Microscale thermophoresis (MST)

S2004
Methods for characterization of biomolecular interactions – classical versus modern

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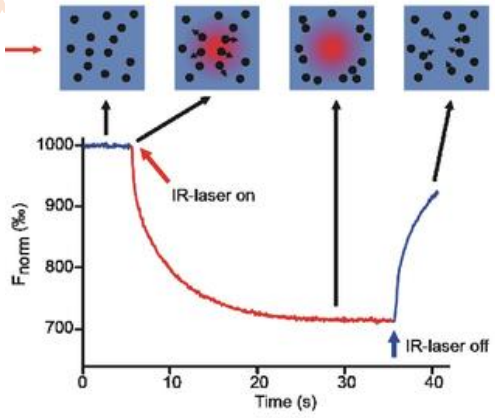
Microscale thermophoresis

- Method used for determination of the binding affinity of a wide range of interactions
- Samples from small ions to big cells
- Affinities pM – mM
- Little buffer limitation
- Small sample consumption
- Quick

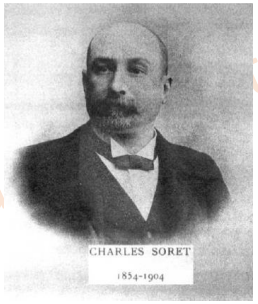
$$S_T = \frac{A}{kT}\, x \left(-\Delta s_{hyd\mathrm{kT}} + \frac{\beta \sigma_{eff}{}^2}{4\varepsilon\varepsilon_0 T}\, x\, \lambda_{DH}\right)$$



History

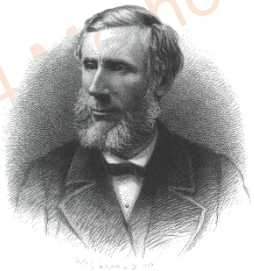
Thermophoresis in liquids (*Carl Ludwig*)

1856



Thermophoresis in liquids (*Charles Soret*)

1870 1879



Thermophoresis in gas (*John Tyndall*)

nanoTEMPER technologies

2006 2008 2010

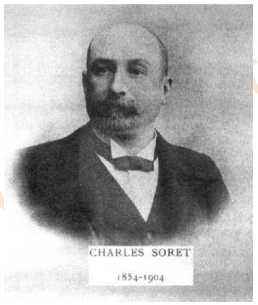
Thermophoresis in solids (*Phillip Schoen*)

First papers on thermophoresis application to affinity of biomacromolecules

History

Thermophoresis in liquids (*Carl Ludwig*)

1856



Thermophoresis in liquids (*Charles Soret*)

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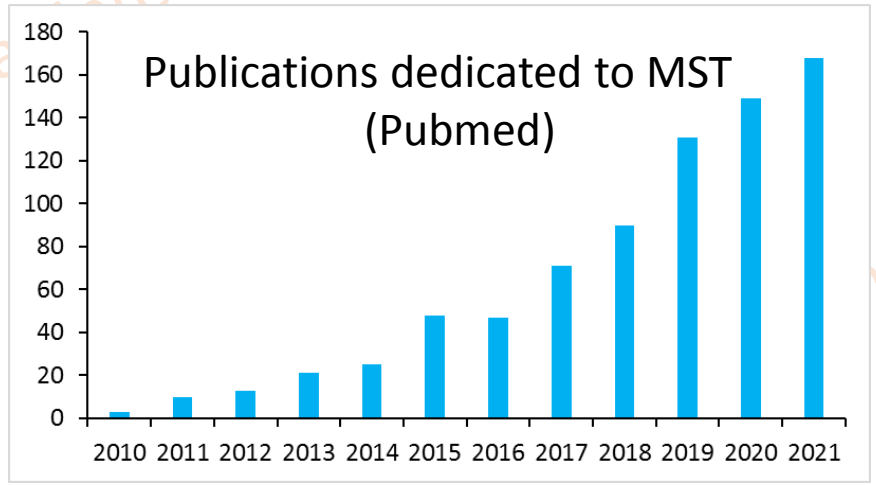
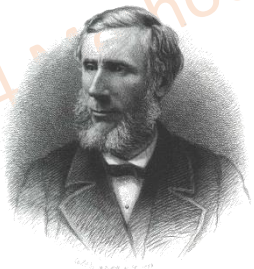


nanoTEMPER technologies

2008

2006 2010

Thermophoresis in gas (*John Tyndall*)

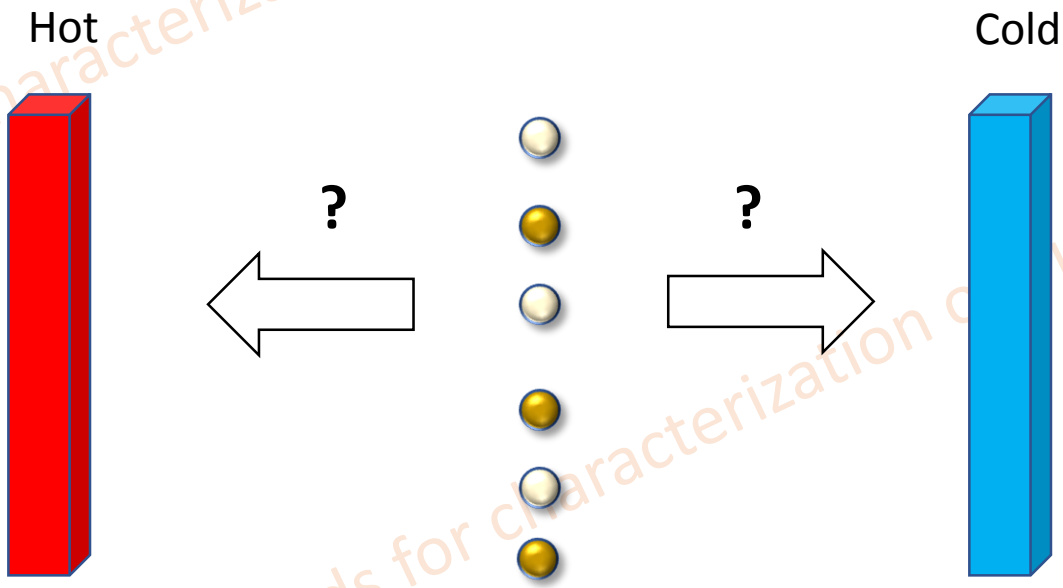


Thermophoresis in solids (*Phillip Schoen*)

