Biomolecular Interaction Analytics using MicroScale Thermophoresis

NanoTemper Technologies GmbH
Flößergasse 4, 81369 München, Germany

www.nanotemper-technologies.com

General Remarks on Sample Handling

- 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 µl
- 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**

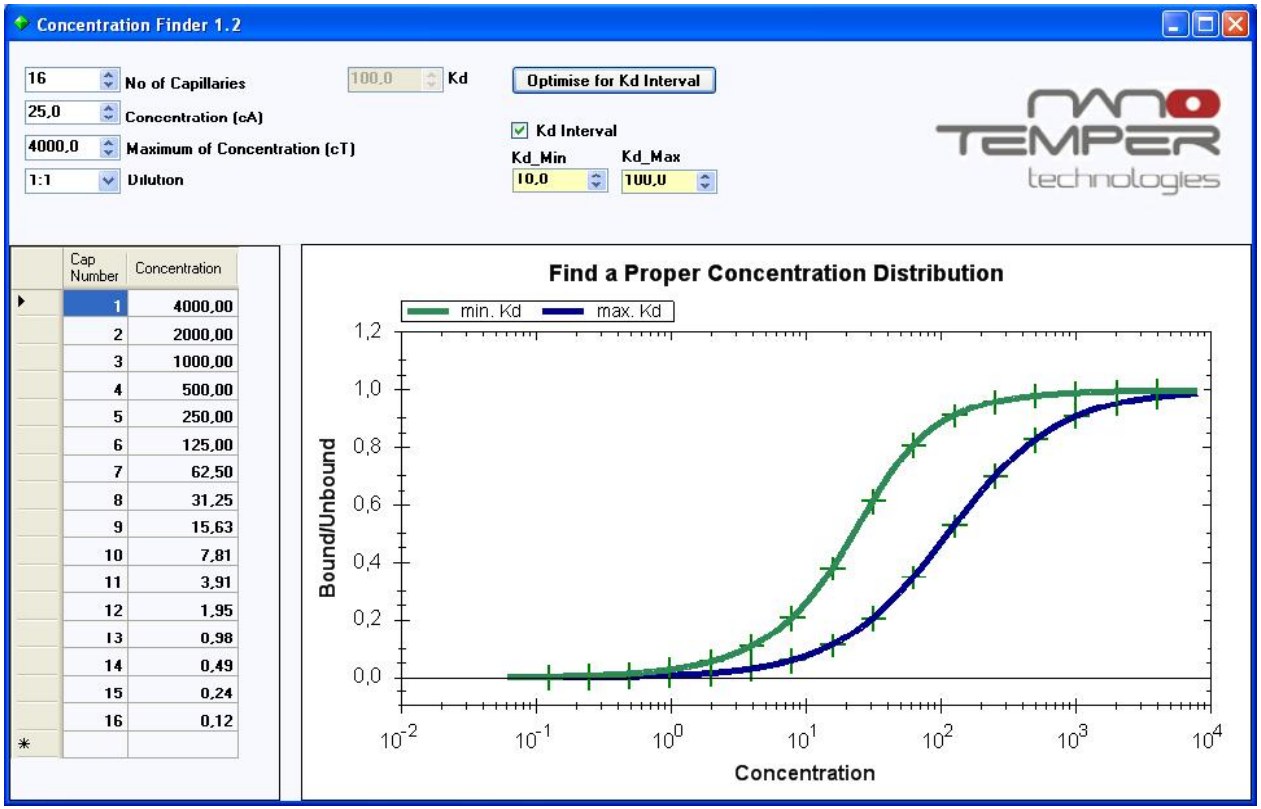
Monolith NT.LabelFree:

- one binding partner contains tryptophans (exitation at 280 nm)
- the other binding partner does not exhibit any fluorescence in this range
- use only high purity samples (> 95 %)

General Remarks for the Monolith NT.LabelFree

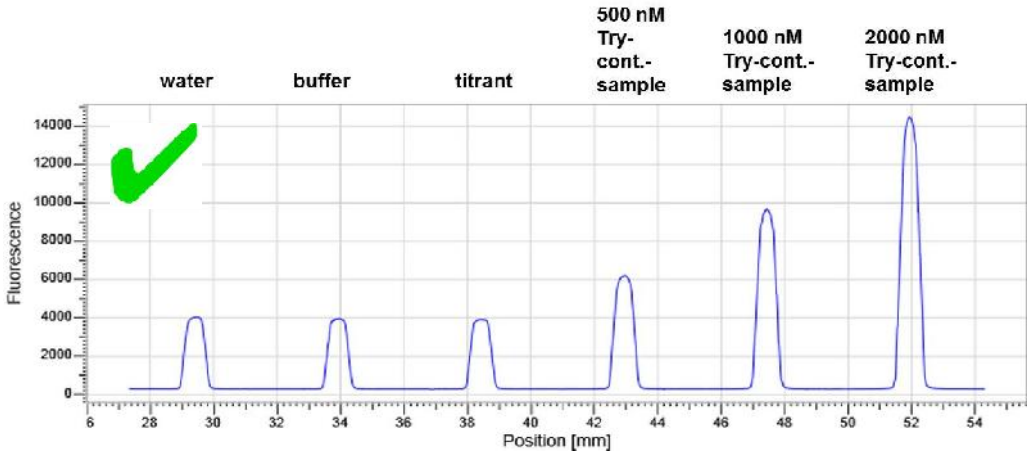
- LOD: depending on number of tryptophans
- adjust LED to obtain 10,000 - 25,000 fluorescence counts
- adjust the concentration to obtain a signal well above the background (+ 2000 counts)
- affinity range: 10 nM – mM
- thermophoresis detected by the NT.LabelFree is very sensitive to changes of the hydration shell

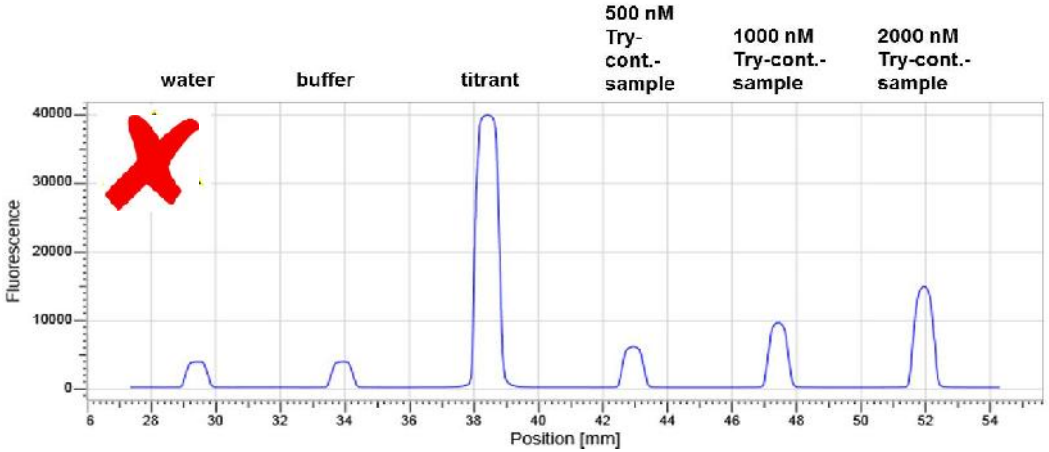
Concentration Finder Tool



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

- ddH$_2$O
- buffer
- highest concentration of titrant
- 3 different concentrations of tryptophan containing sample





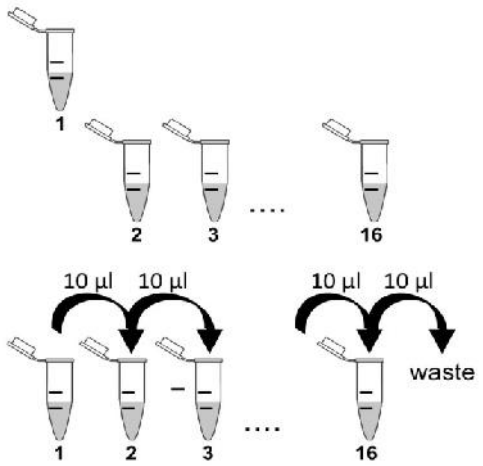
Preparing the Binding Reactions

Prepare a serial titration of the interacting molecule of interest

Highest concentration of ligand (20 µl).

Add 10 µl buffer to 15 reaction tubes.

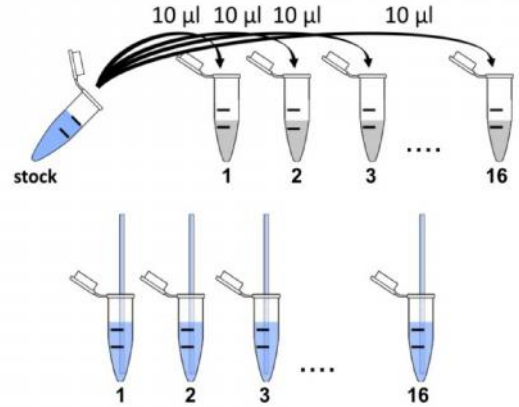
Prepare **dilution series** of unlabeled ligand: Transfer 10 µl from vial 1 to vial 2, mix. Repeat this step for all vials.



Add 10 µl of tryptophan-containing molecule

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

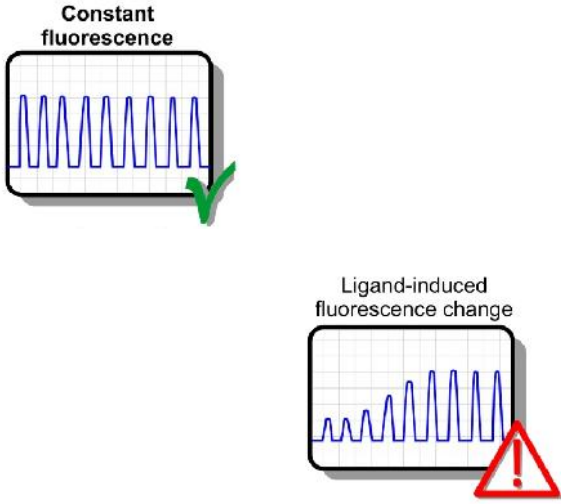
Fill capillaries.



