

Biomolecular Interaction Analytics using MicroScale Thermophoresis

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concentration of **labeled molecule**: in the same range or lower than the expected K_d

highest concentration of **unlabeled molecule**: 20 fold above the expected K_d

final **sample volume** per titration point: ~ 20 μ l

use **small tubes** for the serial dilution: e.g. PCR strips, tubes

dilution buffer **should not vary in composition** in serial dilution: e.g. DMSO

accurate pipetting is essential

mix with pipette instead of vortexing

do not touch **capillaries in the center**

Monolith NT.LabelFree:

one binding partner contains tryptophans (excitation at 280 nm)

the other binding partner does not exhibit any fluorescence in this range

use only high purity samples (> 95 %)



LOD: depending on number of tryptophans

adjust LED to obtain 10,000 - 25,000 fluorescence counts

adjust the concentration to obtain a signal well above the background

(+ 2000 counts)

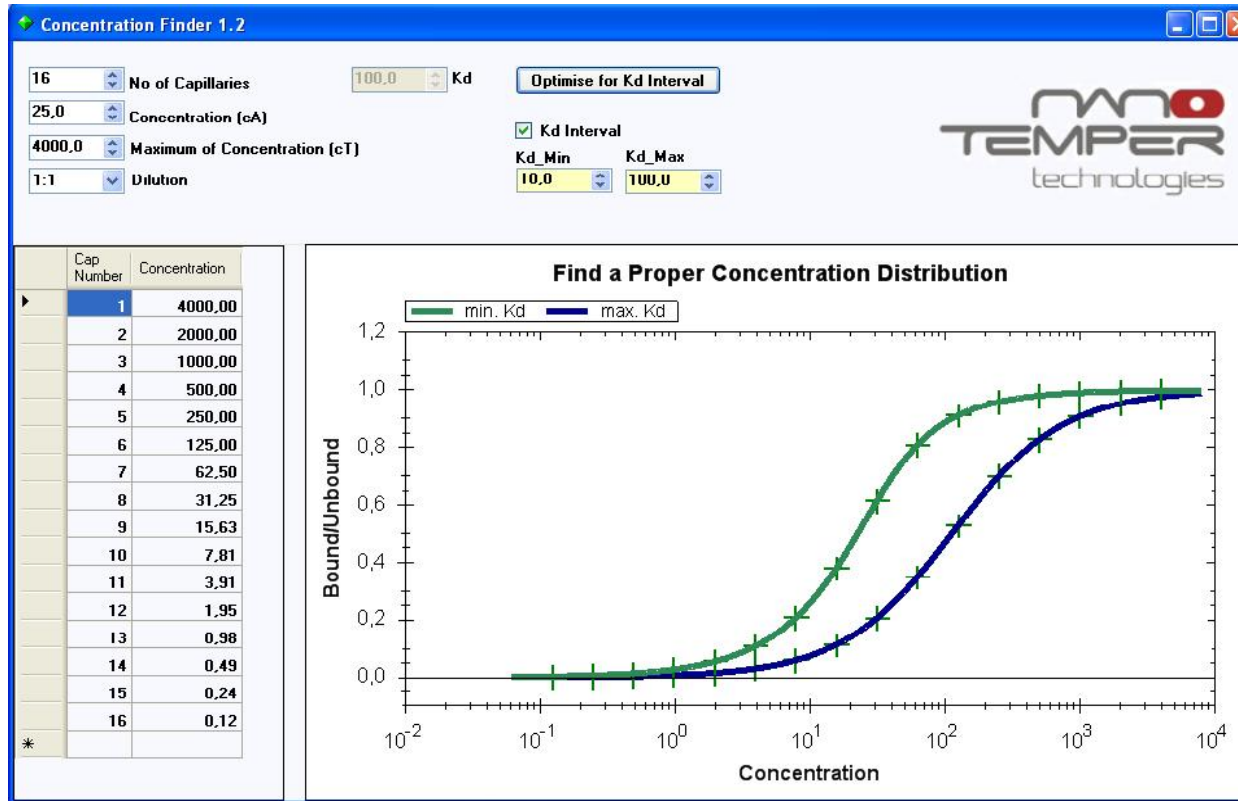
affinity range: 10 nM – mM

thermophoresis detected by the NT.LabelFree is very sensitive to

changes of the hydration shell



Concentration Finder Tool



Note: This tool can be accessed via NT.Control and NT.Analysis software or NanoTemper website

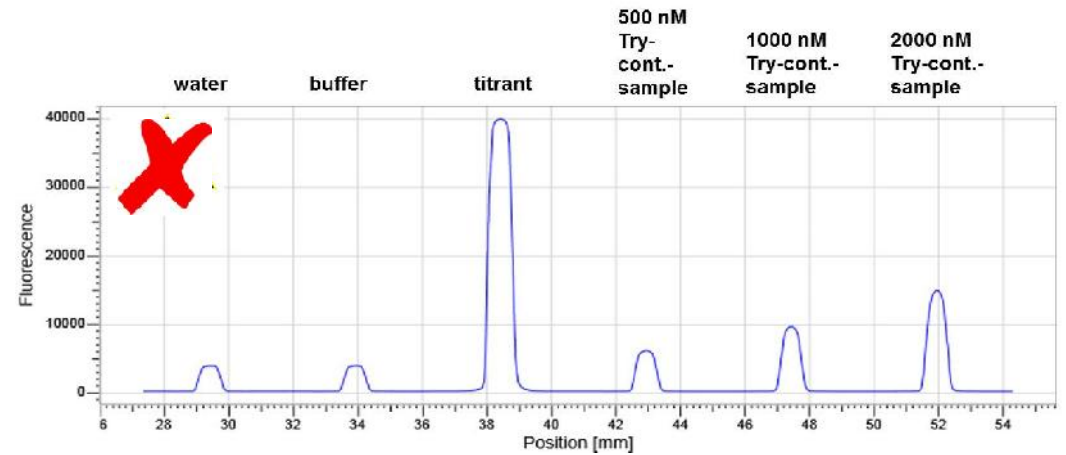
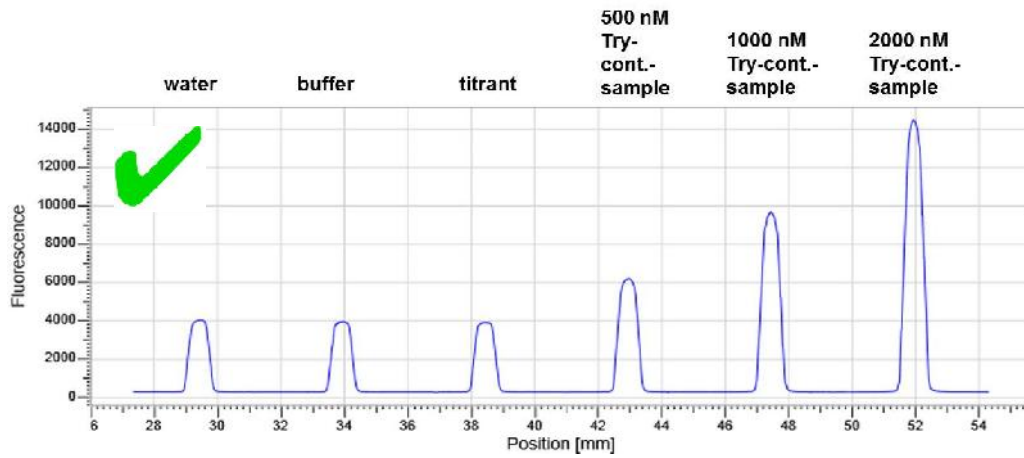
Obligatory Pre-Test

ddH₂O

buffer

highest concentration of titrant

3 different concentrations of tryptophan containing sample

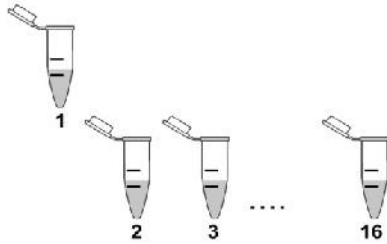


Preparing the Binding Reactions

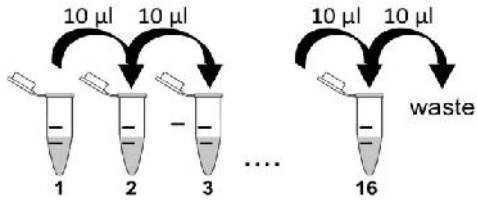
Prepare a serial titration of the interacting molecule of interest

Highest concentration of ligand (20 μ l).

