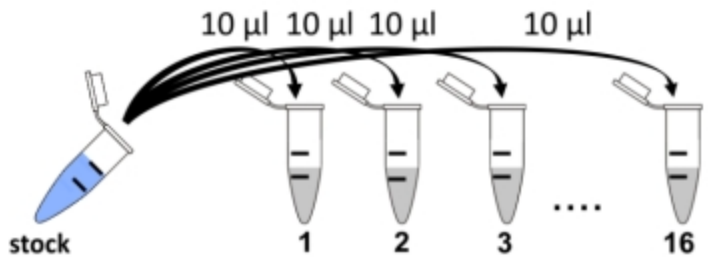
依次完成梯度稀释，最后一管弃去10μl

Binding Affinity

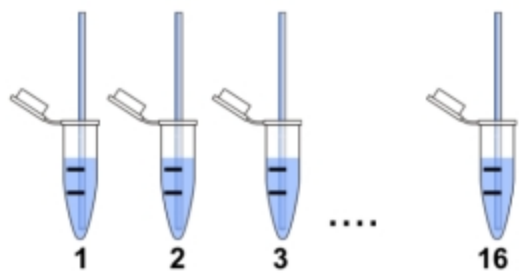
3. 加入Target

准备200μl Target

(浓度<Kd，通常20~100nM)