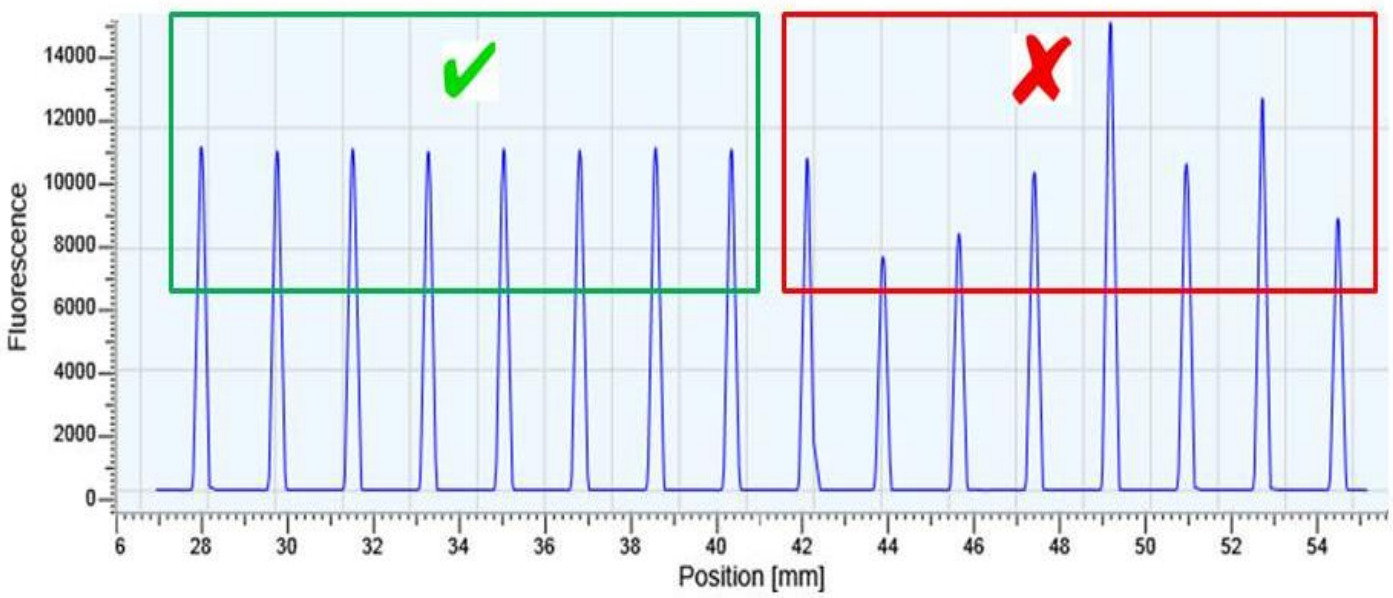
Load samples and start MST analysis

Fluorescence Signal

- Constant fluorescence signal
- Random fluorescence changes
- Titrant-dependent fluorescence changes



Random Fluorescence Changes

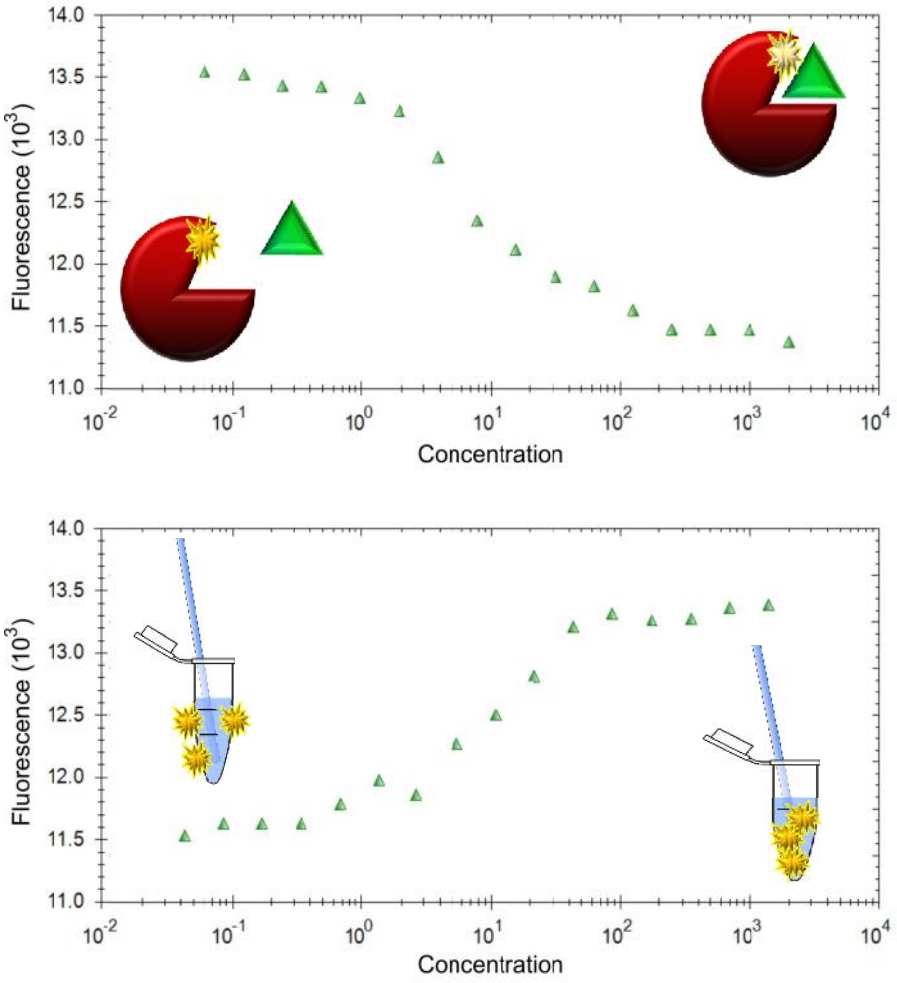


≤ ± 10 %

> ± 10 %

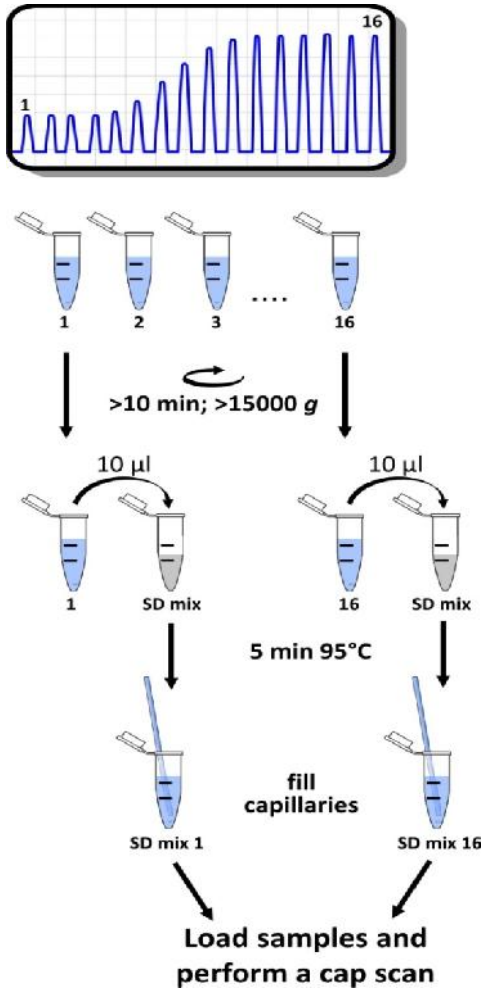
- low sample quality
- poor pipetting

Titrant Dependent Fluorescence Changes

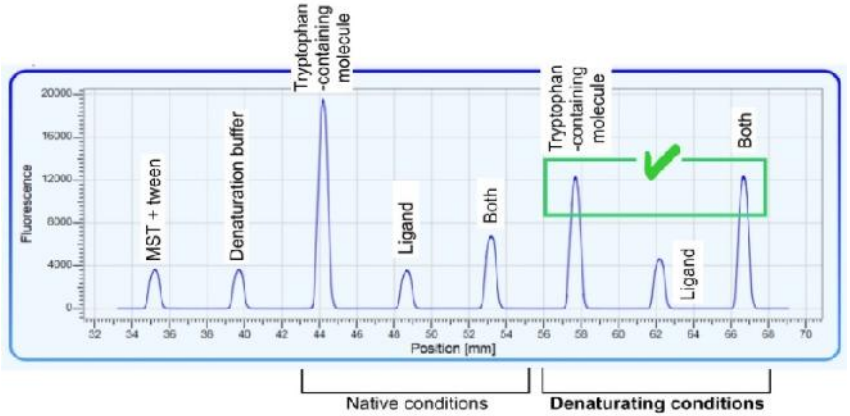


- quenching or enhancement of the fluorescence yield upon binding
- unspecific adsorption to capillaries
- unspecific adsorption to tube walls
- aggregation of the tryptophan containing molecule upon addition of the titrant
- strong absorption of UV light by the ligand