Add 10 μ l buffer to 15 reaction tubes.

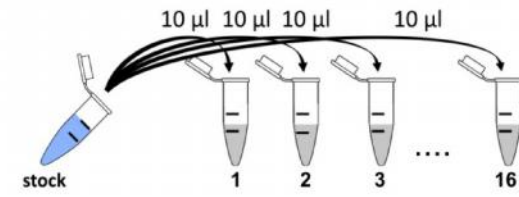


Prepare **dilution series** of unlabeled ligand: Transfer 10 μ l from vial 1 to vial 2, mix. Repeat this step for all vials.

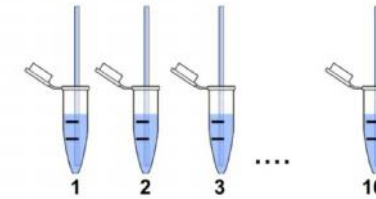


Add 10 μ l of tryptophan-containing molecule

Prepare stock solution of tryptophan-containing molecule (>160 μ l).
Add 10 μ l to each vial of the dilution series. Mix well.



Fill capillaries.



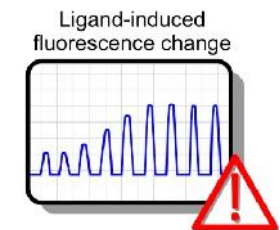
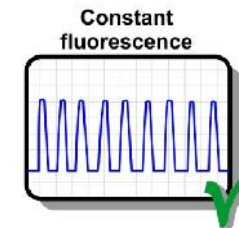
Load samples and start MST analysis



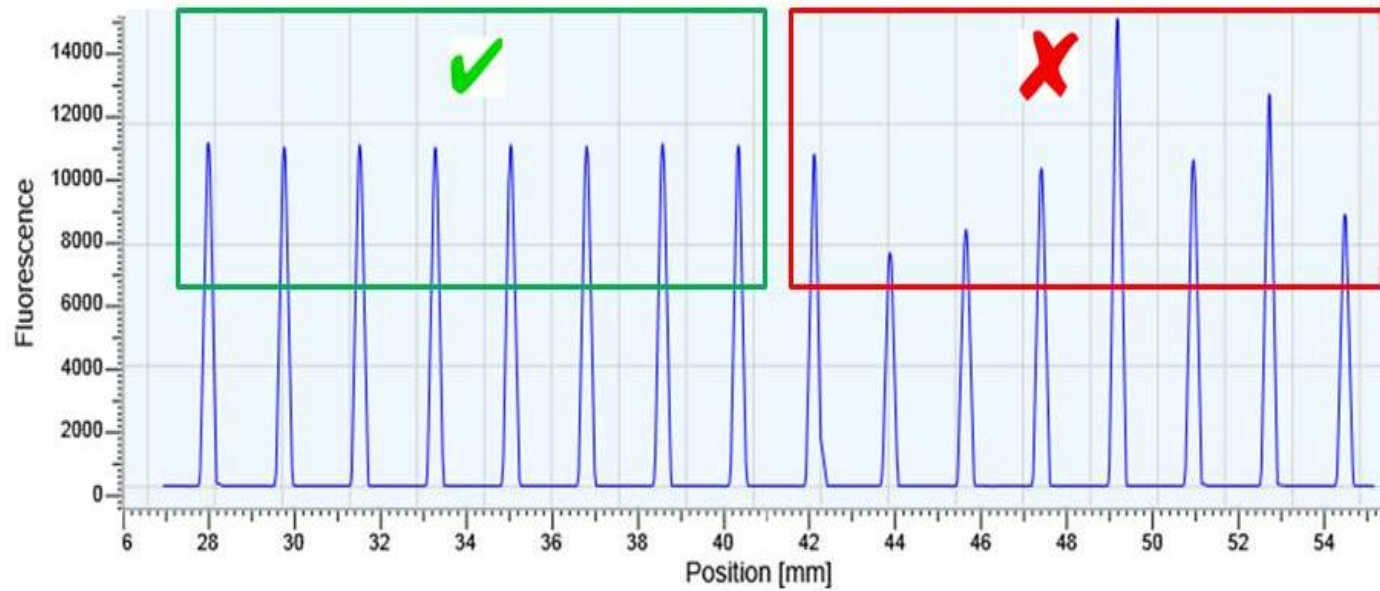
Constant fluorescence signal

Random fluorescence changes

Titrant-dependent fluorescence changes



Random Fluorescence Changes

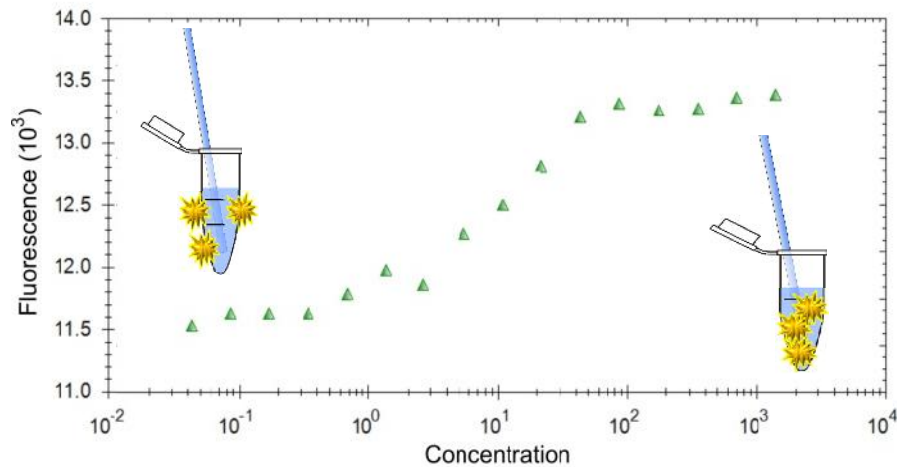
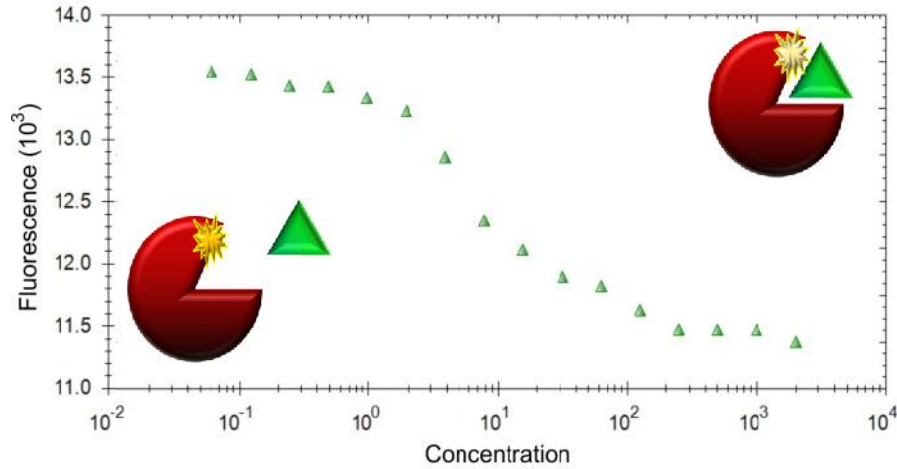