First papers on thermophoresis application to affinity of biomacromolecules

Thermophoresis

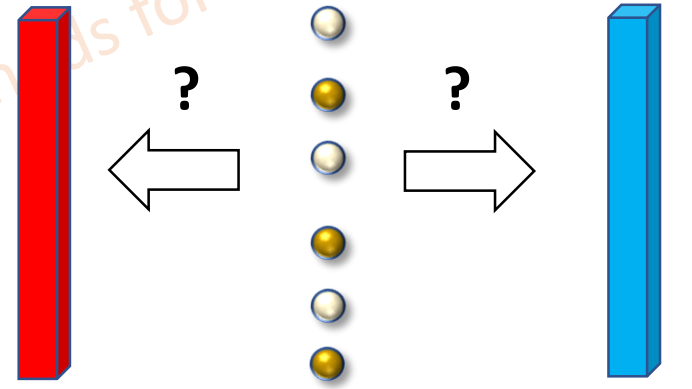
movement of particles in temperature gradient



Thermophoresis vs Electrophoresis

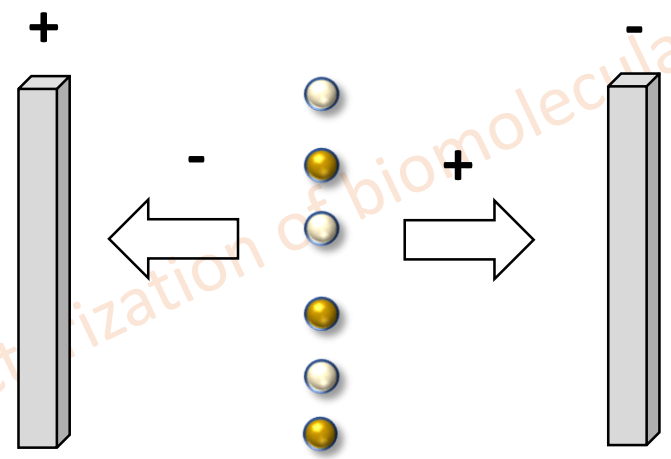
movement of particles

in temperature gradient



Thermophoretic parameters
(Sorret coefficient)

in electric field



Charge, (size)

A bit of theory…

Particle flux j in solution (modified Fick's law)

$$j = -D\Delta\rho - \rho D_T \Delta T$$

mass diffusion ($\overrightarrow{j_m}$)

thermal diffusion ($\overrightarrow{j_T}$)

D… diffusion coefficient

ρ…particle density

D_T…thermal diffusion coefficient

T…temperature

Δ…differenctila value (delta)

Duhr and Braun, PNAS, 2006

A bit of theory…

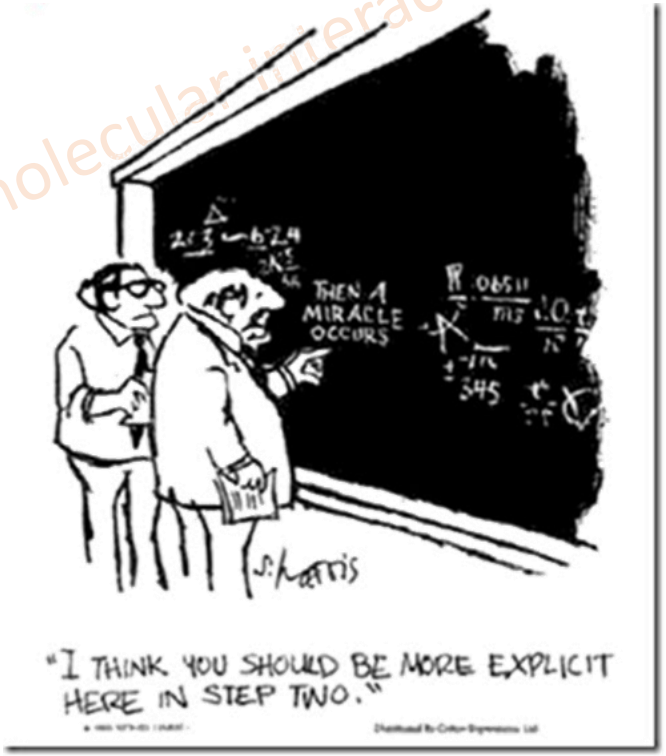
At steady state ("equilibrium"), the flux $j = 0$

thermal diffusion + mass diffusion = 0

$$\Delta\rho = \rho \frac{D_T}{D} \Delta T$$

The difference in molecular density (concentration) depends on:

- Used concentration
- The temperature gradient
- Thermal and mass diffusion coefficitents



Duhr and Braun, PNAS, 2006

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At steady state ("equilibrium"), the flux $j = 0$

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$$\Delta\rho = \rho \frac{D_T}{D} \Delta T$$

Soret coefficient (S_T)

$$S_T = \frac{D_T}{D}$$

Thermal diffusion coefficient
Mass diffusion coefficient

Duhr and Braun, PNAS, 2006

A bit of theory…

At steady state ("equilibrium"), the flux $j = 0$

thermal diffusion + mass diffusion = 0

$$\Delta\rho = \rho \frac{D_T}{D} \Delta T$$

Soret coefficient (S_T)

$$S_T = \frac{D_T}{D}$$

$$\frac{c_{hot}}{c_{cold}} = e^{-S_T.\Delta T}$$

Duhr and Braun, PNAS, 2006

Soret coefficient… for proteins not so easy

$$S_T = \frac{A}{kT}\, x \left(-\Delta s_{hyd}\mathrm{kT} + \frac{\beta \sigma_{eff}{}^2}{4\varepsilon\varepsilon_0 T}\, x\, \lambda_{DH} \right)$$