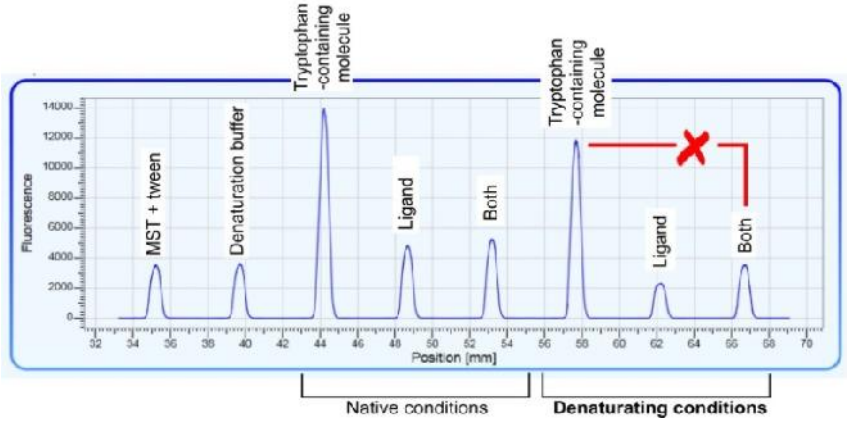
Binding or Material Loss? How to perform the SD-Test



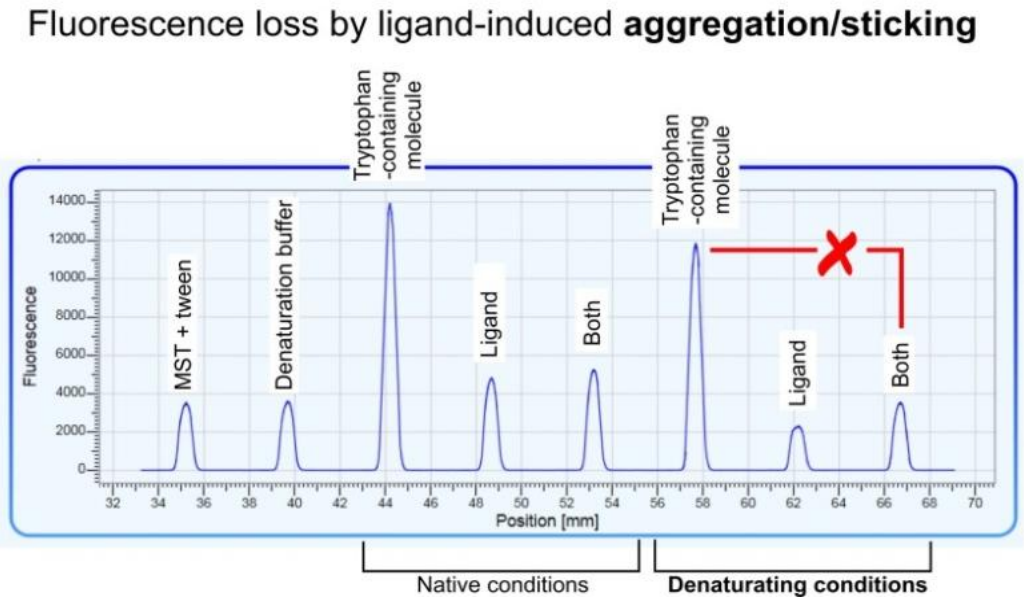
Fluorescence loss by ligand-induced **quenching**



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

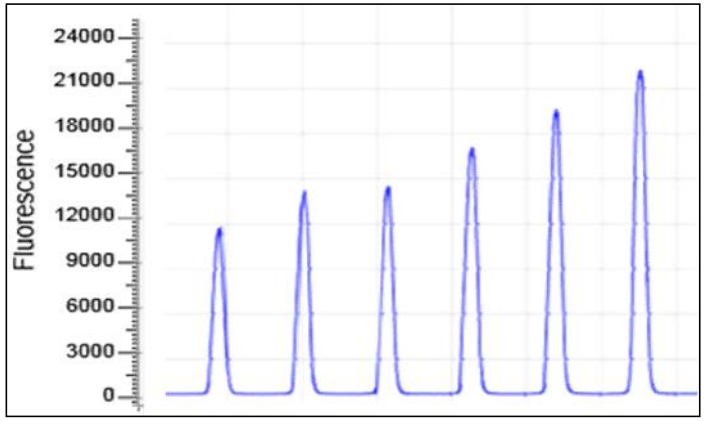


How to avoid Loss of Material

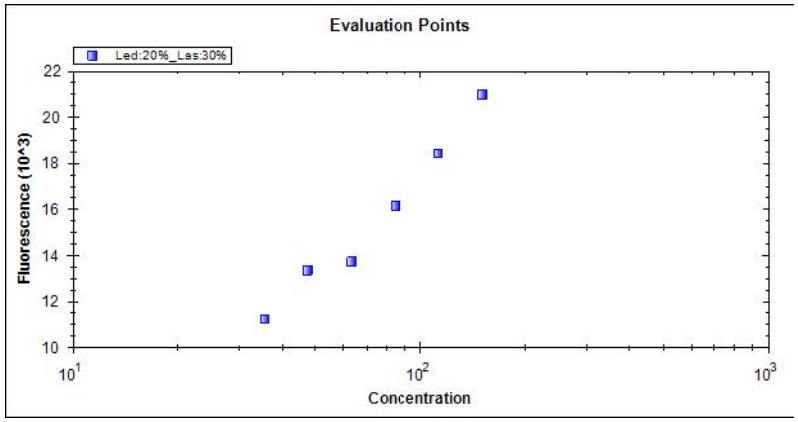


- improve buffer conditions
  - by adding Tween-20 (or other detergents such as Pluronic-F127, Triton, NP-40…)
  - by changing pH
  - by changing ionic strength…

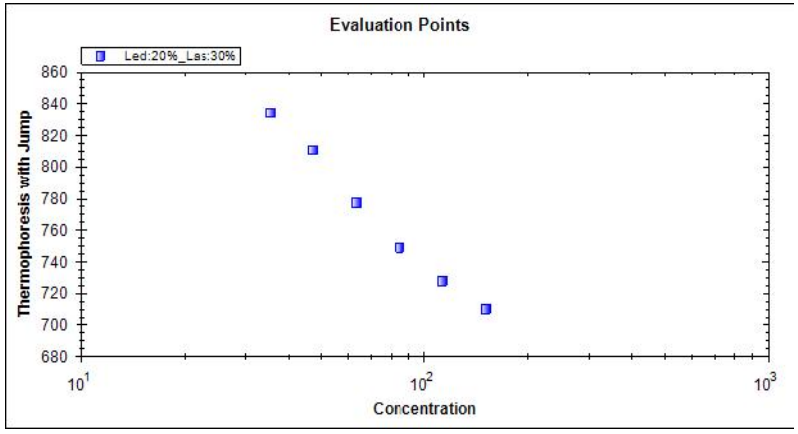
Changes in Fluorescence Intensities



Fluorescence



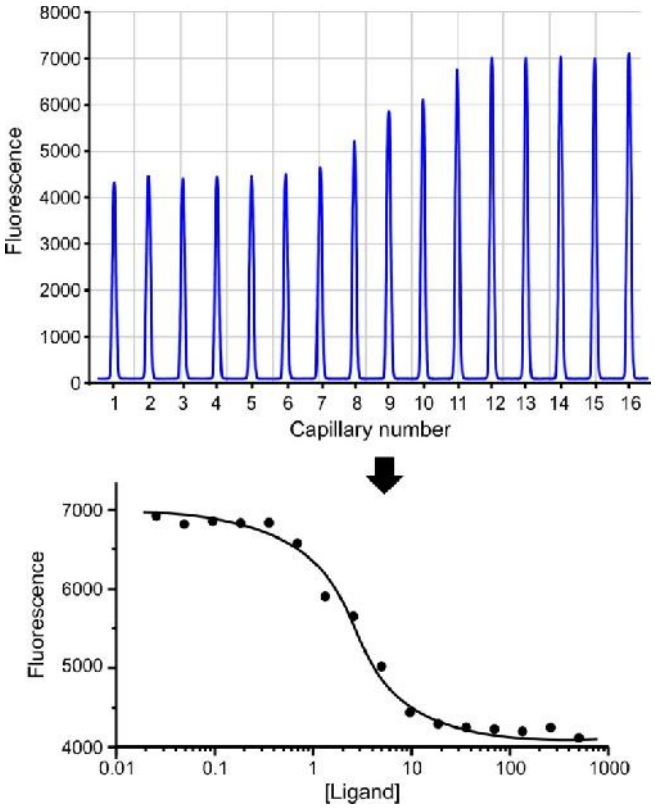
Thermophoresis



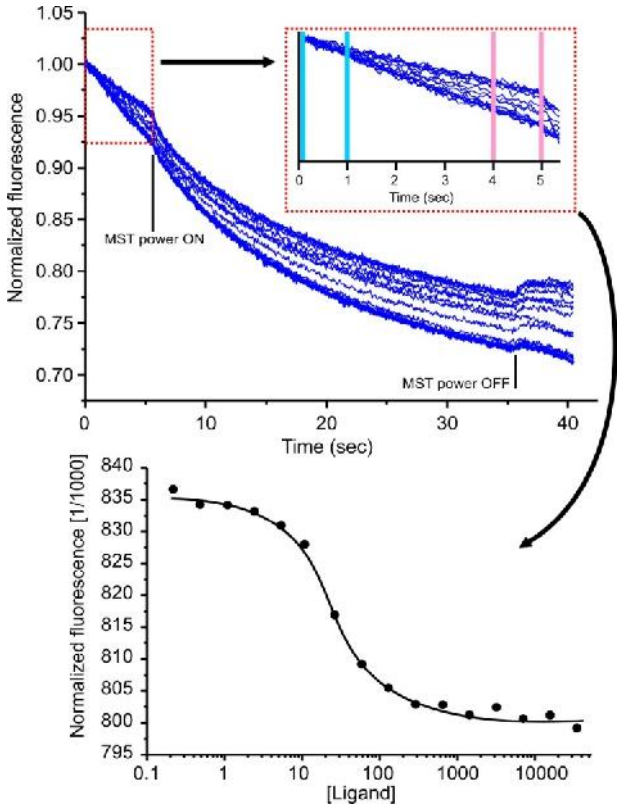
- Please note: Changes in fluorescence intensities will result in a thermophoretic signal!