$\pm 10\%$

$\pm 10\%$

low sample quality
poor pipetting

Titrant Dependent Fluorescence Changes



quenching or enhancement of the fluorescence yield upon binding

unspecific adsorption to capillaries

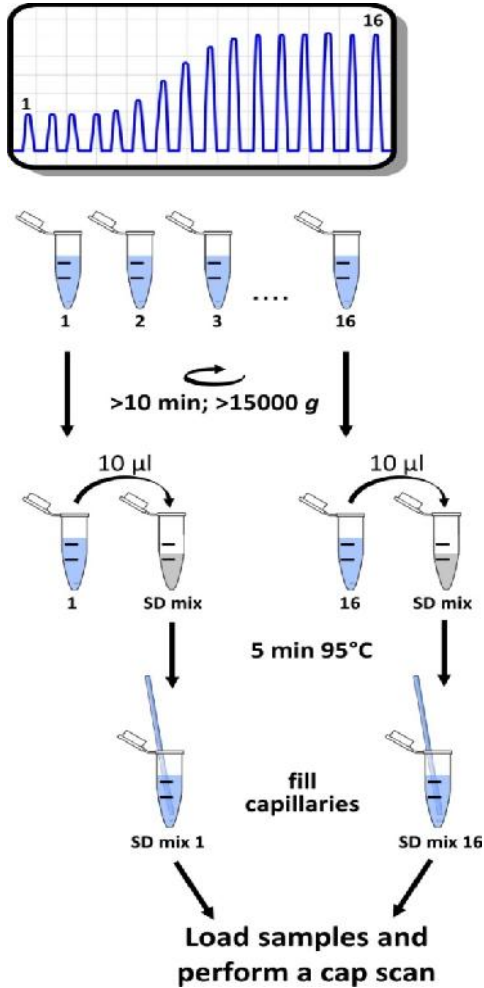
unspecific adsorption to tube walls

aggregation of the tryptophan containing molecule upon

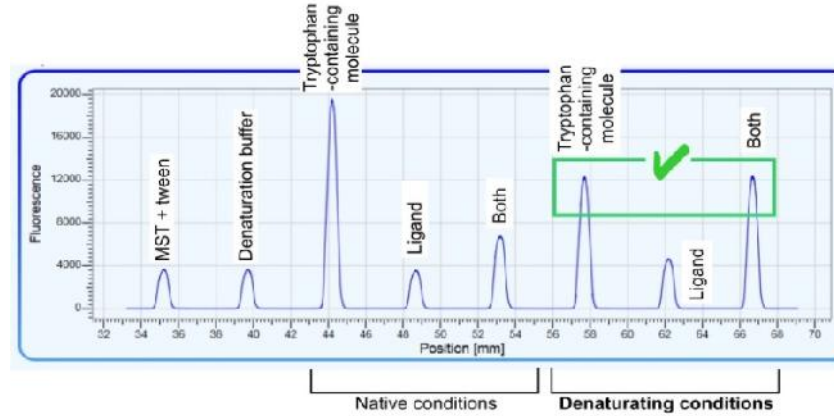
addition of the titrant

strong absorption of UV light by the ligand

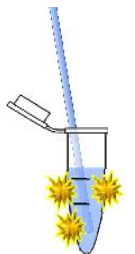
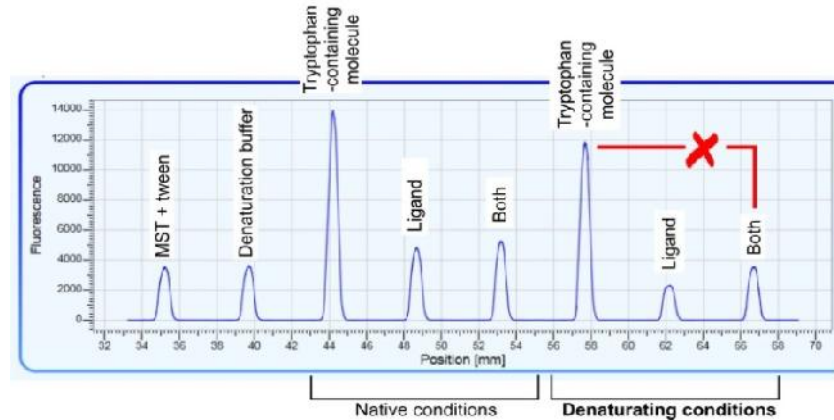
Binding or Material Loss? How to perform the SD-Test



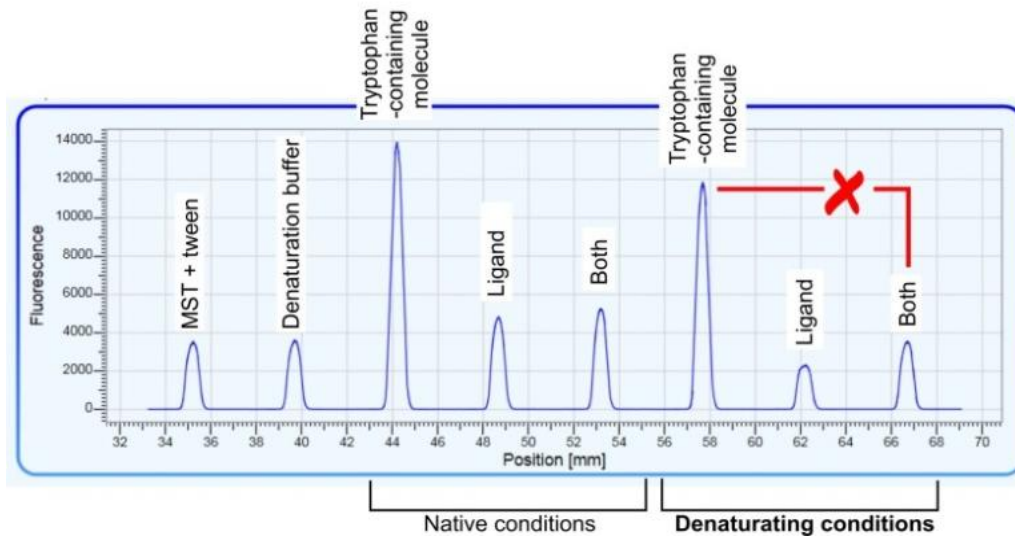
Fluorescence loss by ligand-induced **quenching**



Fluorescence loss by ligand-induced **aggregation/sticking**



Fluorescence loss by ligand-induced **aggregation/sticking**



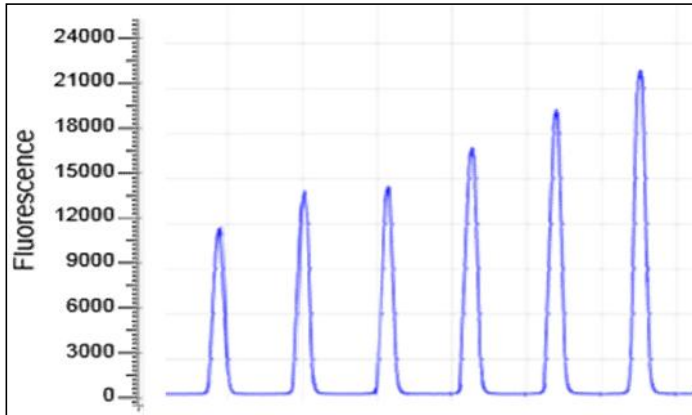
improve buffer conditions

by adding Tween-20 (or other detergents such as Pluronic-F127, Triton, NP-40...)

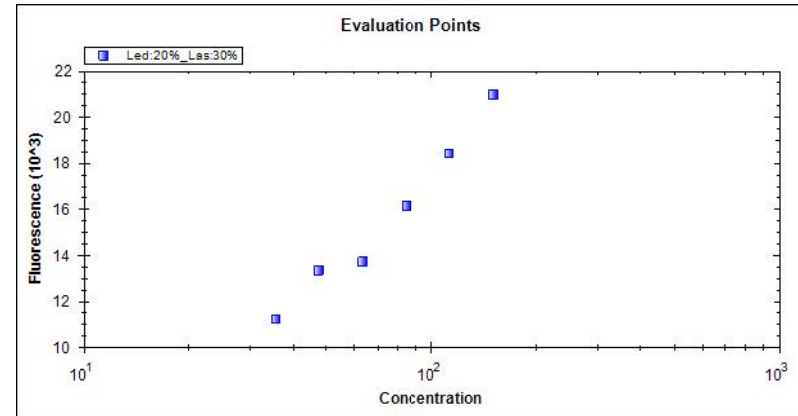
by changing pH

by changing ionic strength...