向所有PCR管中各加入10μl Target并混匀

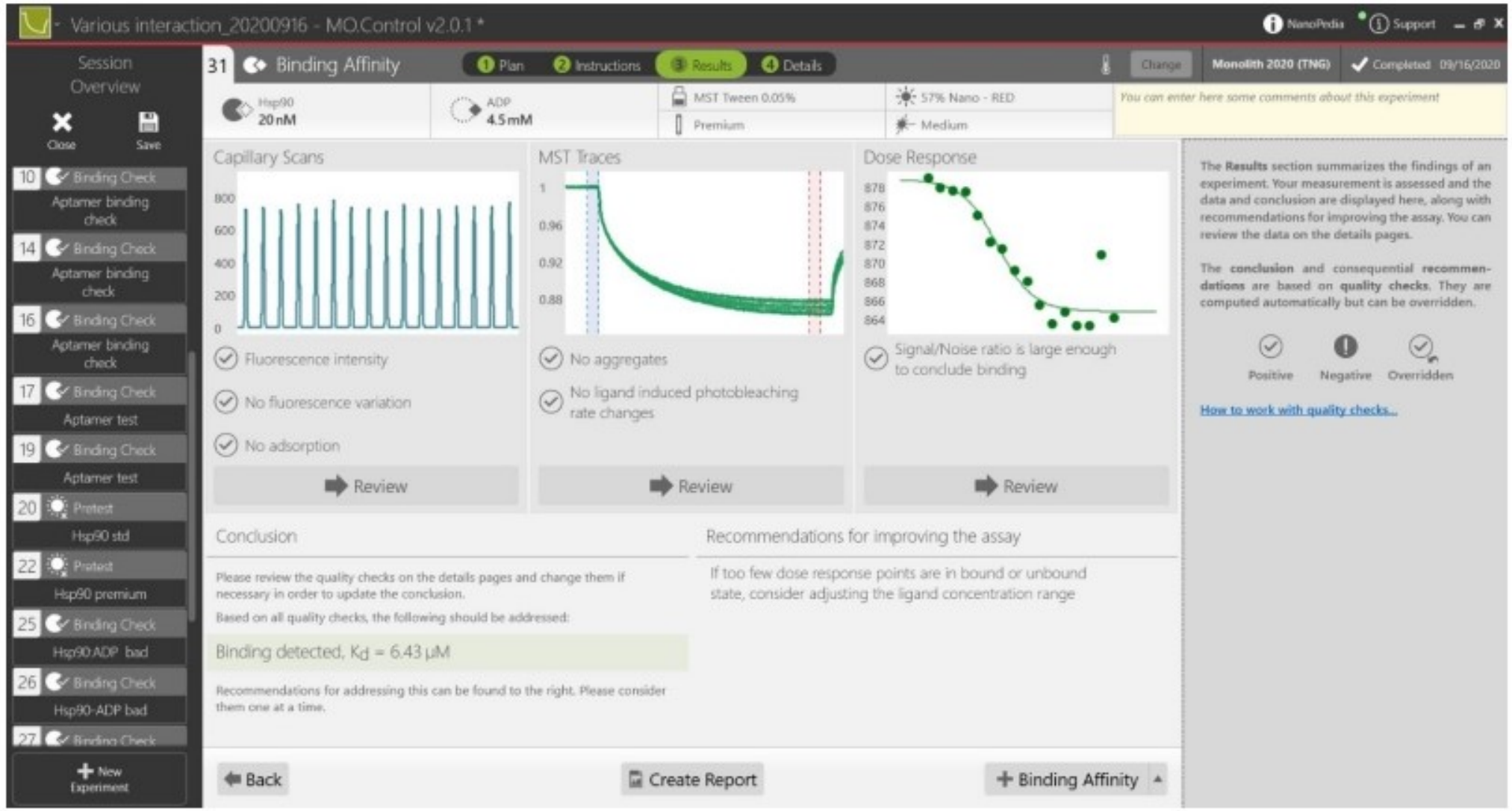


使用毛细管吸取样品

样品处理的注意事项

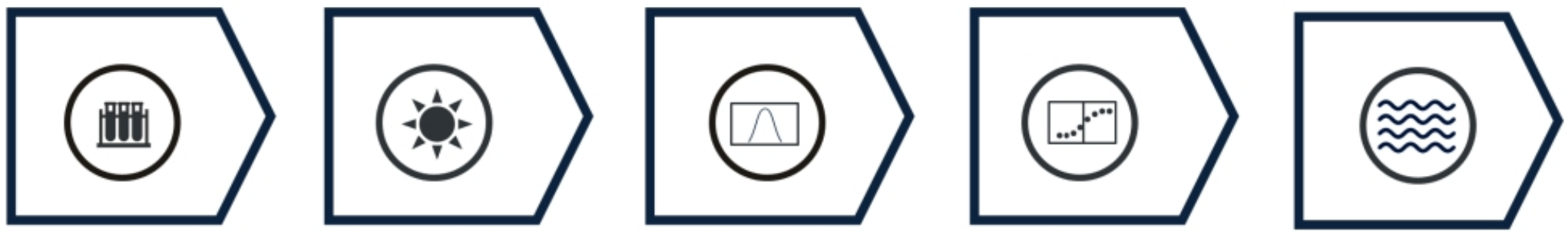
- **荧光分子(target)**的浓度：低于预估的K_d值，一般20-100nM
- **配体(ligand)的最高浓度：预估K_d的**20~50**倍**
- **使用小管进行倍比稀释：** PCR strips, tubes
- **在倍比稀释过程中，稀释缓冲液的成分不应发生变化：** e.g. DMSO
- **准确的移液至关重要**
- **用移液枪混合样品，不要涡旋** (vortex)
- **不要用手触摸毛细管的中间部分**

10 min 得到结果


Various interaction_20200916 - MO.Control v2.0.1 *
NanoPedia
Support
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
New Experiment
31 Binding Affinity
1 Plan
2 Instructions
3 Results
4 Details
Change
Monolith 2020 (TNG)
Completed 09/16/2020
Hsp90 20 nM
ADP 4.5 mM
MST Tween 0.05%
Premium
57% Nano - RED
Medium
You can enter here some comments about this experiment
Capillary Scans
800
600
400
200
0
Fluorescence intensity
No fluorescence variation
No adsorption
Review
MST Traces
1
0.96
0.92
0.88
No aggregates
No ligand induced photobleaching rate changes
Review
Dose Response
878
876
874
872
870
868
866
864
Signal/Noise ratio is large enough to conclude binding
Review
The Results section summarizes the findings of an experiment. Your measurement is assessed and the data and conclusion are displayed here, along with recommendations for improving the assay. You can review the data on the details pages.
The conclusion and consequential recommendations are based on quality checks. They are computed automatically but can be overridden.
Positive
Negative
Overridden
How to work with quality checks...
Conclusion
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, Kd = 6.43 µM
Recommendations for addressing this can be found to the right. Please consider them one at a time.
Recommendations for improving the assay
If too few dose response points are in bound or unbound state, consider adjusting the ligand concentration range
Back
Create Report
Binding Affinity