Selection of the Evaluation Mode

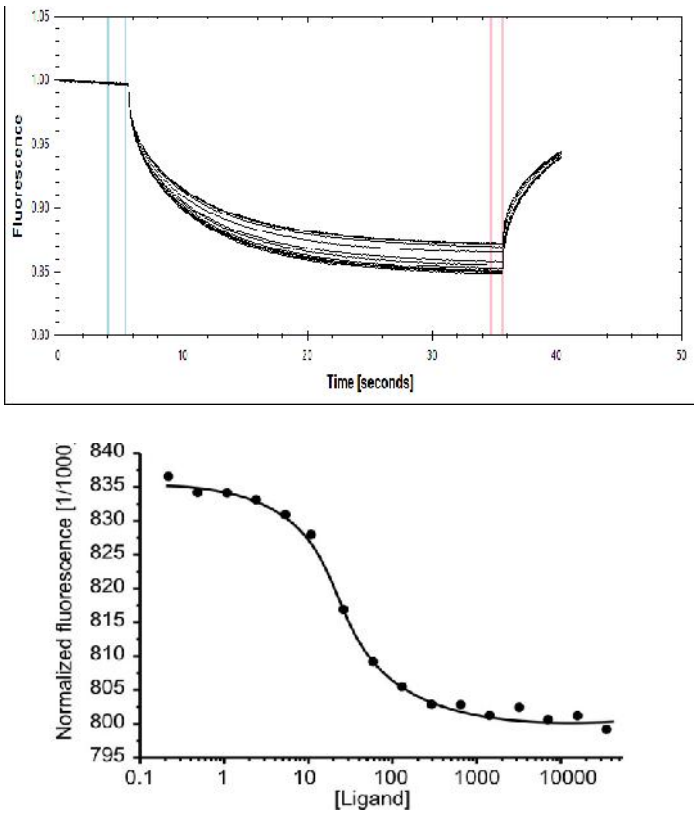
Fluorescence Changes



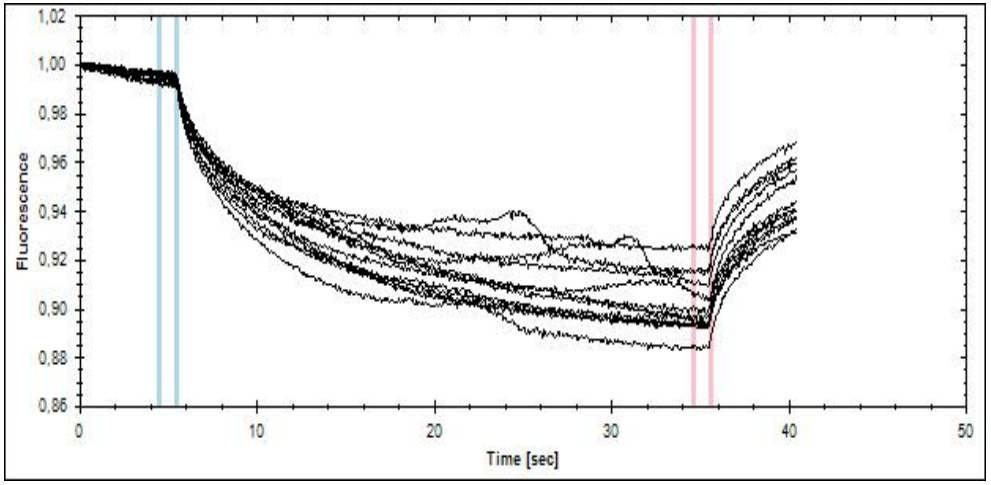
Photobleaching

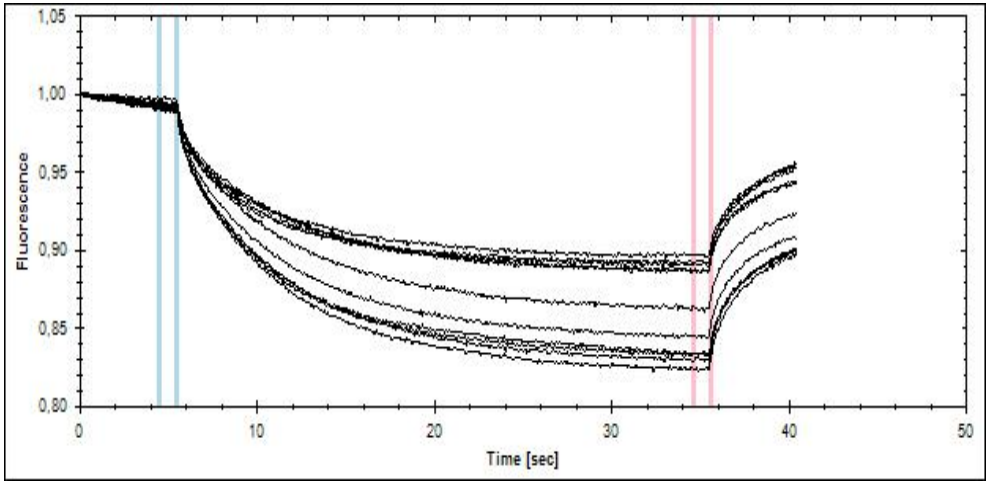


MST



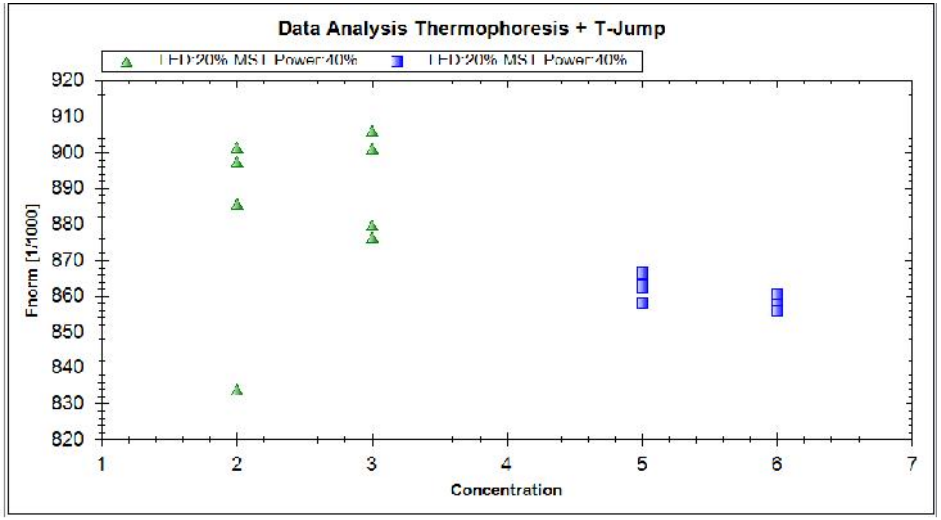
Improving Assay Conditions





- 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…

Improving Assay Conditions



Capillary: std. hydrophilic std. hydrophilic

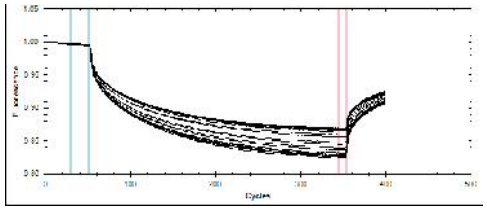
Assay buffer

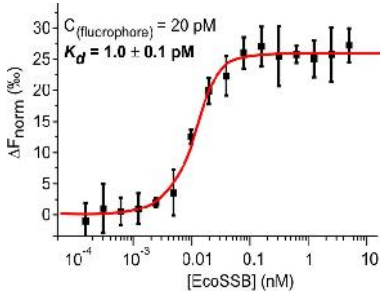
MST optimized buffer

- Load same sample in your assay buffer and in MST buffer (4 capillaries each) and measure with 40 % MST power
- test different types of capillaries (standard, hydrophilic, hydrophobic)
- test/improve buffer conditions
  - by adding Tween-20 (or other detergents such as Pluronic-F127,…)
  - by changing pH
  - by changing ionic strength…