Changes in Fluorescence Intensities

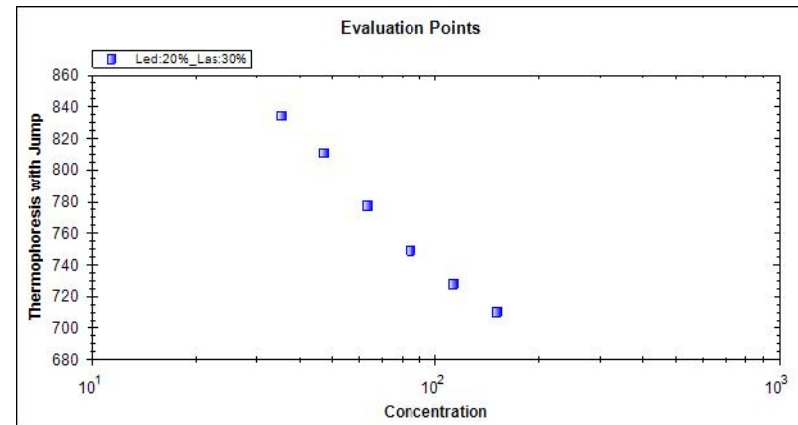


Fluorescence



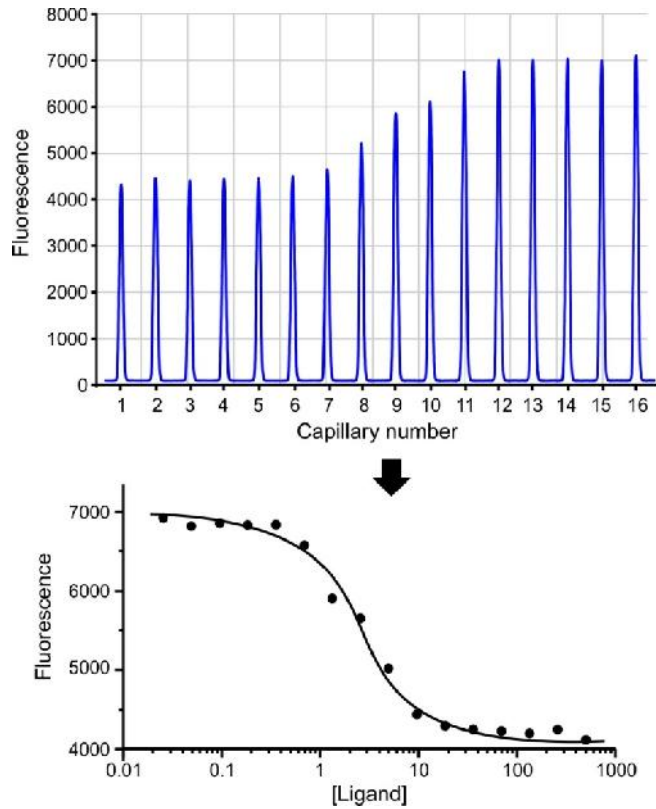
Please note: Changes in fluorescence intensities will result in a thermophoretic signal!

Thermophoresis

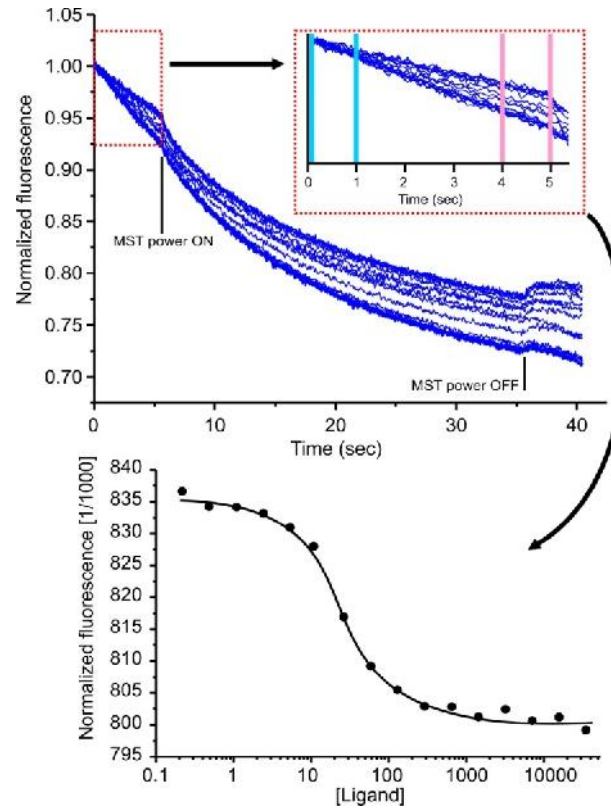


Selection of the Evaluation Mode

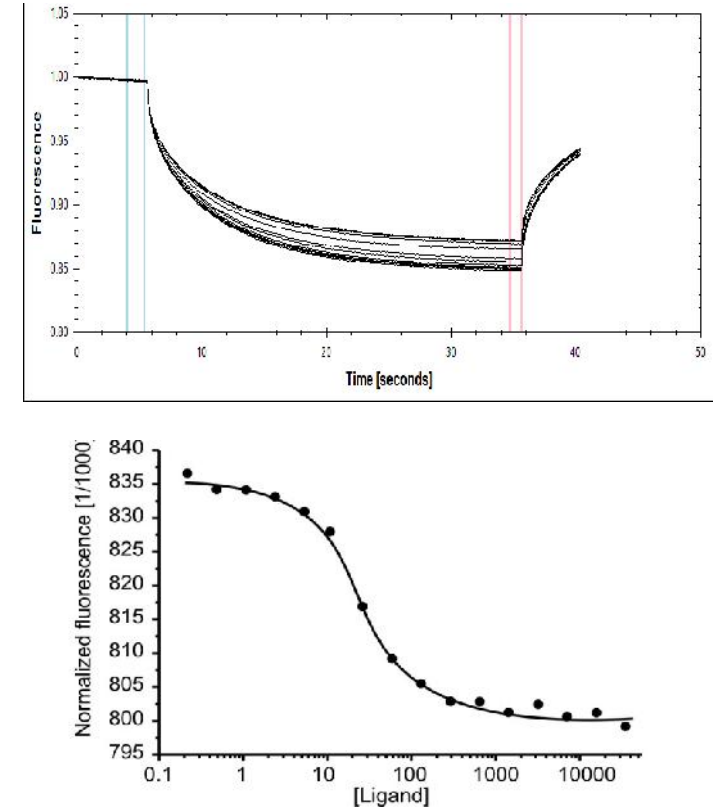
Fluorescence Changes



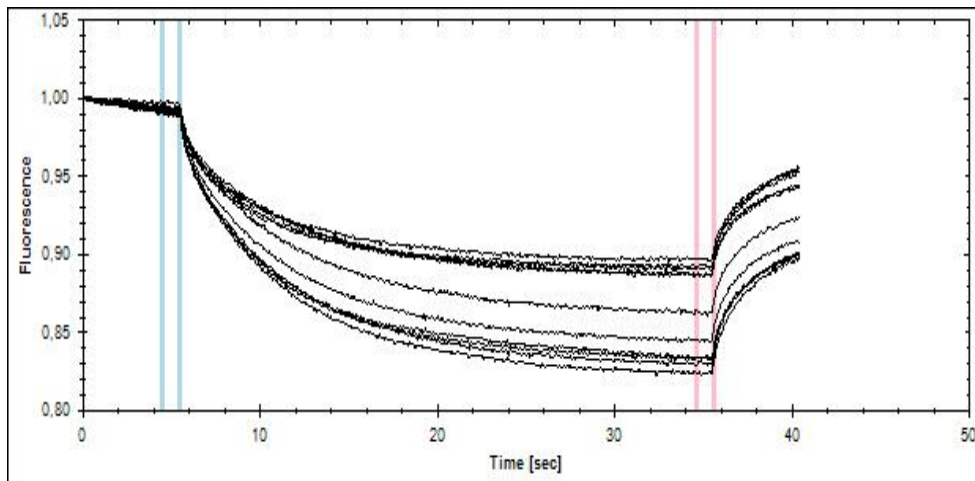
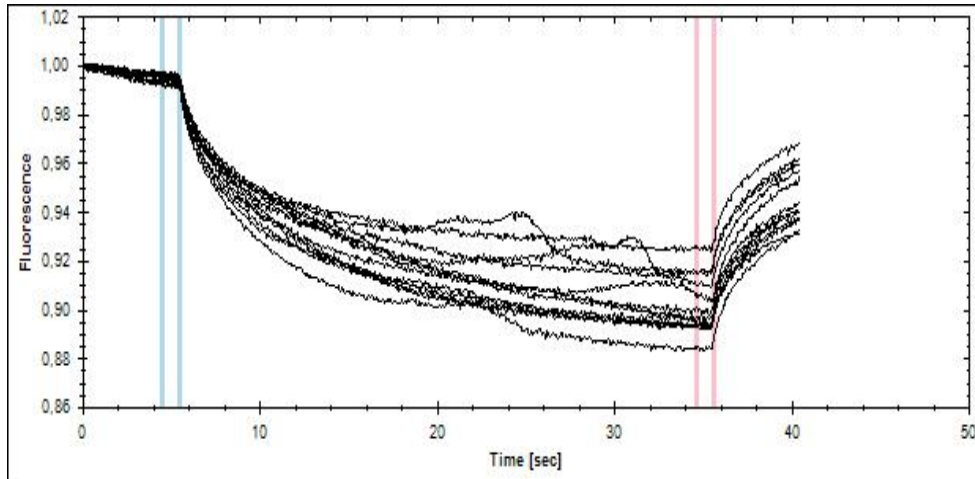
Photobleaching