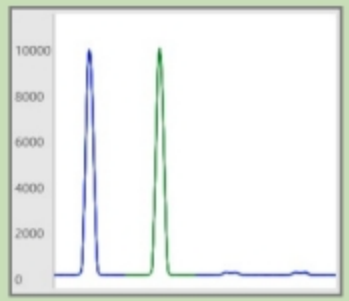
MST 实验流程

1. 蛋白质控
2. Target制备
3. Pretest
4. 亲和力检测
5. 实验优化

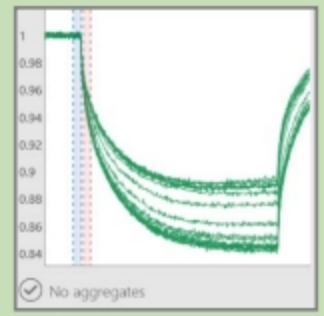


常见的样品问题类型

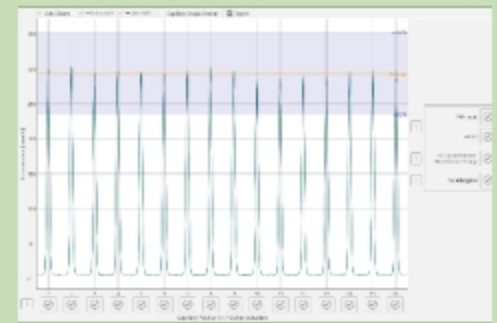
无吸附



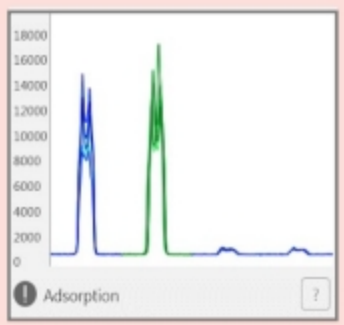
无聚集



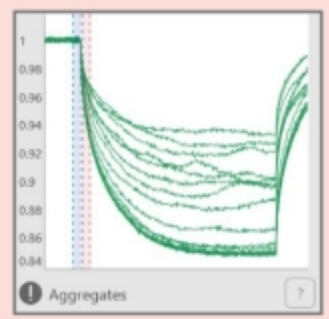
均一性好



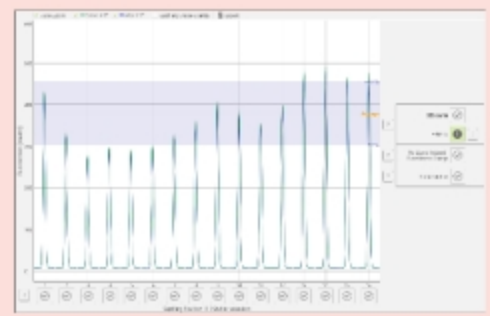
样品吸附



聚集



样品不均一



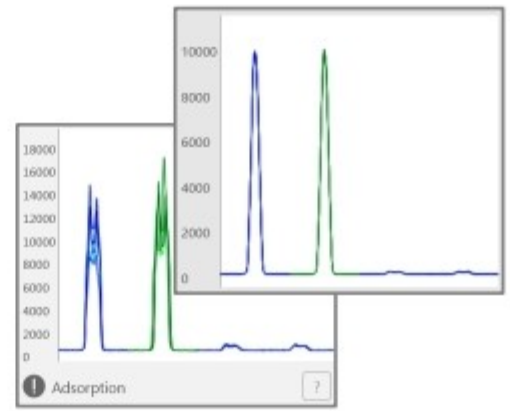
吸附和聚集

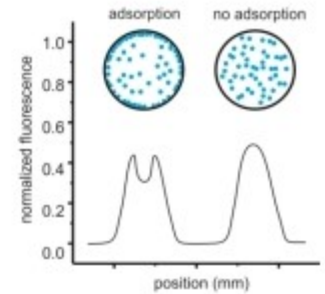
吸附

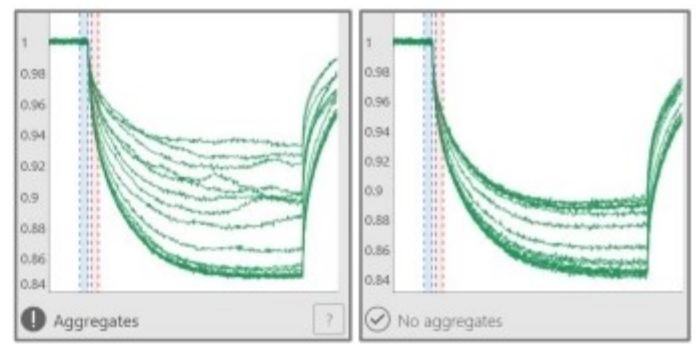
- 更换毛细管 (Standard → Premium)
- 添加去垢剂 (0.05% Tween 20, 0.1% Pluronic F-127)
- 调整缓冲液成分 (pH, salt concentration...)

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...)