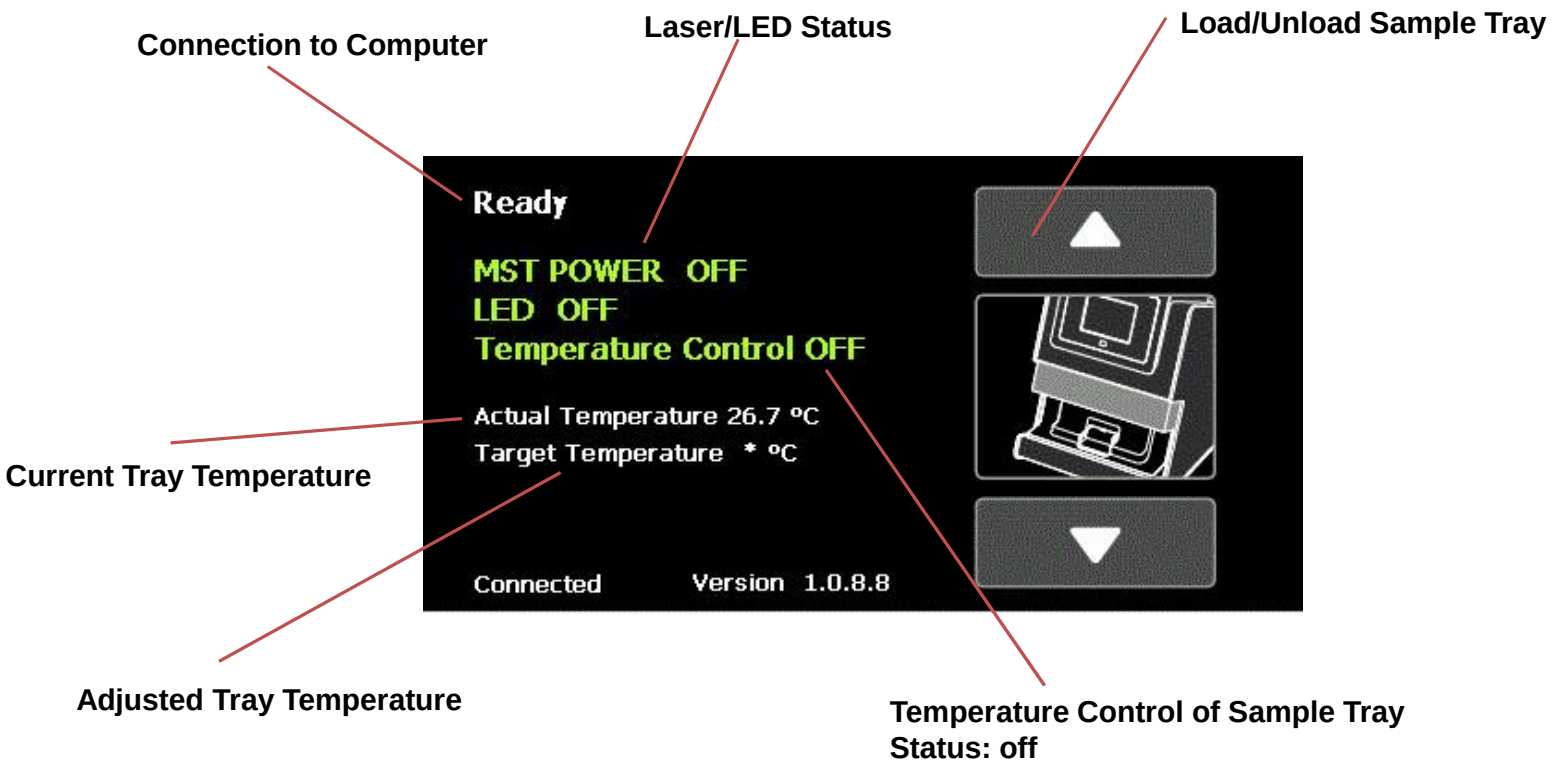
Software and Data Analysis - Overview

- Running the MST Experiment: NT.Control Software
- Analyzing the MST Data: NT.Analysis Software
- MST Data Analysis