MST



Improving Assay Conditions



spin down your sample

improve buffer conditions

by adding Tween-20 (or other

detergents such as Pluronic-F127,...)

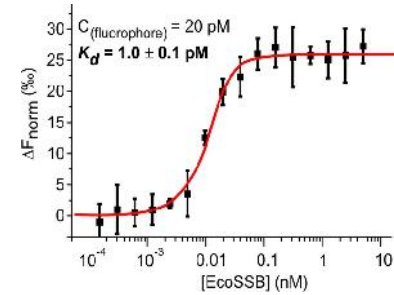
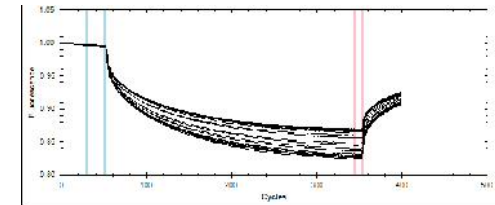
by changing pH

by changing ionic strength...

Running the MST Experiment: NT.Control Software

Analyzing the MST Data: NT.Analysis Software

MST Data Analysis



Running the MST Experiment

The screenshot shows a control panel with the following elements and annotations:

- Connection to Computer:** Points to the 'Ready' status.
- Laser/LED Status:** Points to the 'MST POWER OFF' and 'LED OFF' indicators.
- Load/Unload Sample Tray:** Points to the up and down arrow buttons.
- Current Tray Temperature:** Points to the 'Actual Temperature 26.7 °C'.
- Adjusted Tray Temperature:** Points to the 'Target Temperature * °C'.
- Temperature Control of Sample Tray Status: off:** Points to the 'Temperature Control OFF' indicator.

The control panel displays the following text:

Ready
MST POWER OFF
LED OFF
Temperature Control OFF
Actual Temperature 26.7 °C
Target Temperature * °C
Connected Version 1.0.8.8

Running the MST Experiment

MST parameter table

The screenshot shows the NT Control 2.0.2.29 software interface. Key components are labeled as follows:

- Menu bar:** File, Settings, Capillaries, Temperature Control, Tools, Expert
- LED filterset:** Green-Red Type, LED Color Red
- Defining capillary-number:** From Cap No 01, To Cap No 12
- Capillary scan:** LED Power (%) 20
- Start Measurement:** Start Cap Scan, Start CapScan + MST Measurement, Start MST Measurement
- MST window:** Graph showing Fluorescence vs Position
- Connection /running-Status:** Ready, Position 0.01 mm, Temperature 24.2°C, Temperature Control Off, LED Off, MST Power Off
- Setting temperature:** Manual Target Temp 27.0, Actual Temperature 24.2
- Defining ligand concentration:** Table of Capillaries

Table of Runs (MST parameter table):

No	LED Power [%]	MST Power [%]	Fluo. Before [s]	MST On [s]	Fluo. After [s]	Delay [s]	Name
01	20	20	5	30	5	25	
02	20	40	5	30	5	25	

Table of Capillaries:

No	Concentration	Position	Name	Used
01	5000.0000		uM AMP	<input type="checkbox"/>
02	2500.0000		uM AMP	<input type="checkbox"/>
03	1250.0000		uM AMP	<input type="checkbox"/>
04	625.0000		uM AMP	<input type="checkbox"/>
05	312.5000		uM AMP	<input type="checkbox"/>
06	156.2500		uM AMP	<input type="checkbox"/>
07	78.1250		uM AMP	<input type="checkbox"/>
08	39.0625		uM AMP	<input type="checkbox"/>
09	19.5313		uM AMP	<input type="checkbox"/>
10	9.7656		uM AMP	<input type="checkbox"/>
11	4.8828		uM AMP	<input type="checkbox"/>
12	2.4414		uM AMP	<input type="checkbox"/>
13				<input type="checkbox"/>
14				<input type="checkbox"/>
15				<input type="checkbox"/>
16				<input type="checkbox"/>

Capillary Scan

NT Control 2.0.2.29

File Settings Capillaries Temperature Control Tools Expert

LED Settings

Green-Rec Type
LED Color Red

Capillary Scanning

From Cap No 01
To Cap Nu 12
LED Power [%] 10

Start Cap Scan Start CapScan + MST Measurement

Start MST Measurement

Table of Runs

No	LED Power [%]	MST Power [%]	Fluo. Before [s]	MST On [s]	Fluo. After [s]	Delay [s]	Name
01	10	20	5	30	5	25	

Temperature Loop

No	Temp. [°C]	Delay [s]

Manual Temperature Control

Manual Target Temp ***
On/Off Actual Temperature 26.9

Stop

Connected ✓

Actual Experiment's Name Aptamer vs AMP
Project File: C:\Users\nanotemper\Desktop\Aptamer130207.ntp
Concentration Fluor. Mol. ***

Dilution 1:1

Graphic

Ready
Position 0.00 mm Temperature 26.9°C Temperature Control Off LED Off MST Power Off

Table of Capillaries

No	Concentration	Position	Name	Used
01	5000.0000	23.33	uM AMP	<input checked="" type="checkbox"/>
02	2500.0000	27.82	uM AMP	<input checked="" type="checkbox"/>
03	1250.0000	32.32	uM AMP	<input checked="" type="checkbox"/>
04	625.0000	36.82	uM AMP	<input checked="" type="checkbox"/>
05	312.5000	41.32	uM AMP	<input checked="" type="checkbox"/>
06	156.2500	45.82	uM AMP	<input checked="" type="checkbox"/>
07	78.1250	50.32	uM AMP	<input checked="" type="checkbox"/>
08	39.0625	54.84	uM AMP	<input checked="" type="checkbox"/>
09	19.5313	59.33	uM AMP	<input checked="" type="checkbox"/>
10	9.7656	63.83	uM AMP	<input checked="" type="checkbox"/>
11	4.8828	68.34	uM AMP	<input checked="" type="checkbox"/>
12	2.4414	72.80	uM AMP	<input checked="" type="checkbox"/>
13				<input type="checkbox"/>
14				<input type="checkbox"/>
15				<input type="checkbox"/>
16				<input type="checkbox"/>

Parameter Setup and Running the Measurement

The screenshot displays the NT Control 2.0.2.29 software interface. The main window is titled "NT Control 2.0.2.29" and has a menu bar with "File", "Settings", "Capillaries", "Temperature Control", "Tools", and "Expert".

LED Settings: Type is "Green-Red", LED Color is "Red".

Capillary Scanning: From Cap No. is "01", To Cap No. is "12", LED Power [%] is "10".