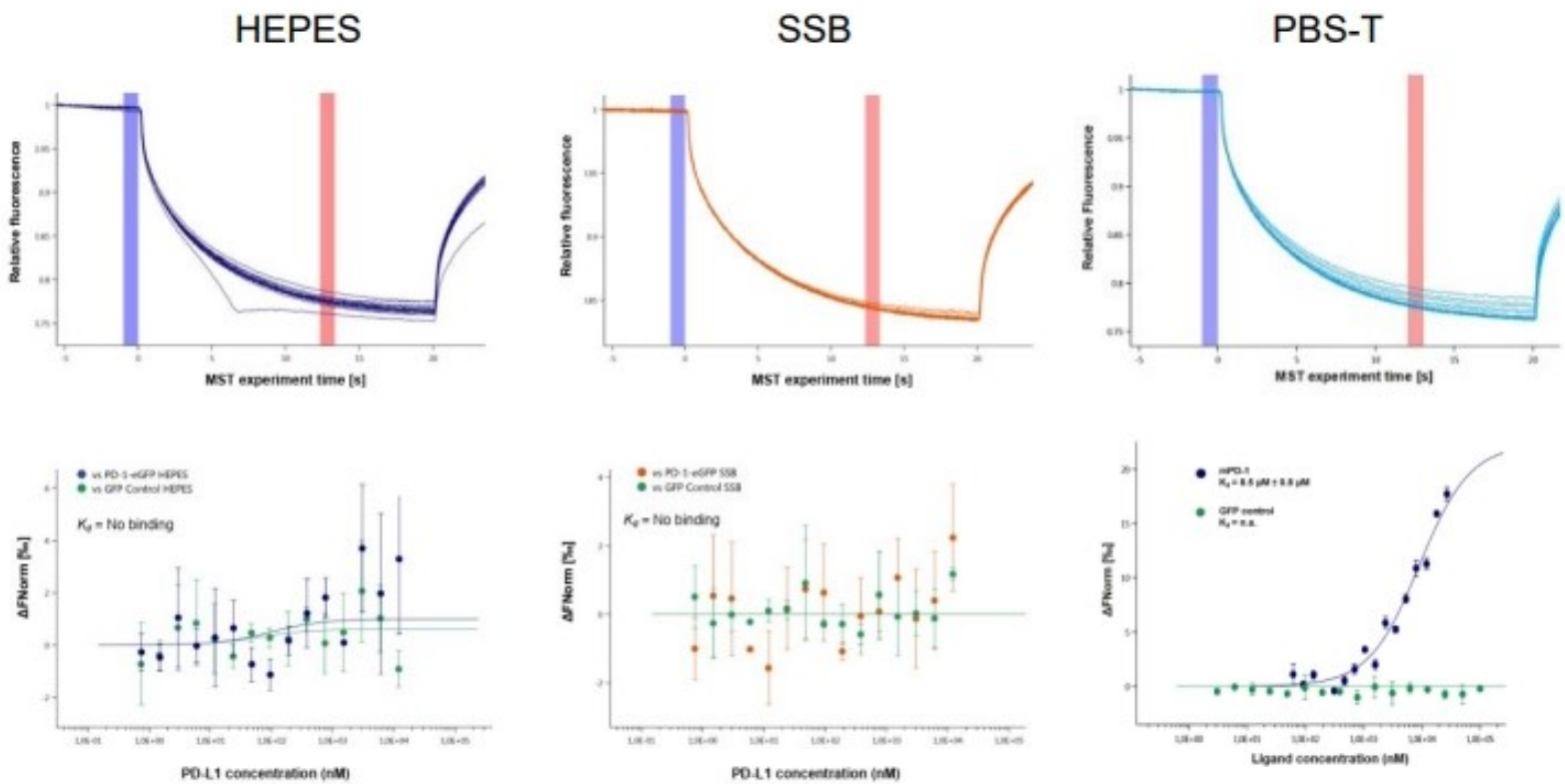






缓冲液优化

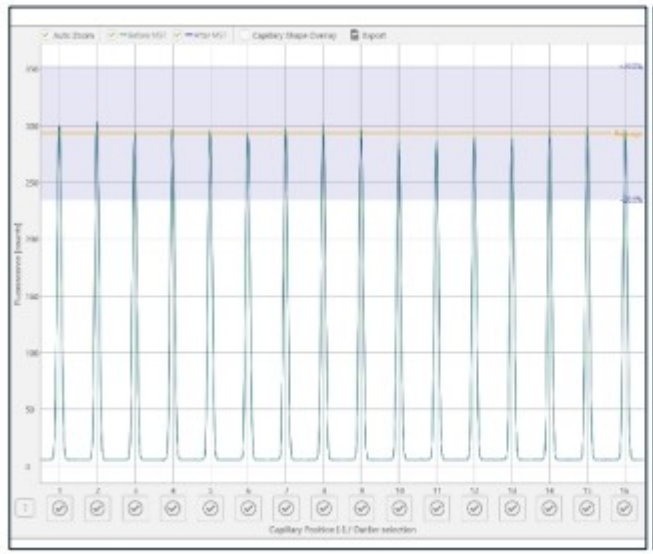
缓冲液对互作影响(盐浓度，pH值，去垢剂和添加剂..)



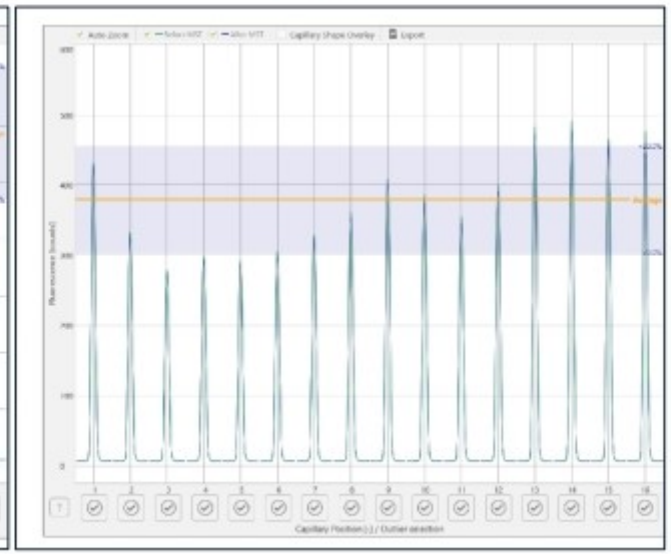
Magnez, R., Thiroux, B., Taront, S., Segaoula, Z., Quesnel, B., & Thuru, X. (2017). PD-1/PD-L1 binding studies using microscale thermophoresis. *Scientific Reports*, *7*(1), 1–8.