A … surface area of the molecule
T… temperature (Kelvins)
s_{hyd} … hydratation enthropy of the molecule – solution interface
σ_{eff} … the effective charge
ε … dielectric constant
β … temperature derivative
λ_{DH} … Debey-Hueckel length

S 2004 Methods for characterization of biomolecular interactions
S 2004 Methods for characterization of biomolecular interactions

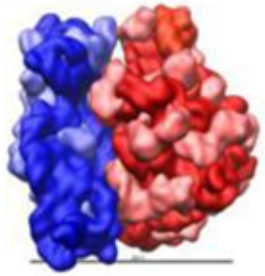
A bit of theory…

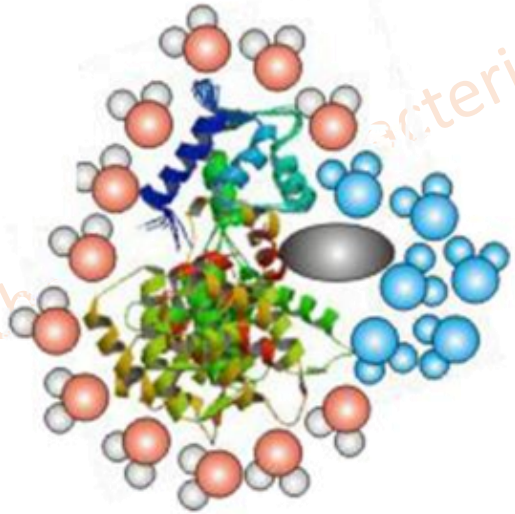
$$S_T = \frac{A}{kT}\left(-\Delta s_{hyd}(T) + \frac{\beta\sigma_{eff}^{2}}{4\varepsilon\varepsilon_0 T} \times \lambda_{DH}\right)$$

size

hydration shell

charge ^{2}





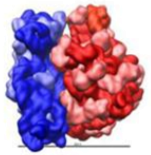
A bit of theory…

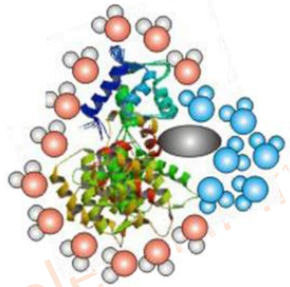
$$S_T = \frac{A}{kT}\left(-\Delta s_{hyd}(T) + \frac{\beta\sigma_{eff}^2}{4\varepsilon\varepsilon_0 T} \times \lambda_{DH}\right)$$

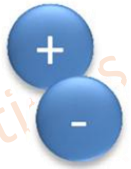
size — hydration shell — charge ²

Soret coefficient S_T depends on:

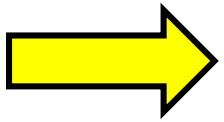
- mean temperature
- particle size (surface area)
- hydration shell entropy (solvation, conformation)
- electrostatic potential (~ charge)







Strength of MST – almost every interaction causes changes in one of these parameters (not in mean temperature)



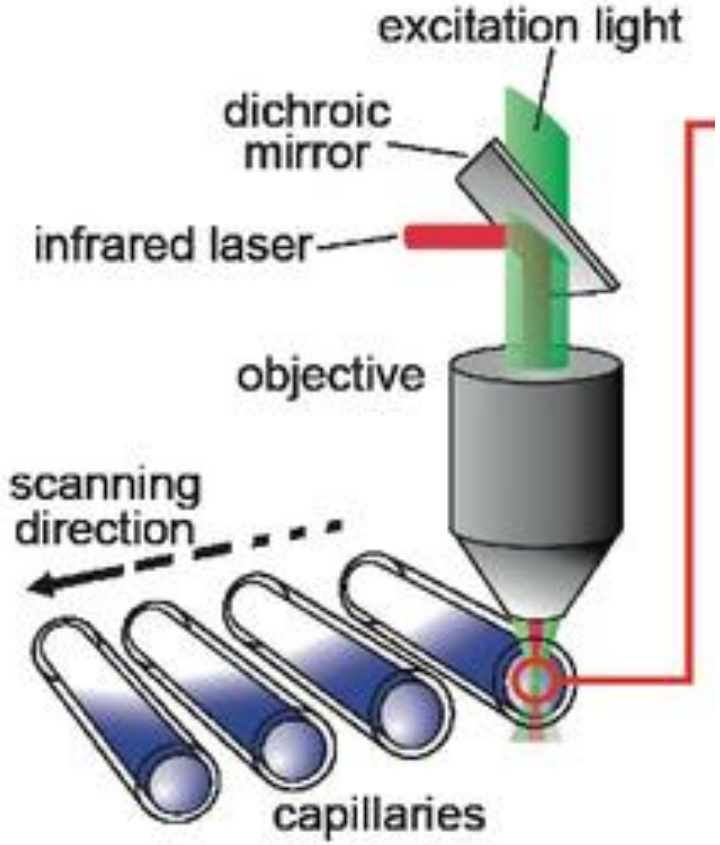
is measurable by MST

MST measurement

- Measures fluorescence – one of the binding partners must be fluorescent (e.g. target), constant concentration
- Serial dilution of the other partner (e.g. ligand) in capillaries