Table of Runs: A table with columns: No, LED Power [%], MST Power [%], Fluor. Before [s], MST On [s], Fluor. After [s], Delay [s], Name. Row 01 is highlighted with values: 01, 10, 20, 5, 30, 5, 25.

Temperature Loop: A table with columns: No, Temp. [°C], Delay [s].

Manual Temperature Control: Manual Target Temp is "***", Actual Temperature is "26.9".

Buttons: Start Cap Scan, Start CapScan + MST Measurement, Start MST Measurement, Stop.

Actual Experiment's Name: Aptamer vs AMP

Project File: C:\Users\nanotemper\Desktop\Aptamer190207.ntp

Concentration Fluor. Mcl.: ***

Graph: A line graph showing Fluorescence vs Time [sec]. The y-axis ranges from 720 to 820, and the x-axis ranges from 0 to 42. The curve shows a sharp drop in fluorescence at approximately 5 seconds, followed by a gradual decrease to a baseline around 35 seconds, and then a slight rise.

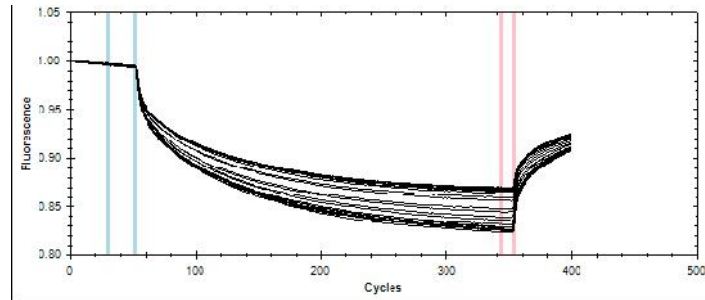
Table of Capillaries: A table with columns: No, Concentration, Position, Name, Used. Row 05 is highlighted with values: 05, 156.2500, 45.02, uM AMP, [checked].

Status: Connected ✓

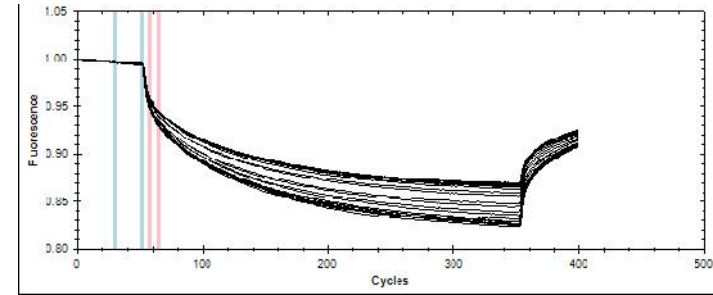
Dilution: 1:1

Footer: Thermoprecis Measurement - Hw Initialization
Position 47.54 mm | Temperature 26.9°C | Led Power 10% | MST Power 20.0% | Temperature Control Off | LED On | MST Power Off

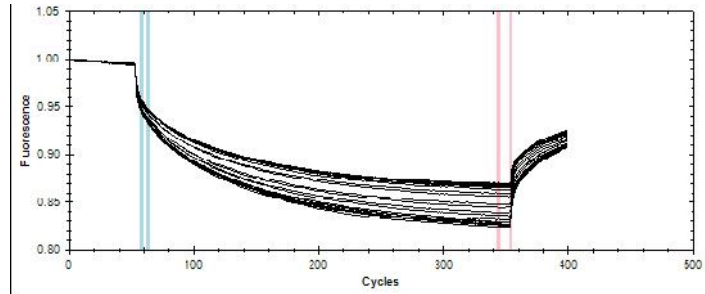
Thermophoresis with MST T-Jump



Only MST T-Jump



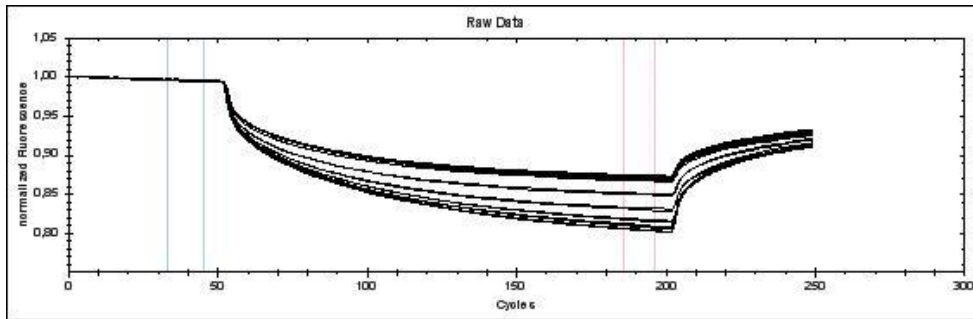
Only Thermophoresis



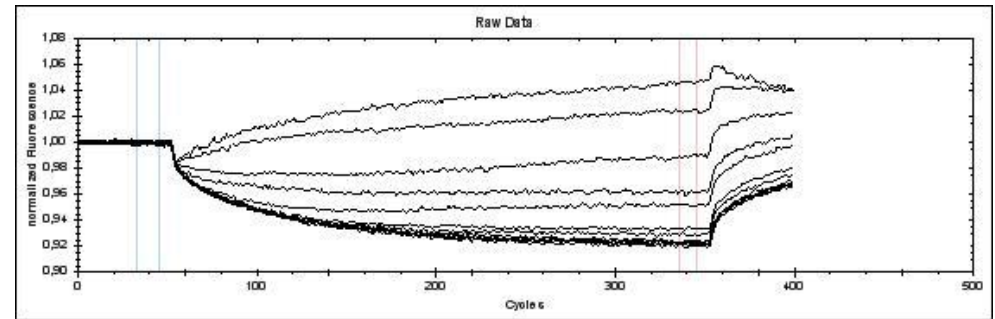
Information from T-Jump:
binding close to fluorophore / tryptophane

Information from Thermophoresis:
overall structure

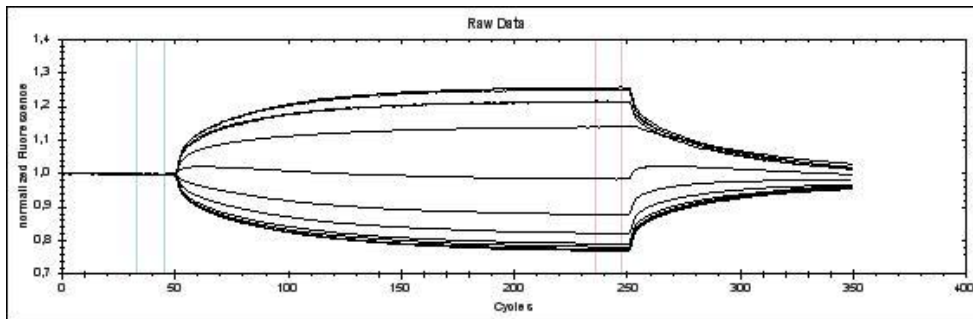
T-Jump (+) and Thermophoresis (+)



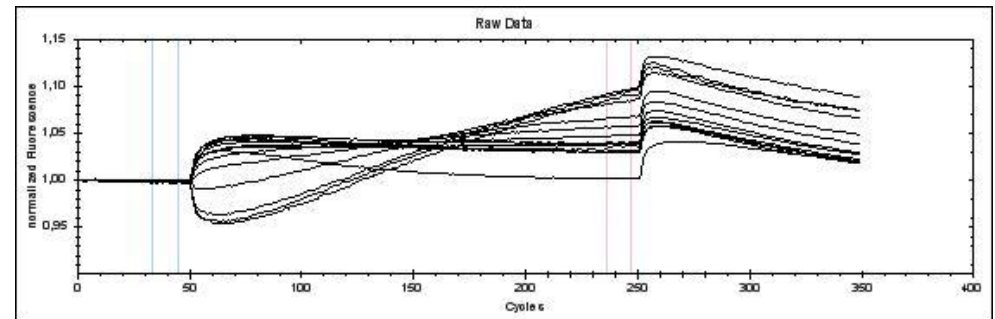
T-Jump (+) and Thermophoresis (+/-)



T-Jump (+/-) and Thermophoresis (+/-)