初始荧光

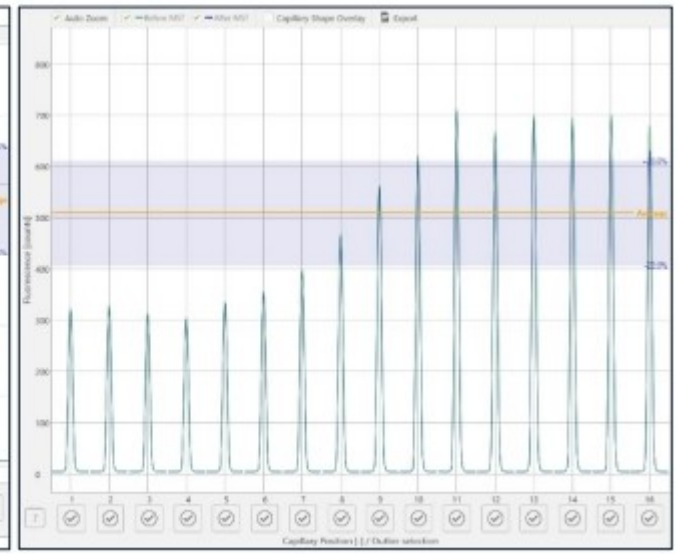
强度



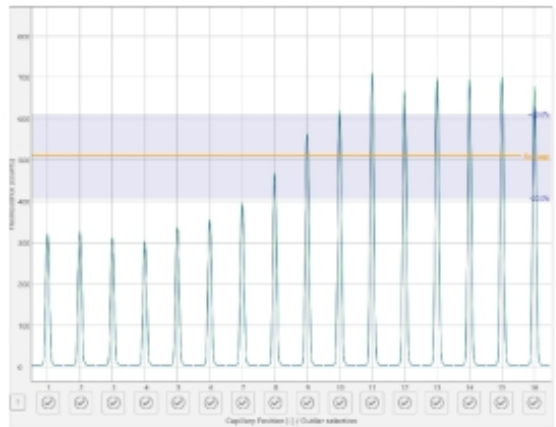
均一性



Ligand浓度依赖变化



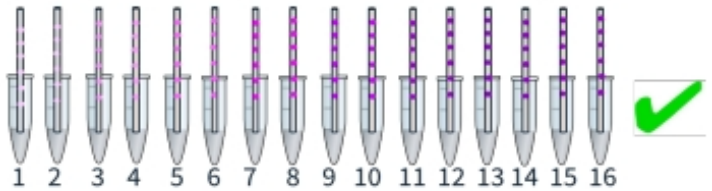
Ligand浓度依赖的初始荧光变化



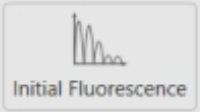
Specificity test

(SD-Test or ECP-Test)