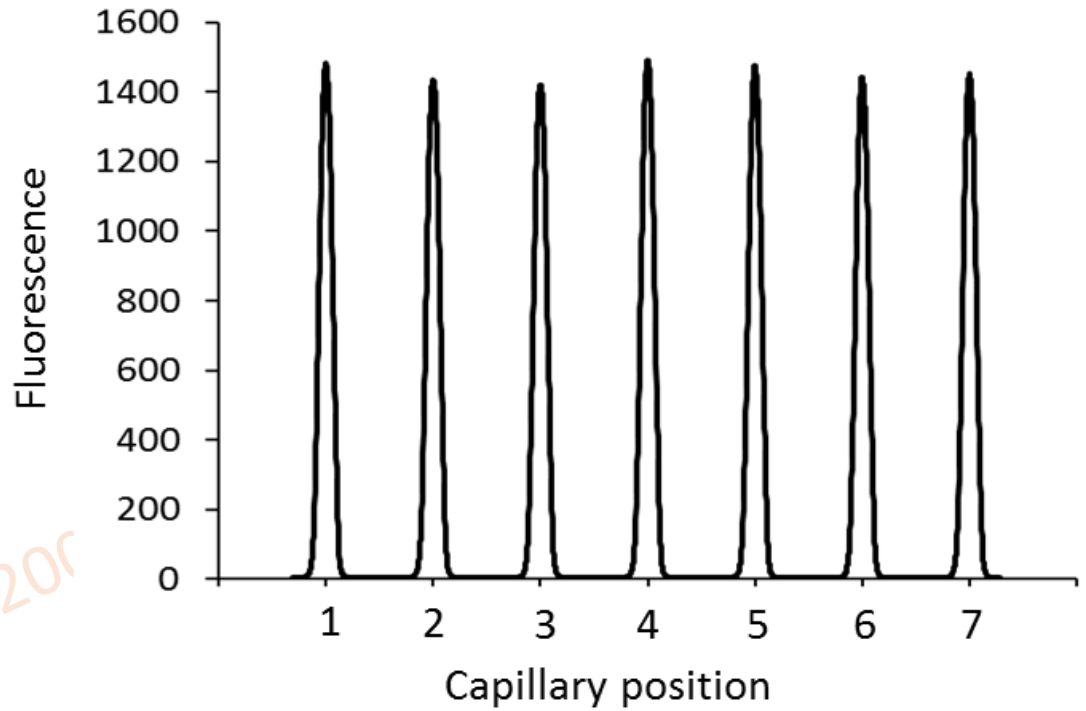
- Two types of lasers
  - **Infrared laser** – creates the temperature gradient
  - ΔT depends on the laser power and time (>10 K after 5 s for 40% laser power)

  - **Excitation laser** – excites the fluorescence
  - Red, blue or green laser
  - Dye needs to be compatible



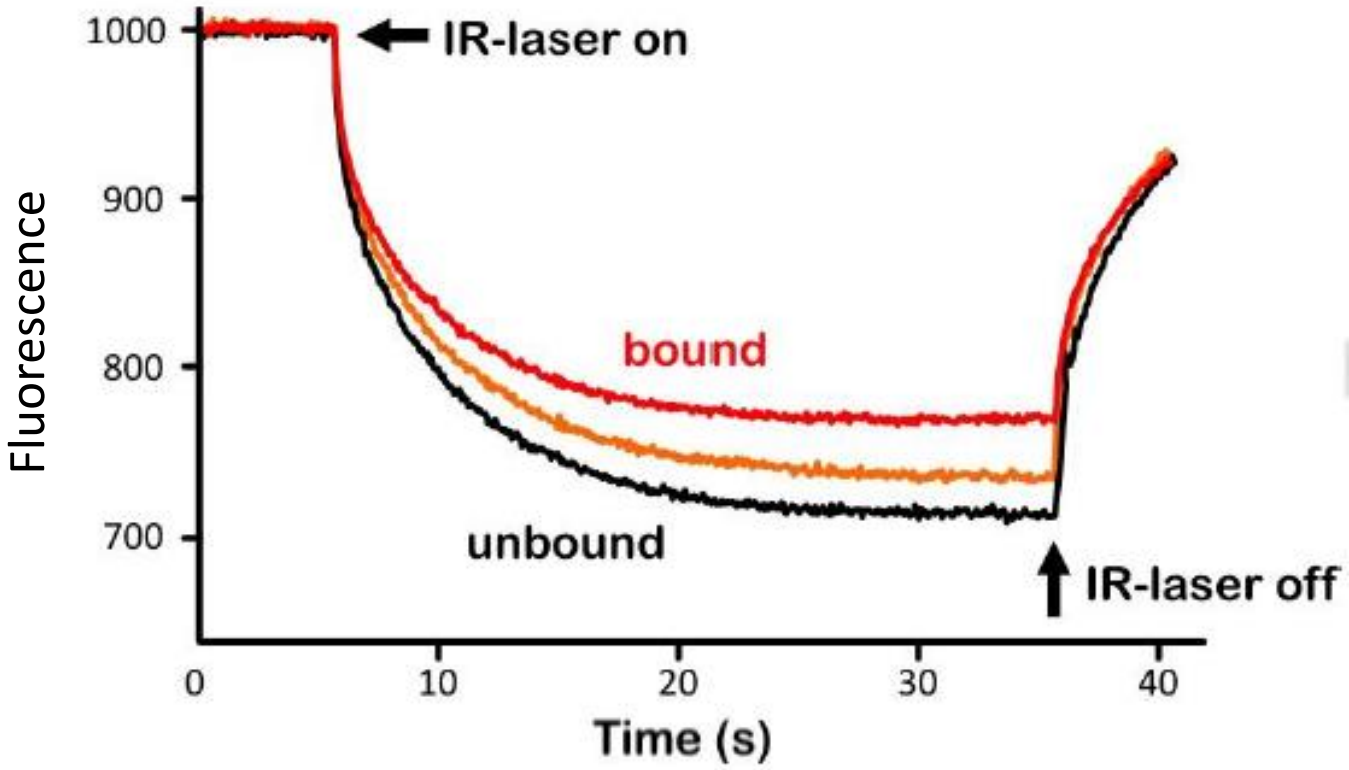
MST measurement

- Capillary scan
  - Fluorescence for each capillary similar
  - 10% deviance from average is acceptable