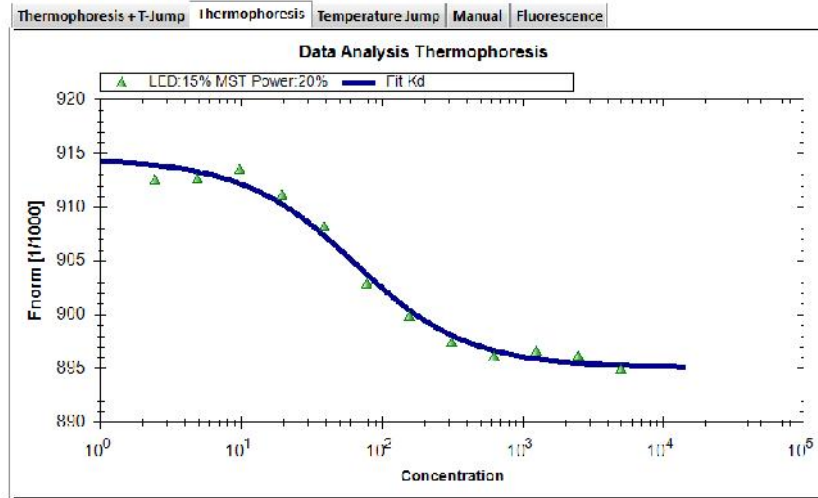


T-Jump (+/-) and Thermophoresis (+/-)



K_d Curve vs. Hill Curve Fitting

K_d Curve Fitting:

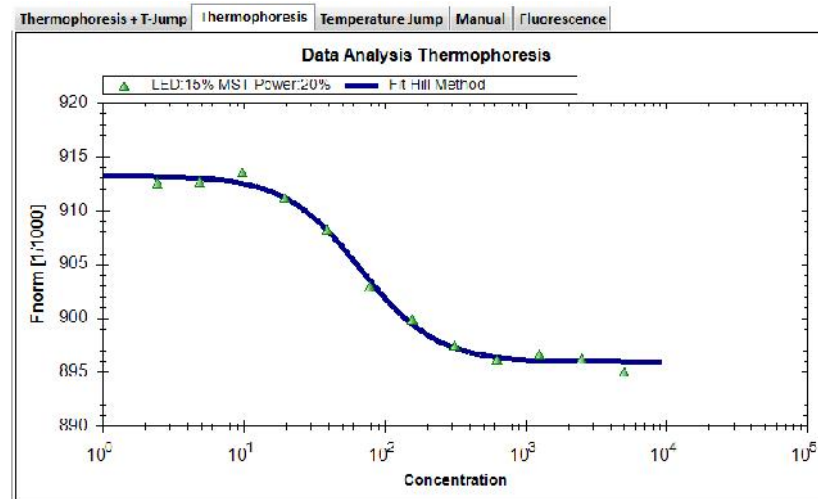


Kd Formula (law of mass action):
 $f(c) = \text{unbound} + (\text{bound} - \text{unbound}) / (1 + \sqrt{(\text{FluoConc} + c + Kd - \sqrt{(\text{FluoConc} + c + Kd)^2 - 4 * \text{FluoConc} * c})}$

Kd Fit	Hill Method	Unbound	Bound
54	20	914.55	895.10
<input type="checkbox"/> Fix	<input checked="" type="checkbox"/> Fix	<input type="checkbox"/> Fix	<input type="checkbox"/> Fix

Fit

Hill Curve Fitting:

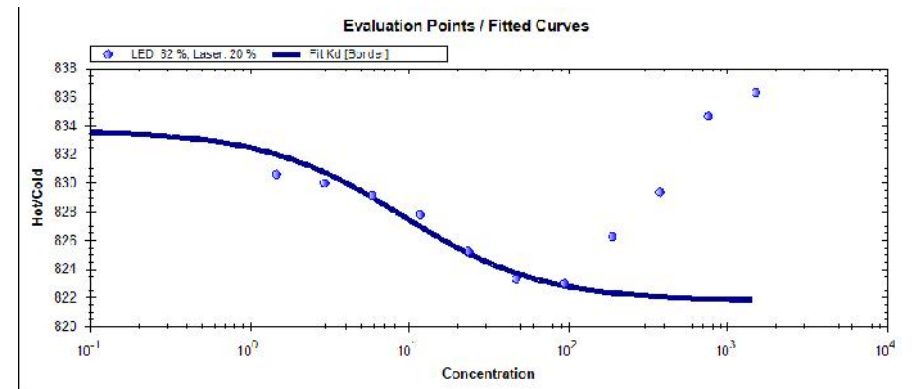
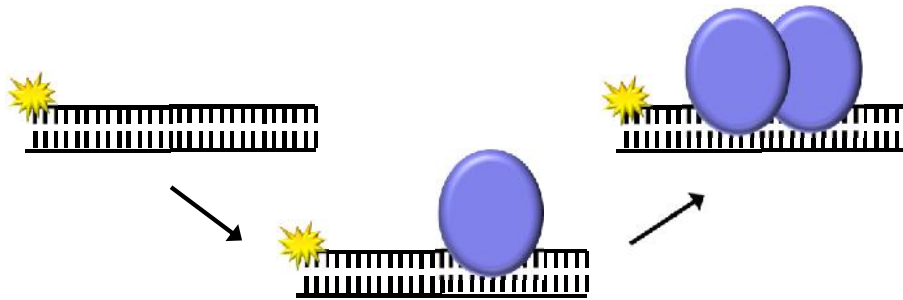
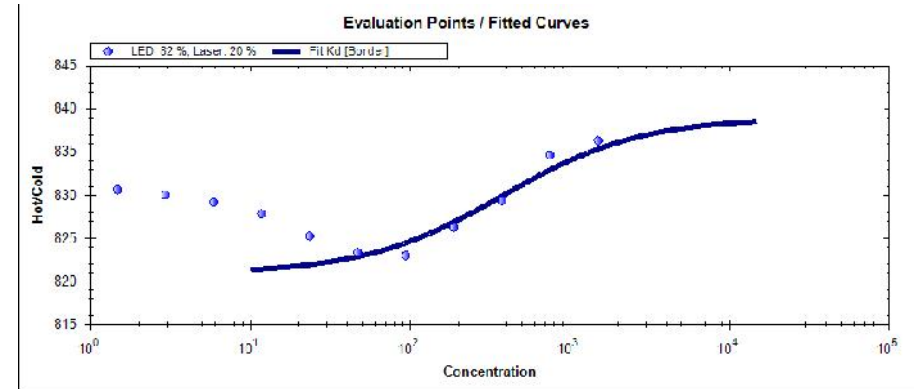
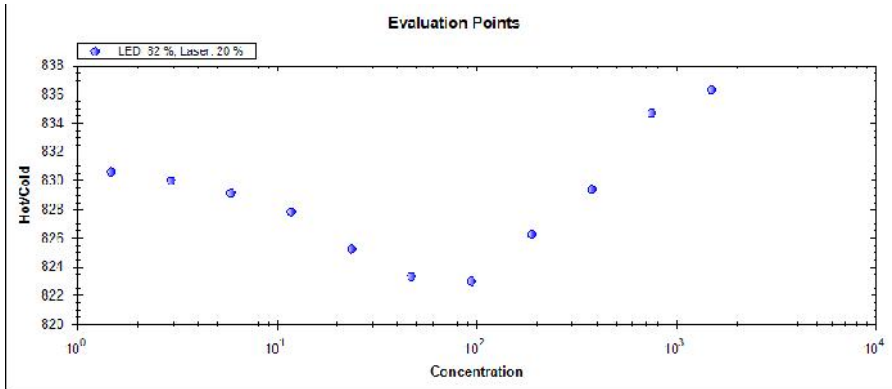


Hill Formula:
 $f(c) = \text{unbound} + (\text{bound} - \text{unbound}) / (1 + (\text{EC50}/c)^n)$