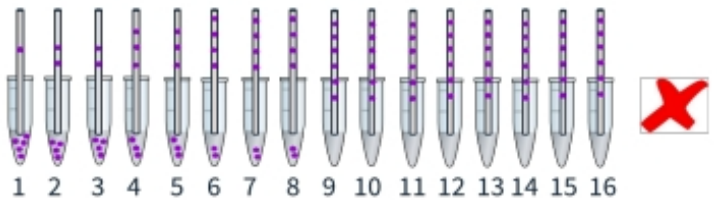
› Ligand特异性结合引起荧光衰减或增强



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



› Ligand引起吸附或聚集产生的非特异性变化



优化反应条件

软件介绍

NANOTEMPER

两个强大的软件帮助您进行MST实验

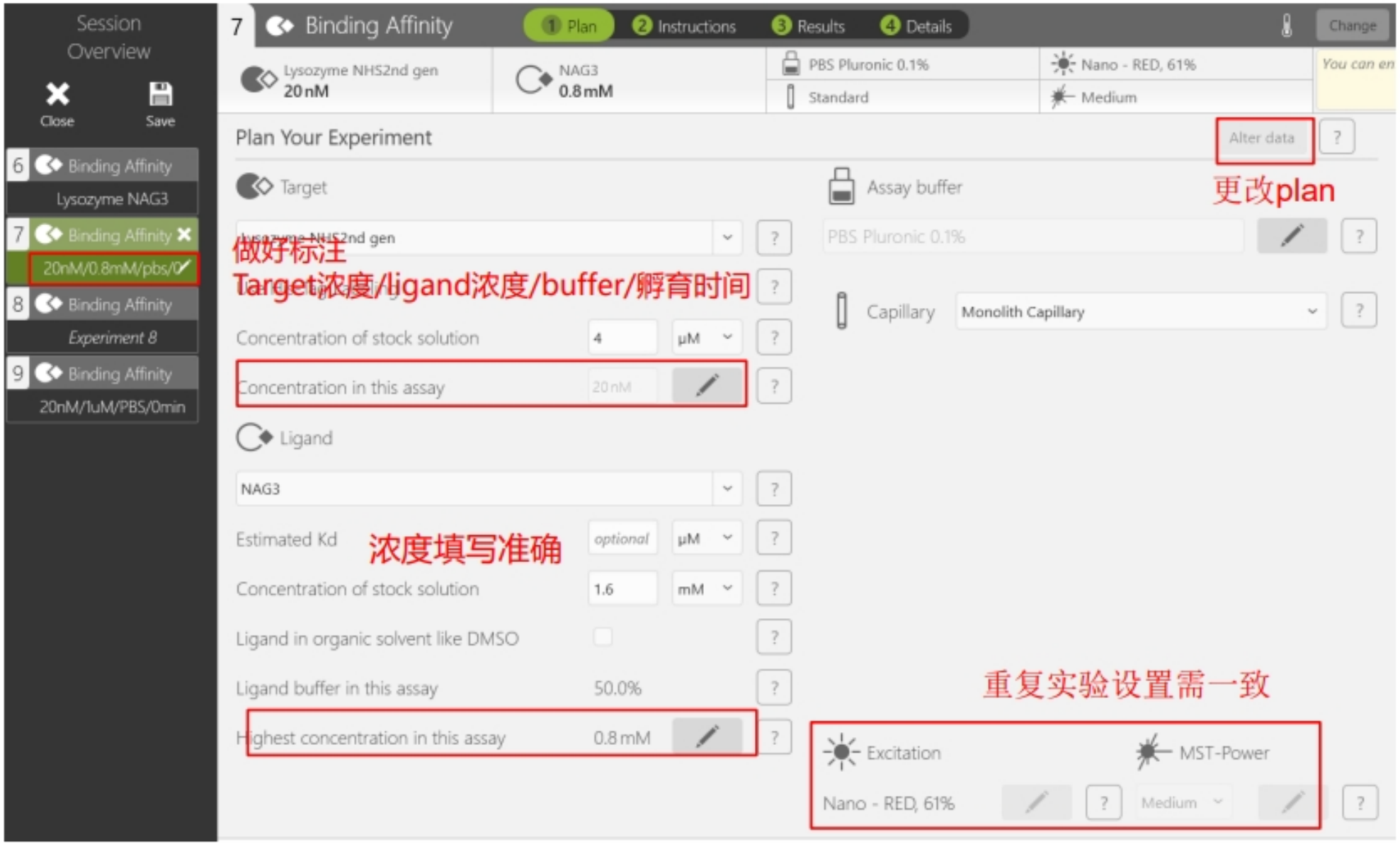
MO.Control

- 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

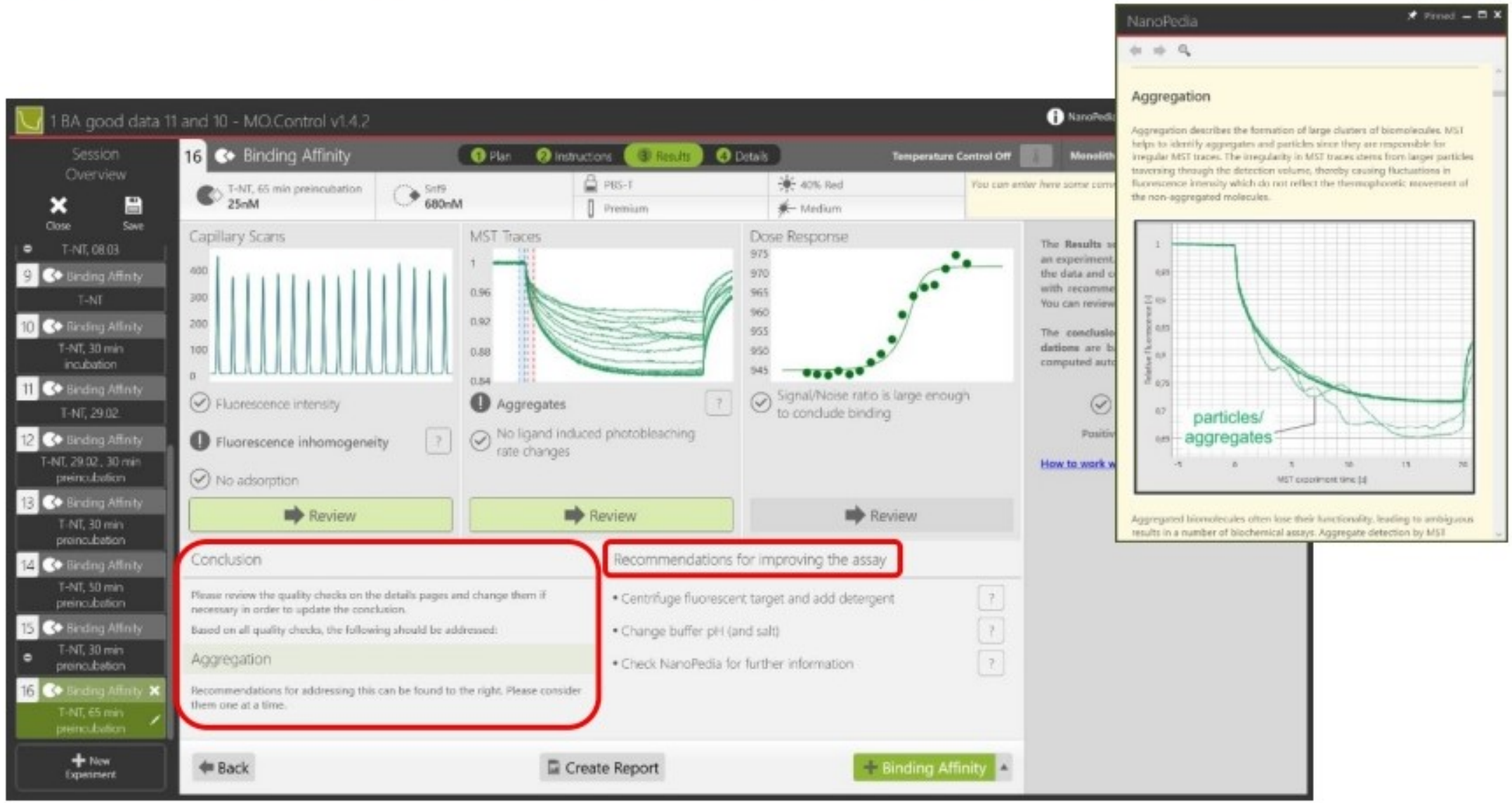
MO.Affinity Analysis

- 数据合并/对比分析
- 导出图片/数据

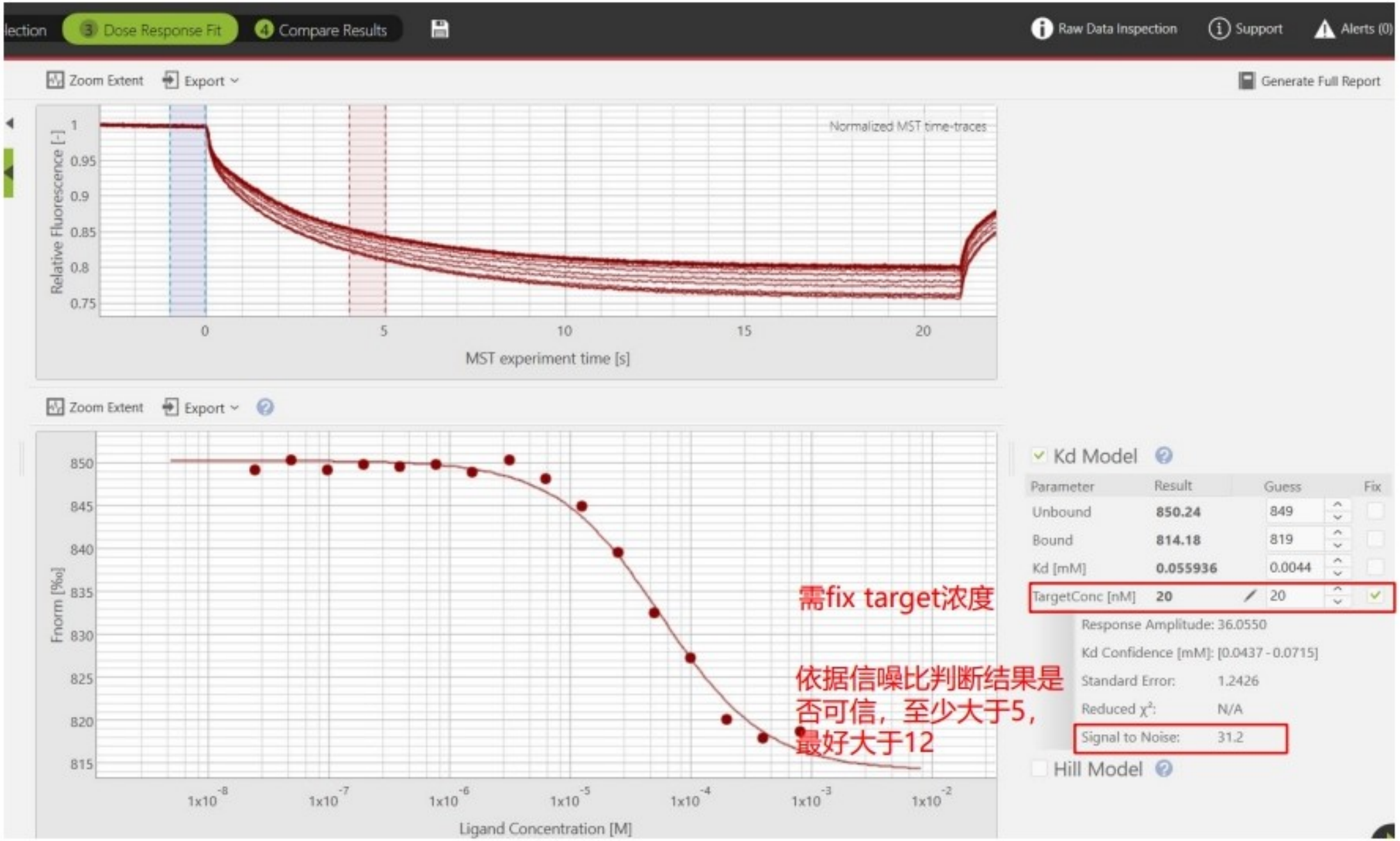