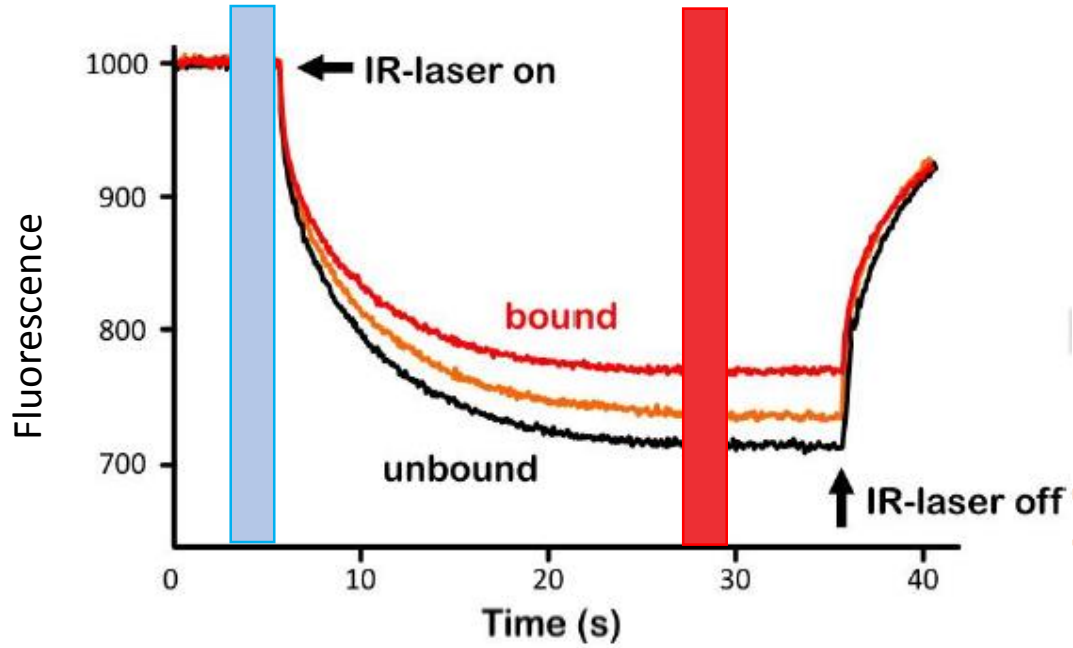
MST measurement

- MST measurement



MST measurement

- Data analyses



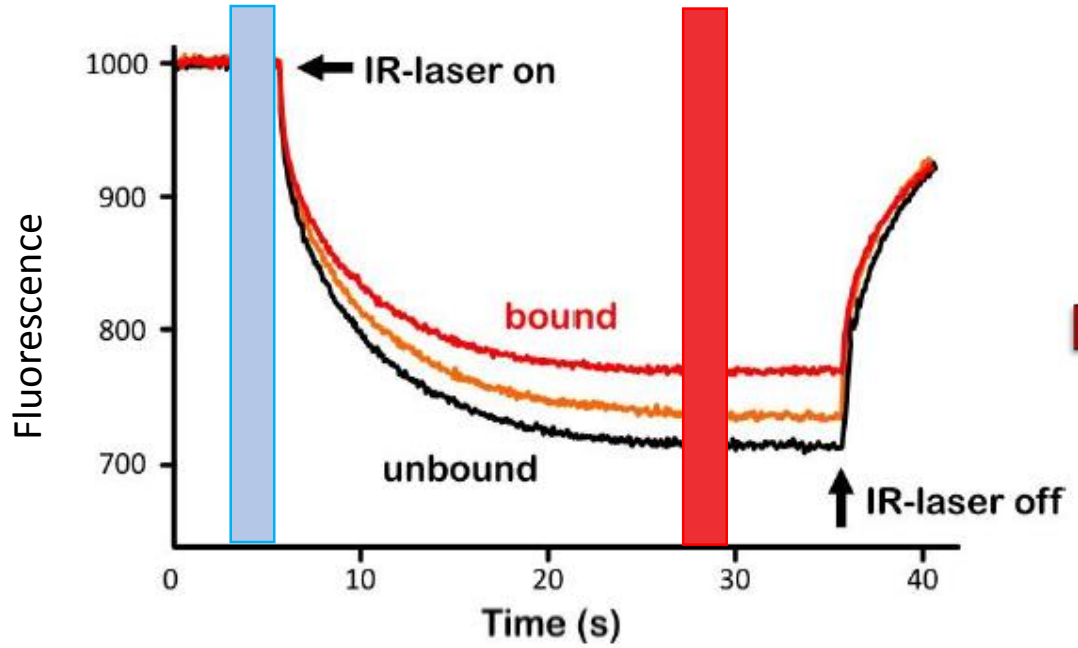
Normalized fluorescence F_N , F_{norm}

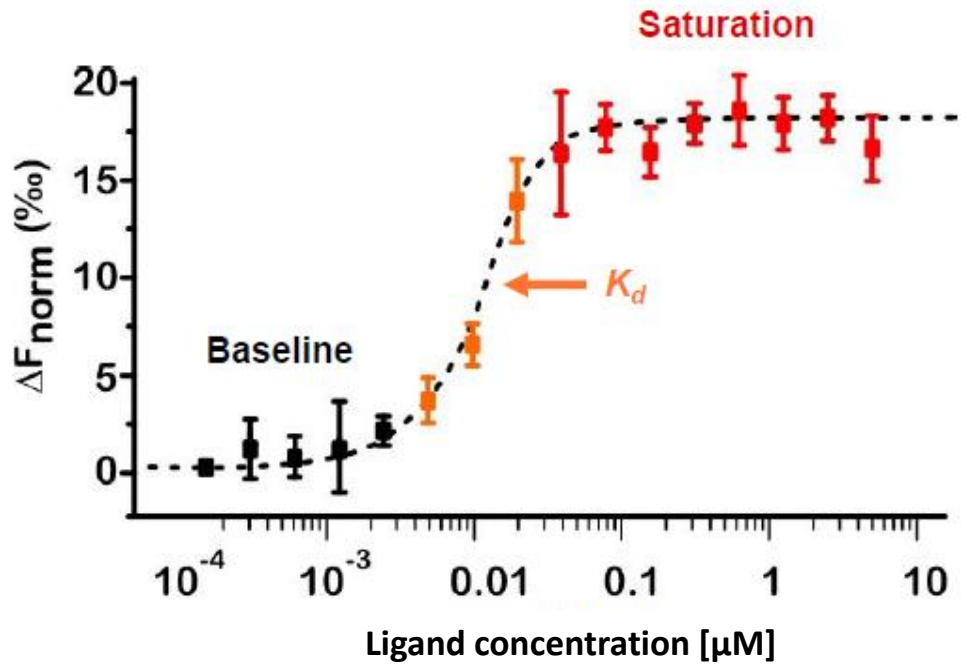
$$F_N = \frac{\langle \mathbf{F_{hot}} \rangle}{\langle \mathbf{F_{cold}} \rangle}$$