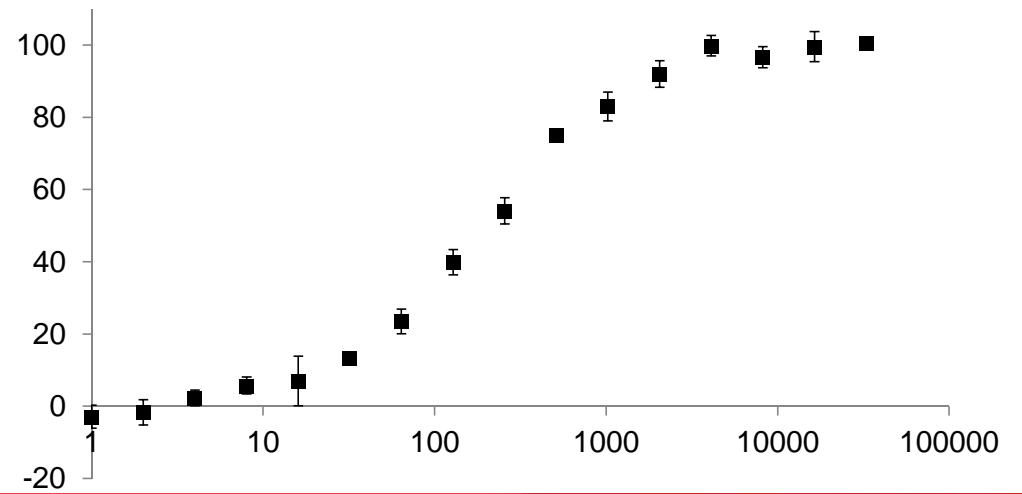
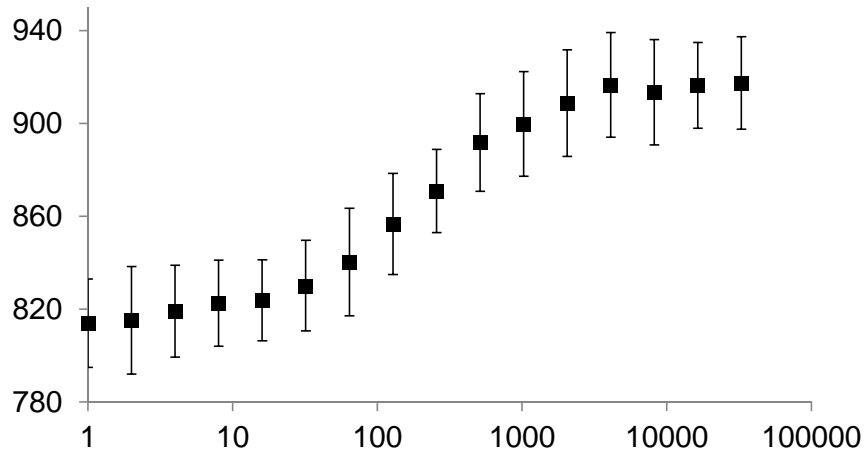
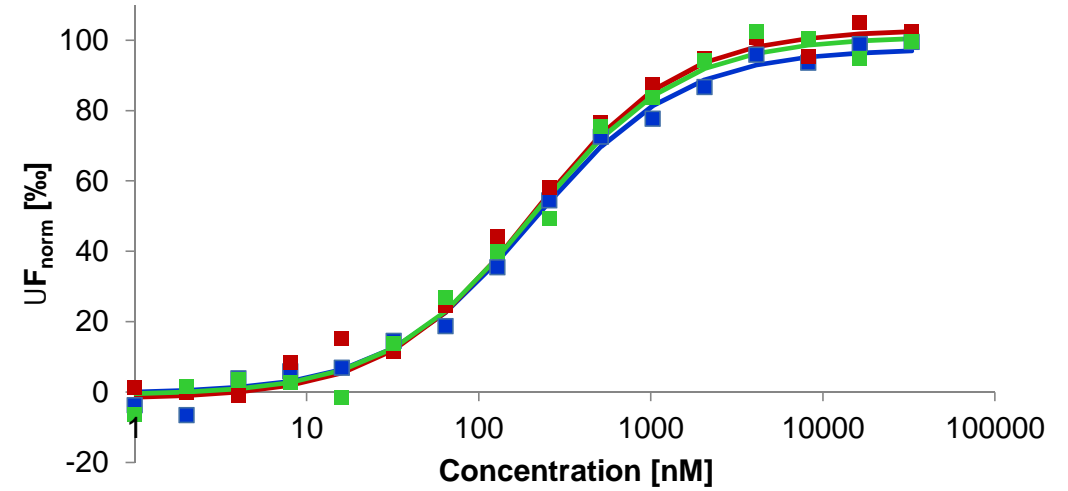
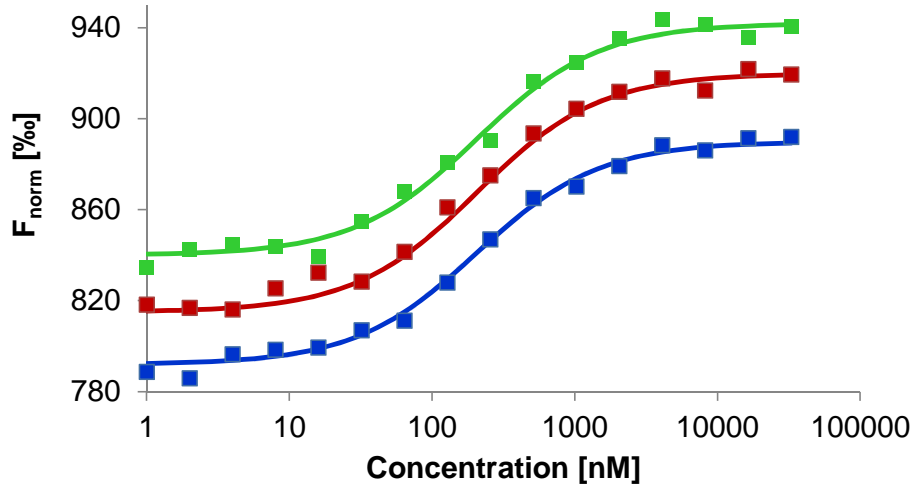
Kd Fit	Hill Method	Unbound	Bound
67.1	1.62	913.77	895.95
<input type="checkbox"/> Fix	<input checked="" type="checkbox"/> Fix	<input type="checkbox"/> Fix	<input type="checkbox"/> Fix

Fit

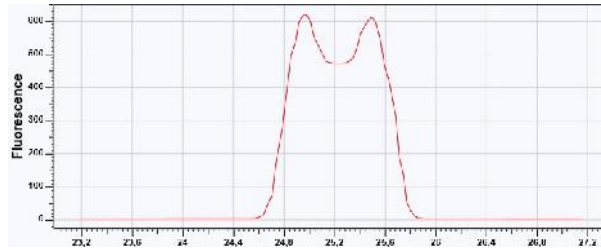
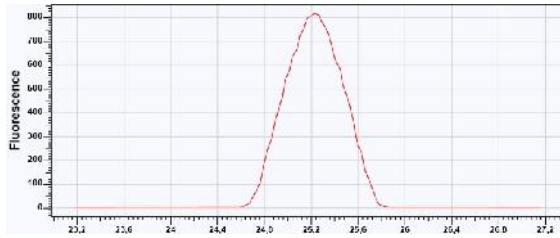
Two Binding Affinities



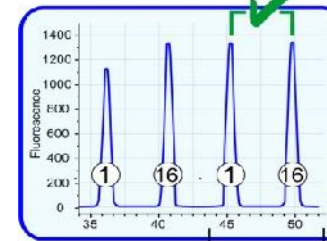
Multiple Measurements – Baseline correction



Assay Optimization – Summary



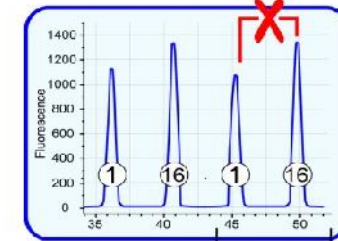
Specific, ligand-induced fluorescence change



Denaturing conditions

Use fluorescence change of K_d -determination.

Fluorescence change due to loss of fluorescent molecule



Denaturing conditions

Assay optimization.

easy and straight forward assay optimization

Thank You For Your Attention!



Please feel free to contact us:

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