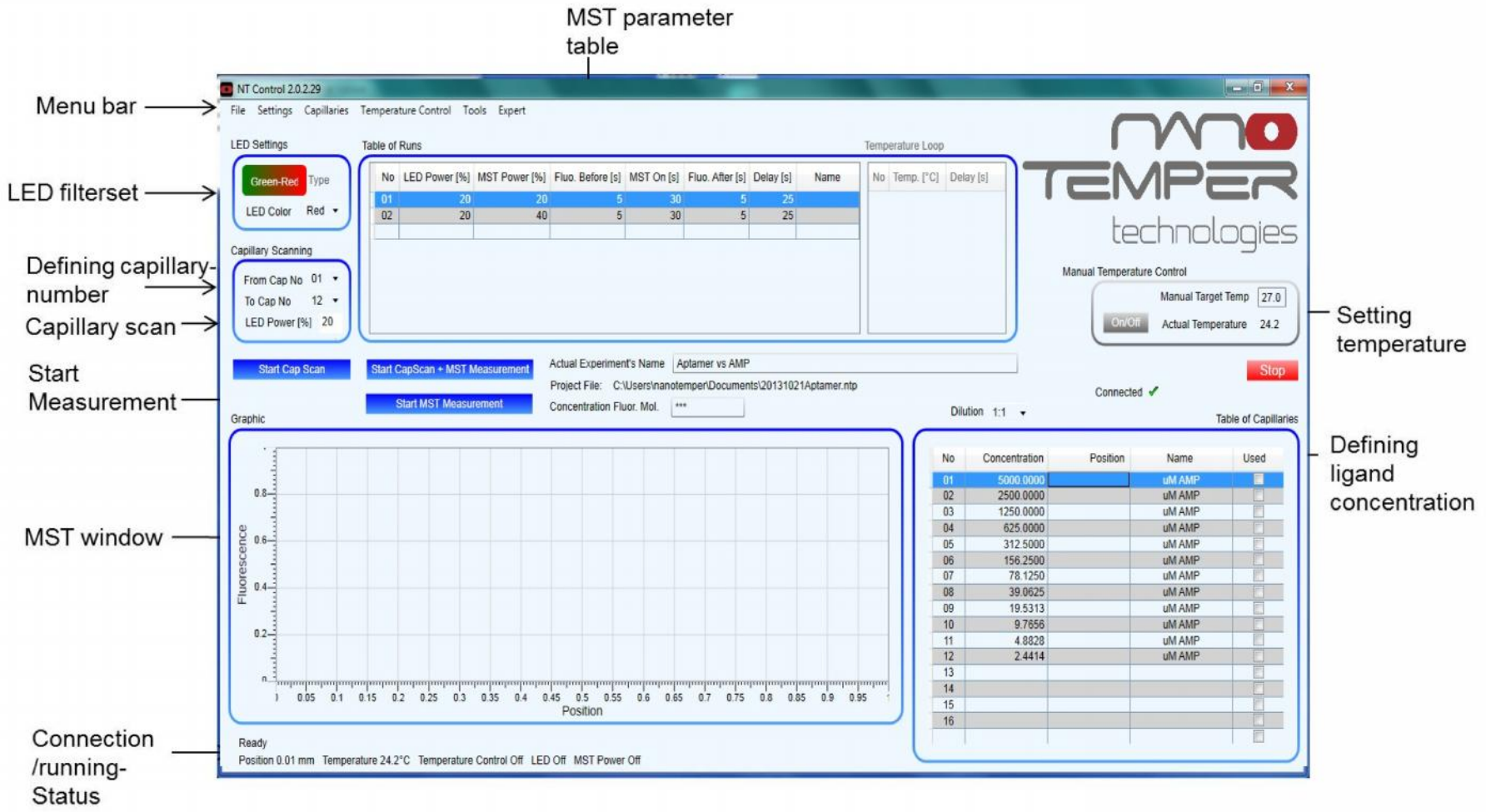




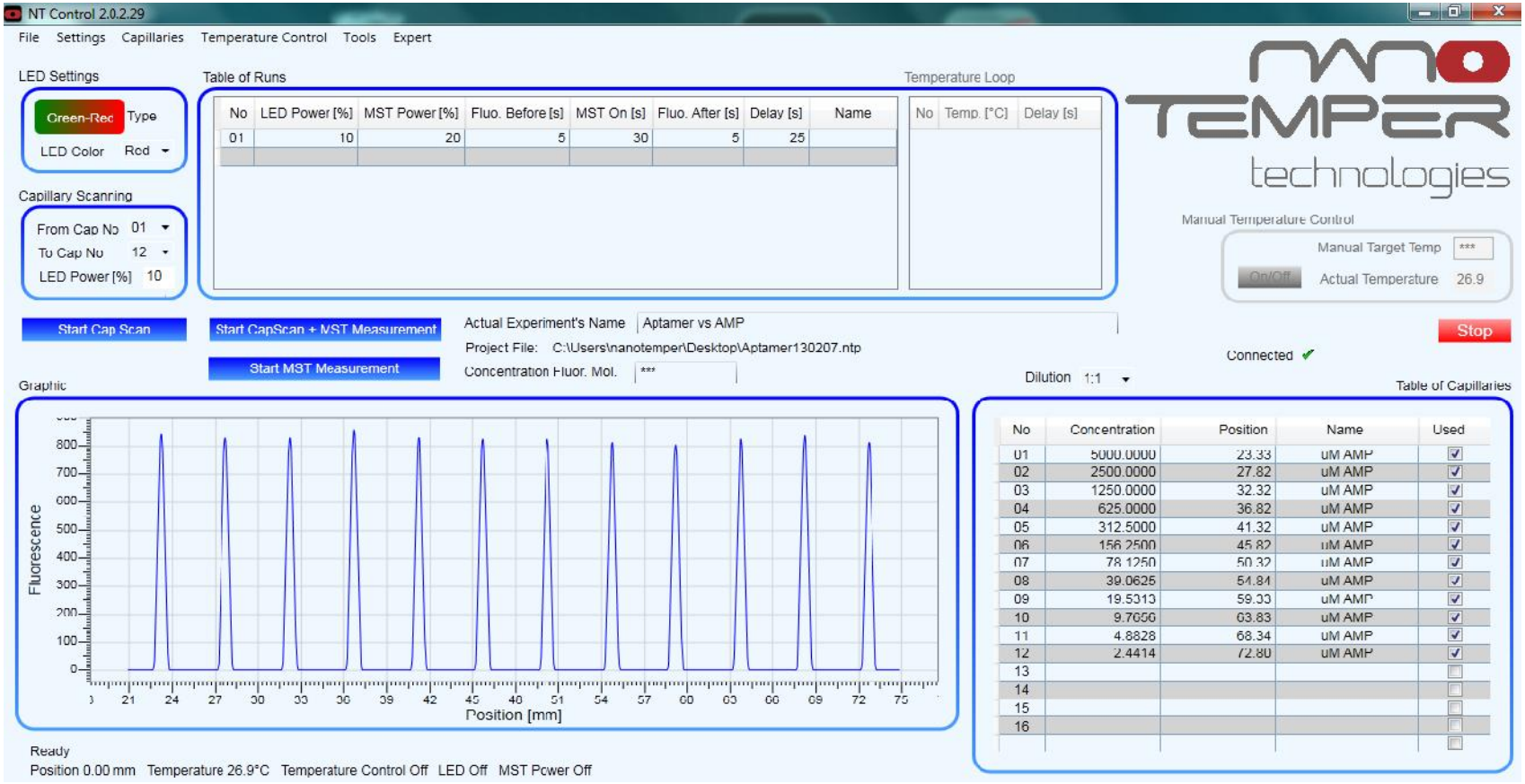
Running the MST Experiment



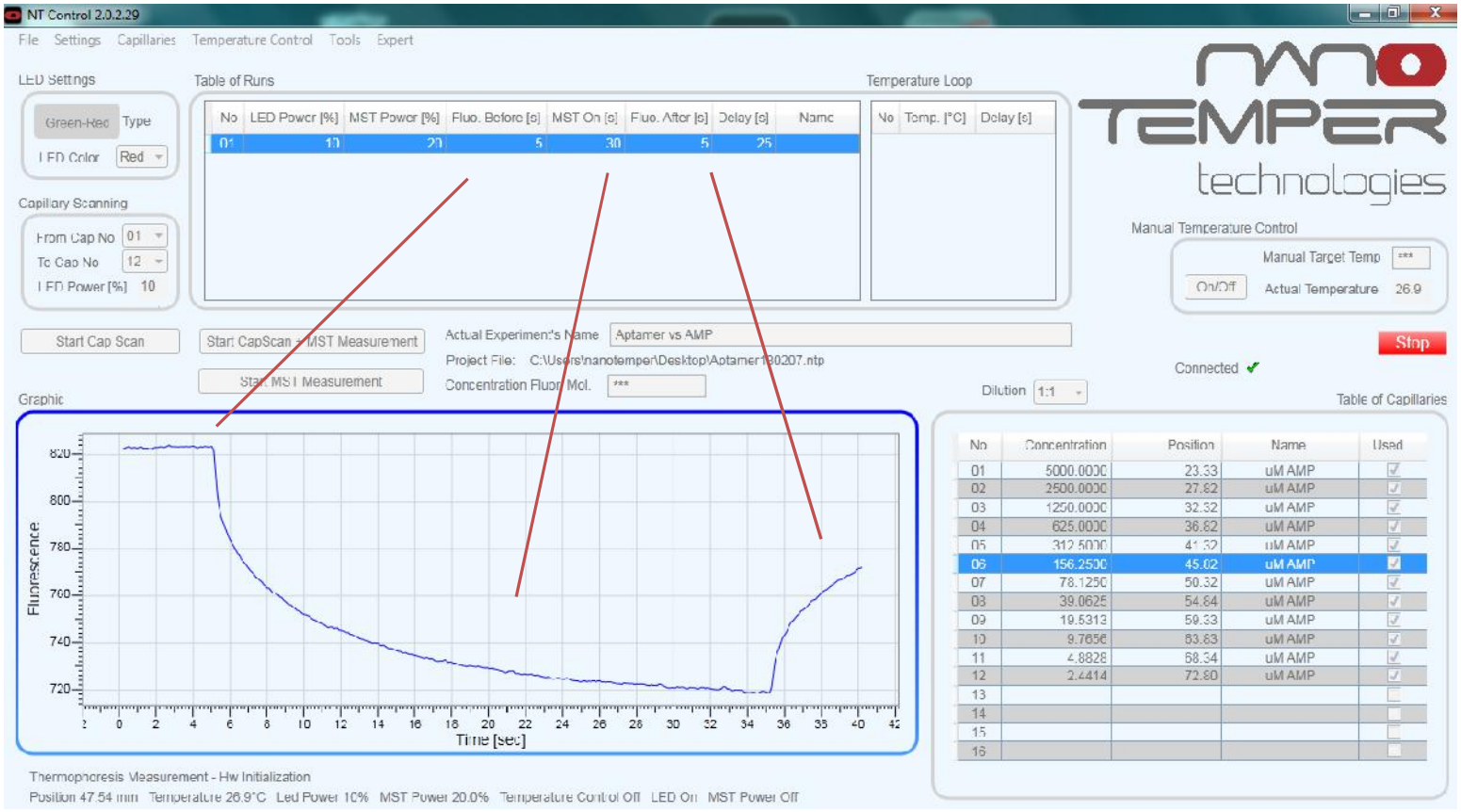
Running the MST Experiment



Capillary Scan

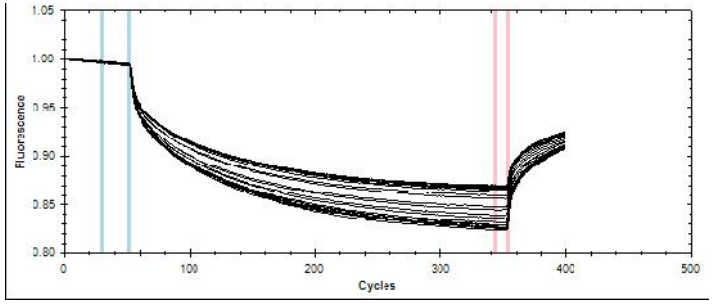


Parameter Setup and Running the Measurement

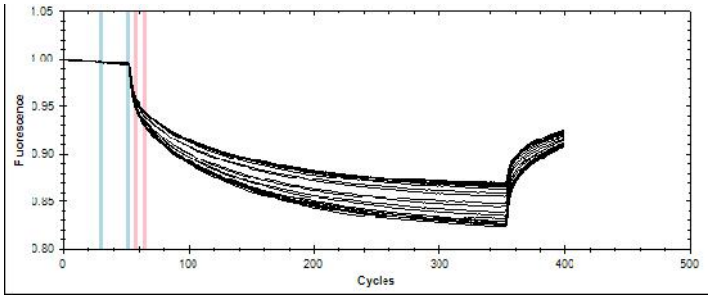


Data Analysis Modes

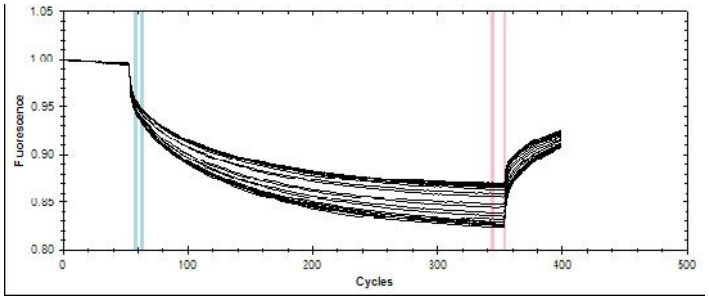
Thermophoresis with MST T-Jump



Only MST T-Jump



Only Thermophoresis

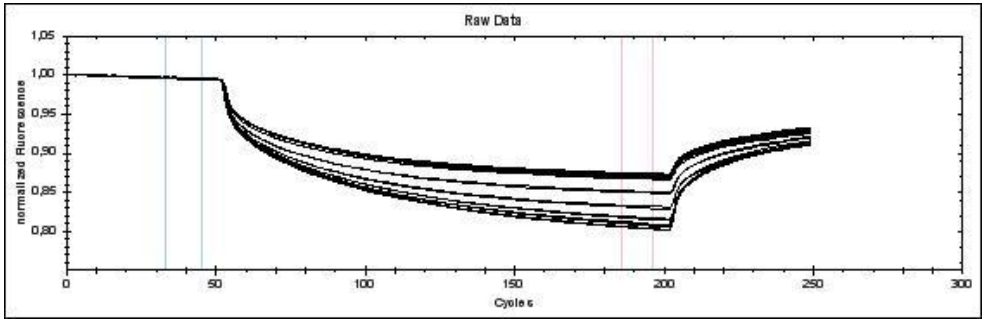


Information from T-Jump:
binding close to fluorophore / tryptophane

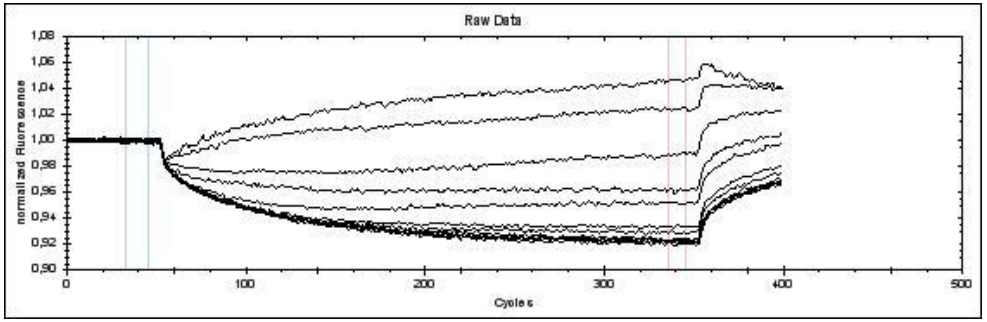
Information from Thermophoresis:
overall structure

Data Analysis Modes

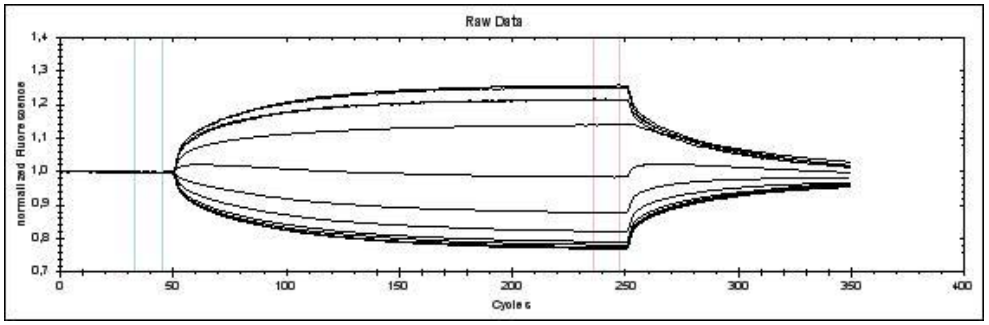
T-Jump (+) and Thermophoresis (+)