$$\frac{c_{hot}}{c_{cold}} = e^{-S_T.\Delta T}$$

MST measurement

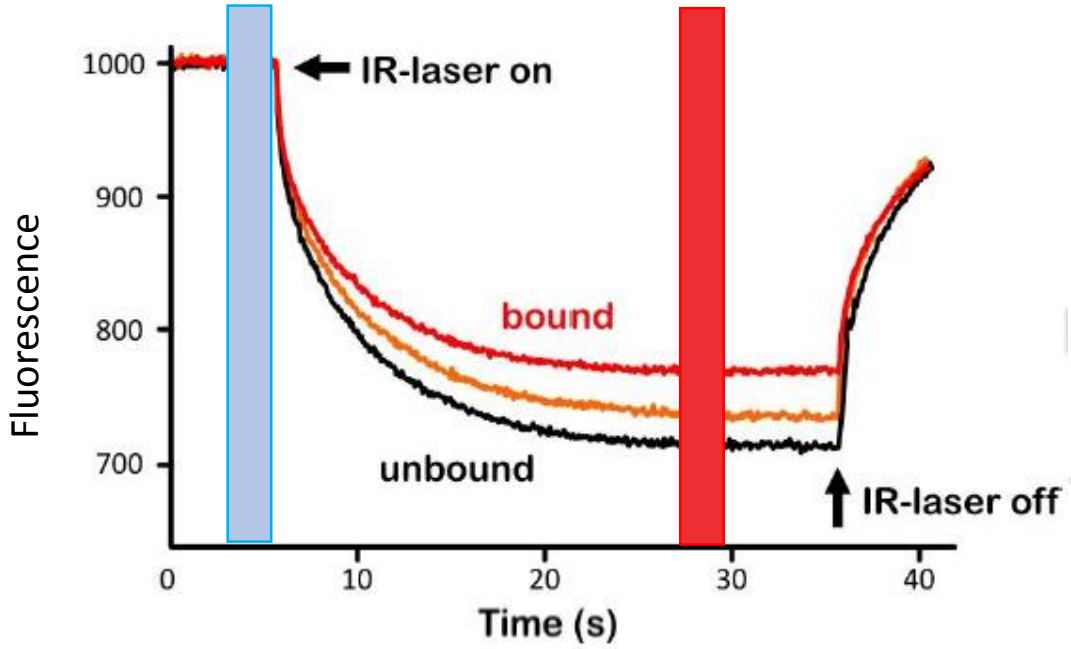
- Data analyses



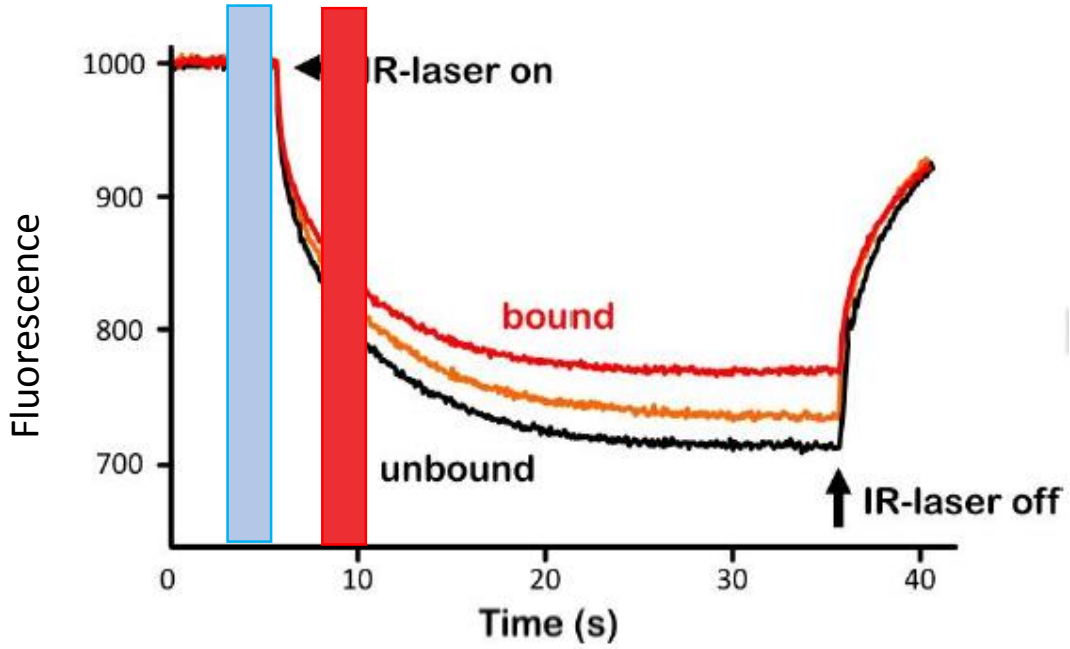


MST measurement