MO.Control 软件



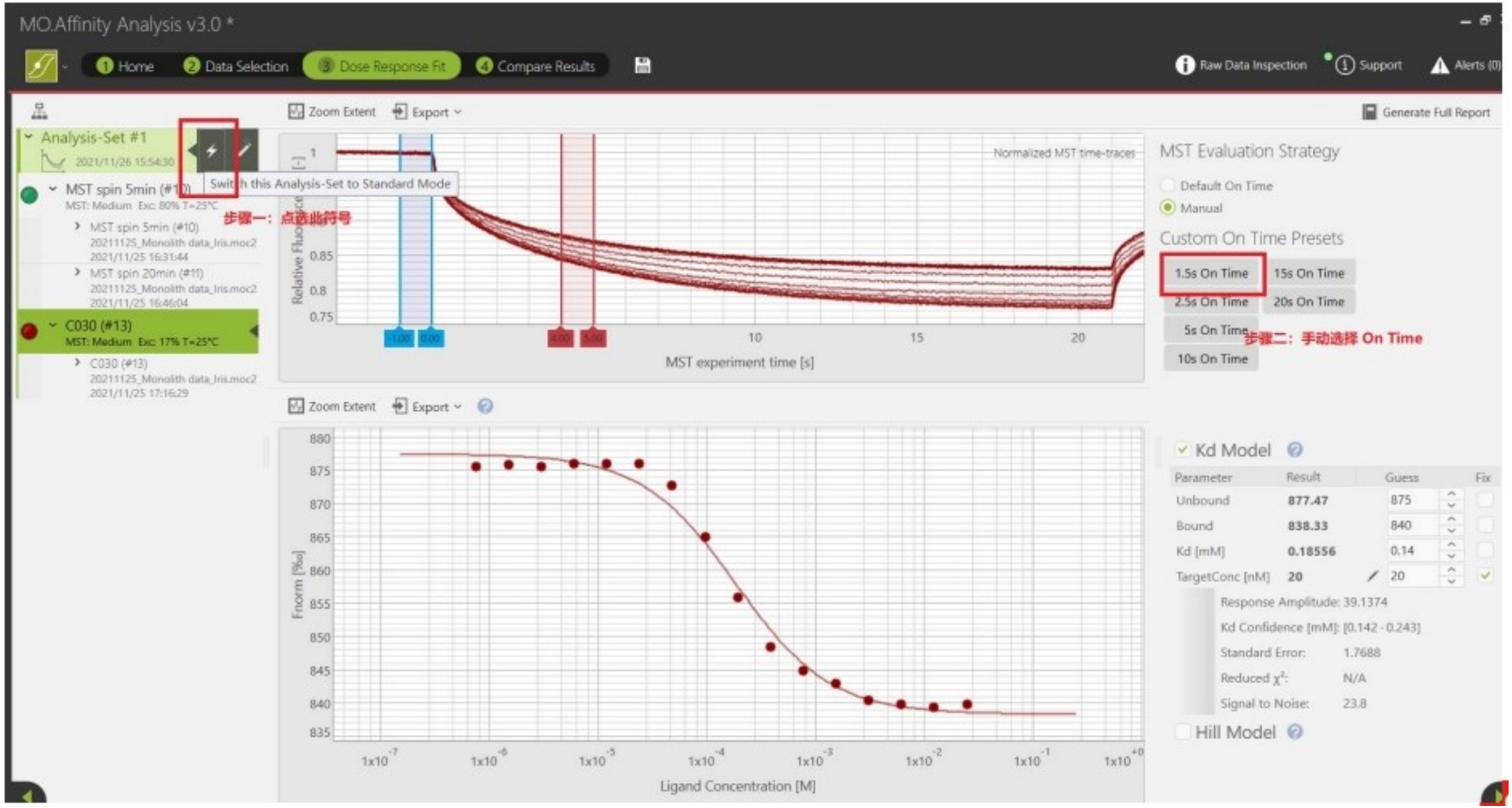
智能化的NanoPedia给您更清楚的结论和优化指南



MO. Affinity Analysis软件


lection
3 Dose Response Fit
4 Compare Results
Raw Data Inspection
Support
Alerts (0)
Zoom Extent
Export
Generate Full Report
Normalized MST time-traces
Relative Fluorescence [-]
1
0.95
0.9
0.85
0.8
0.75
0
5
10
15
20
MST experiment time [s]
Zoom Extent
Export
Fnorm [‰]
850
845
840
835
830
825
820
815
1x10-8
1x10-7
1x10-6
1x10-5
1x10-4
1x10-3
1x10-2
Ligand Concentration [M]
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
Bound
814.18
819
Kd [mM]
0.055936
0.0044
TargetConc [nM]
20
20
Response Amplitude: 36.0550
Kd Confidence [mM]: [0.0437 - 0.0715]
Standard Error: 1.2426
Reduced χ²: 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



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3	单笔订购10万9折，送套装(MO-X001)