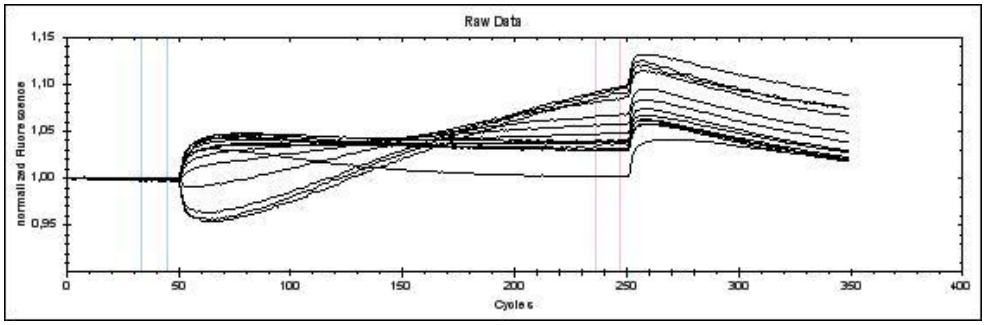
T-Jump (+) and Thermophoresis (+/-)



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

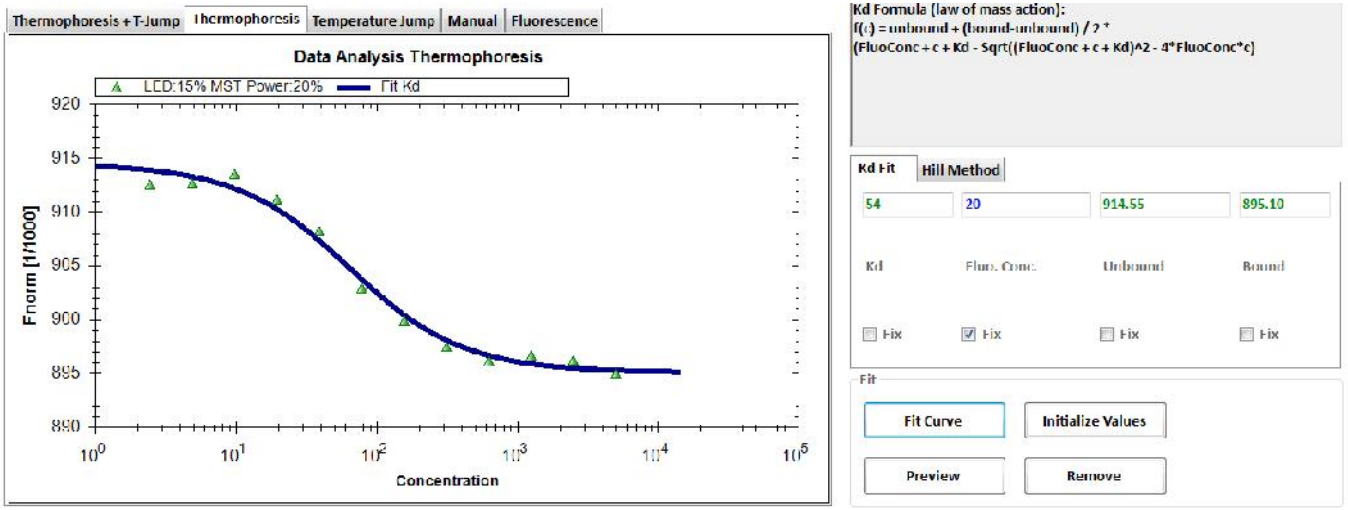


T-Jump (+/-) and Thermophoresis (+/-)

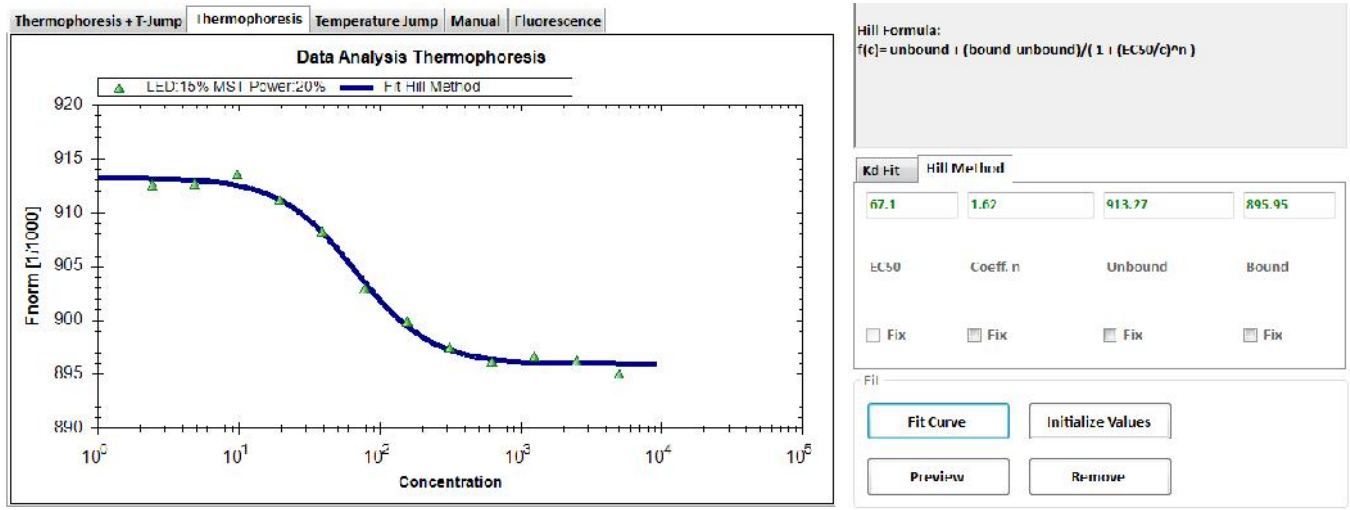


K_d Curve vs. Hill Curve Fitting

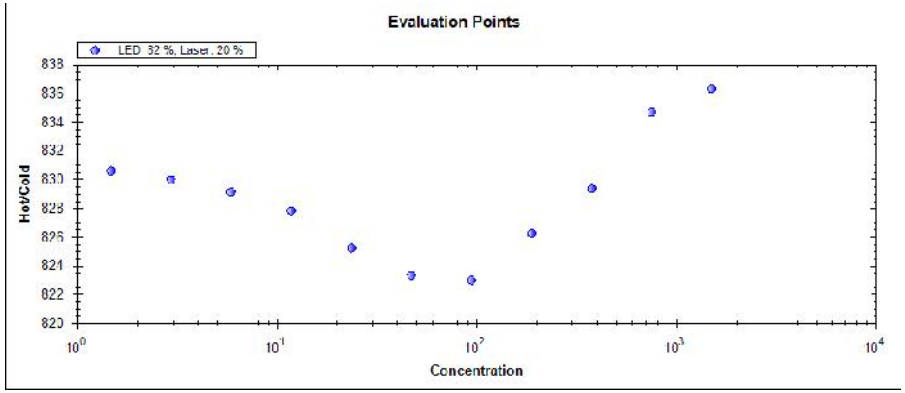
K_d Curve Fitting:

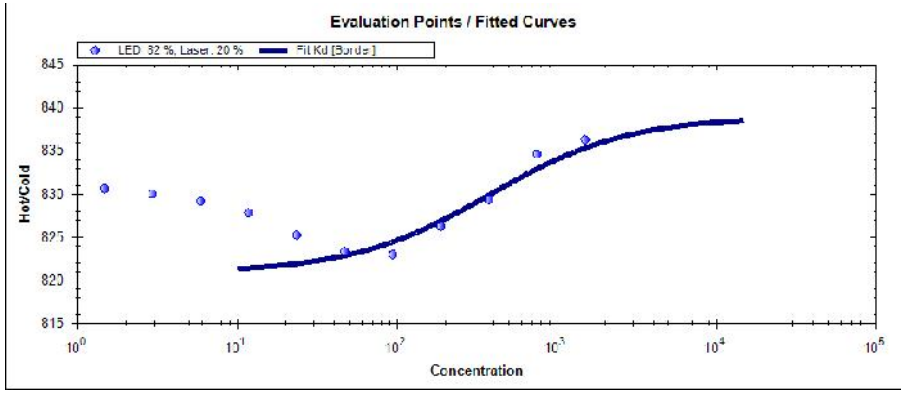


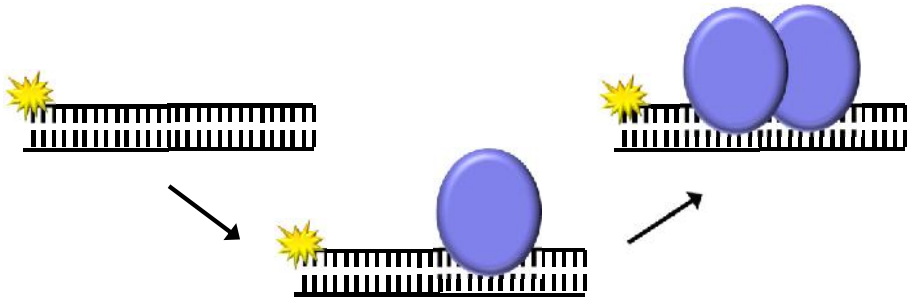
Hill Curve Fitting:

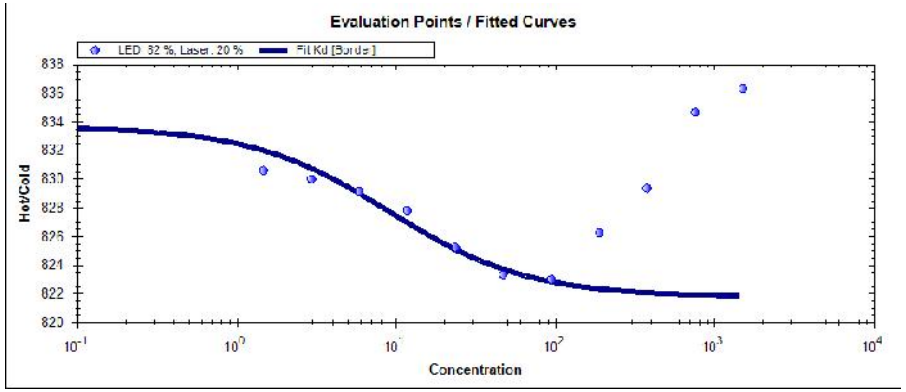


Two Binding Affinities

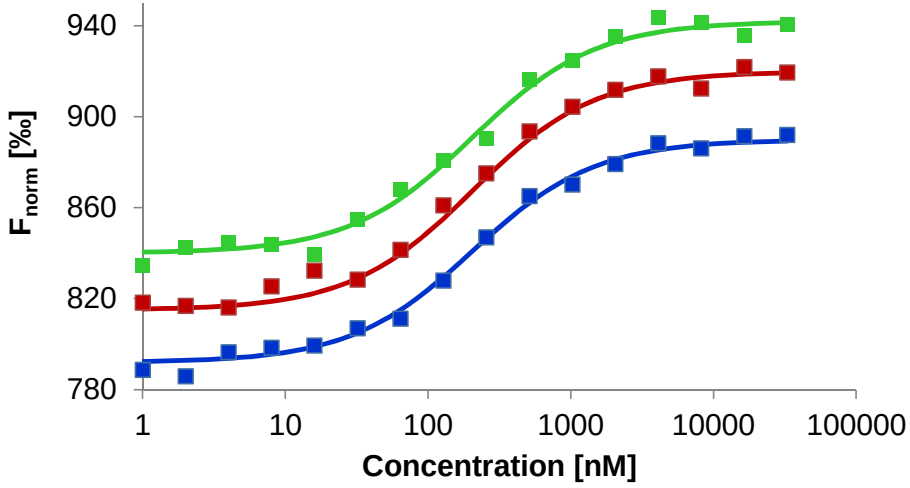


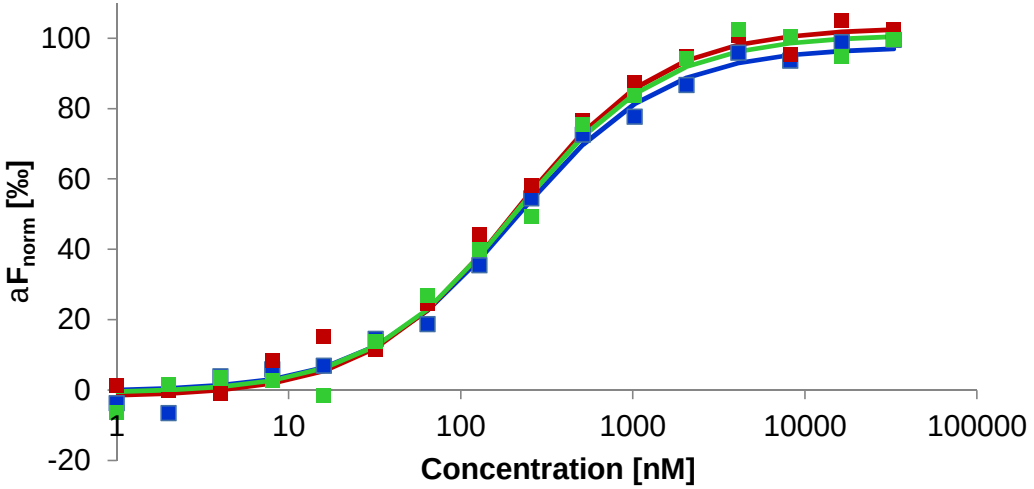


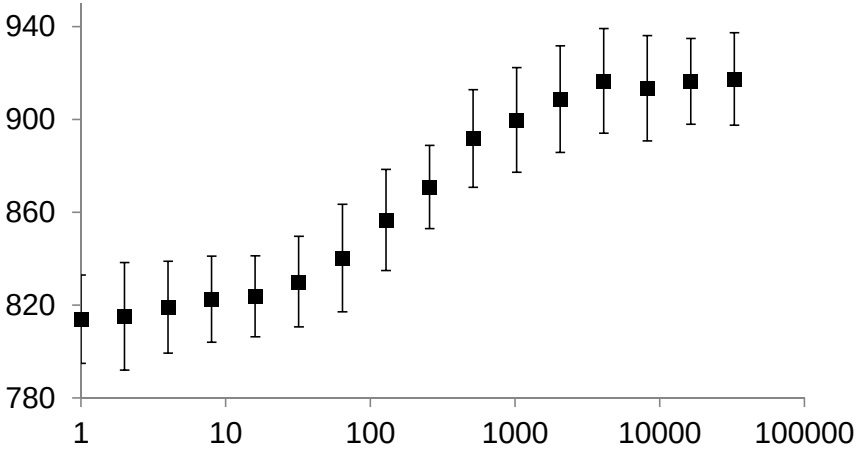


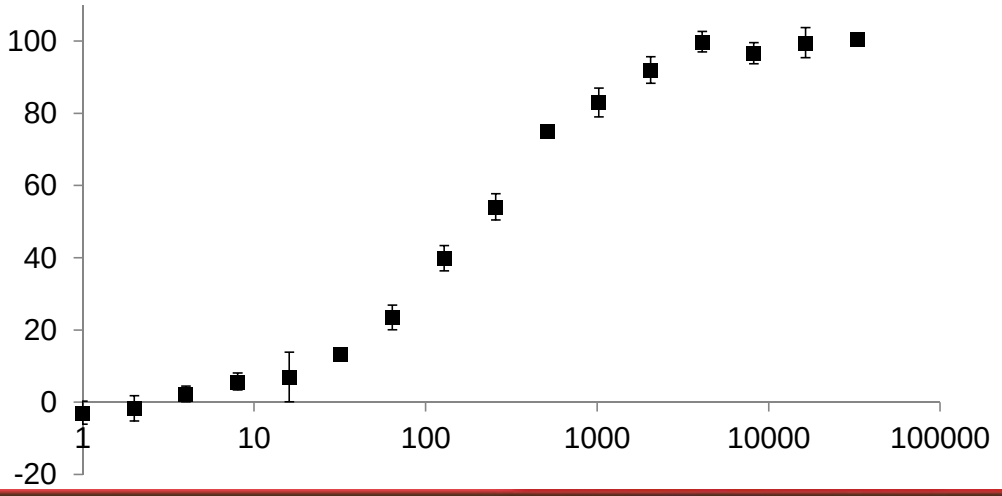


Multiple Measurements – Baseline correction

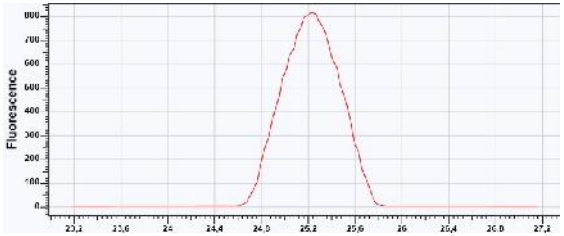


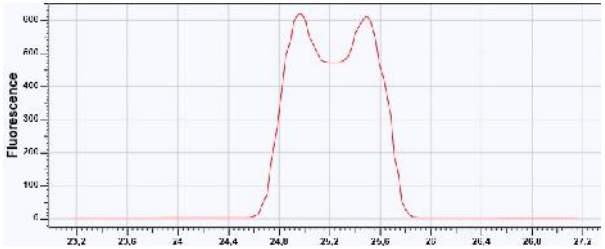


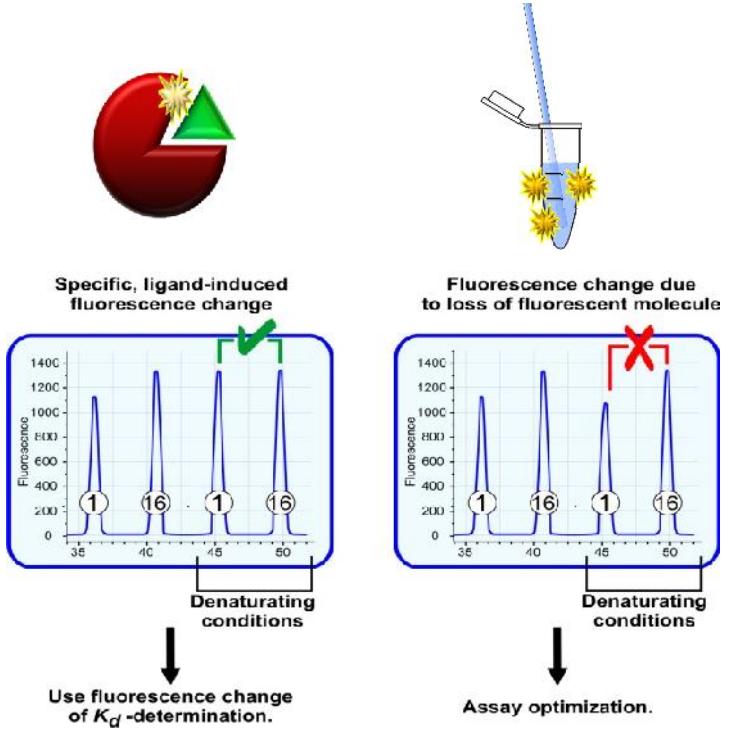




Assay Optimization – Summary







- easy and straight forward assay optimization

Thank You For Your Attention!

Please feel free to contact us:

David Witte

NanoTemper Technologies GmbH
Flößergasse 4, 81369 München, Germany

www.nanotemper-technologies.com

david.witte@nanotemper.de