- Data analyses

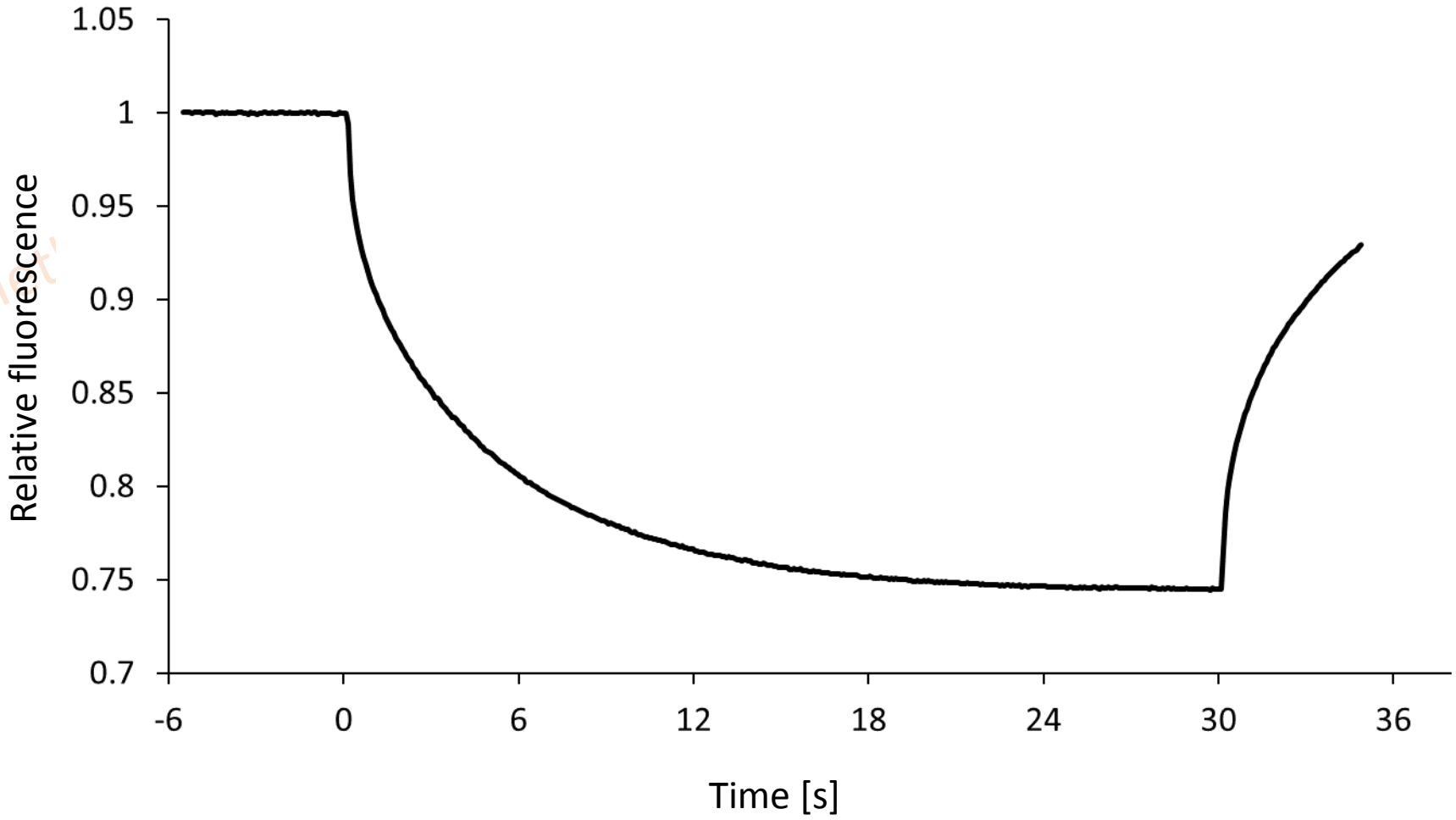


Original approach

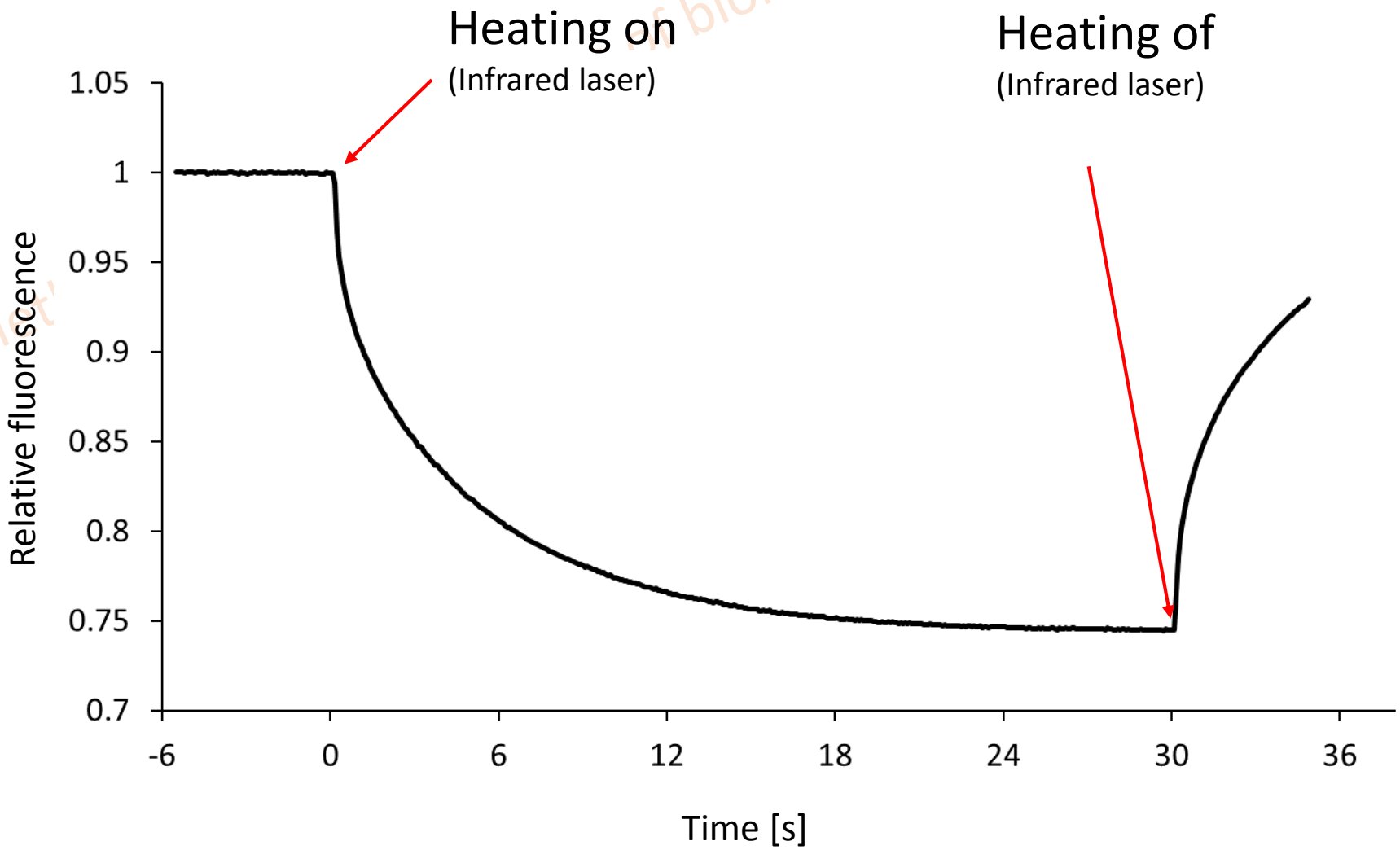


Recent recommendation

Dissecting the MST timetrace



Dissecting the MST timetrace

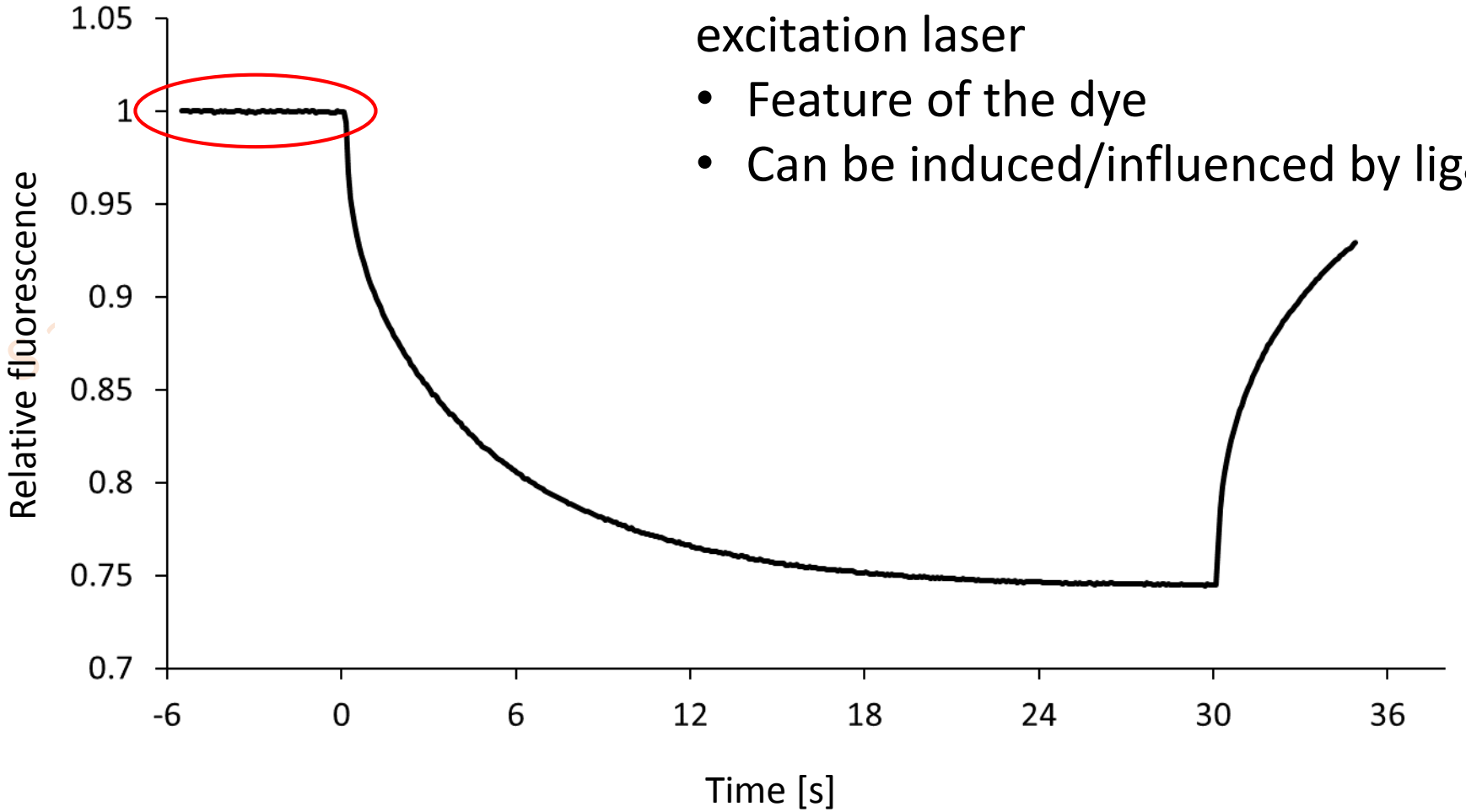


Dissecting the MST timetrace

Initial fluorescence

Bleaching = decrease of fluorescence signal caused by excitation laser

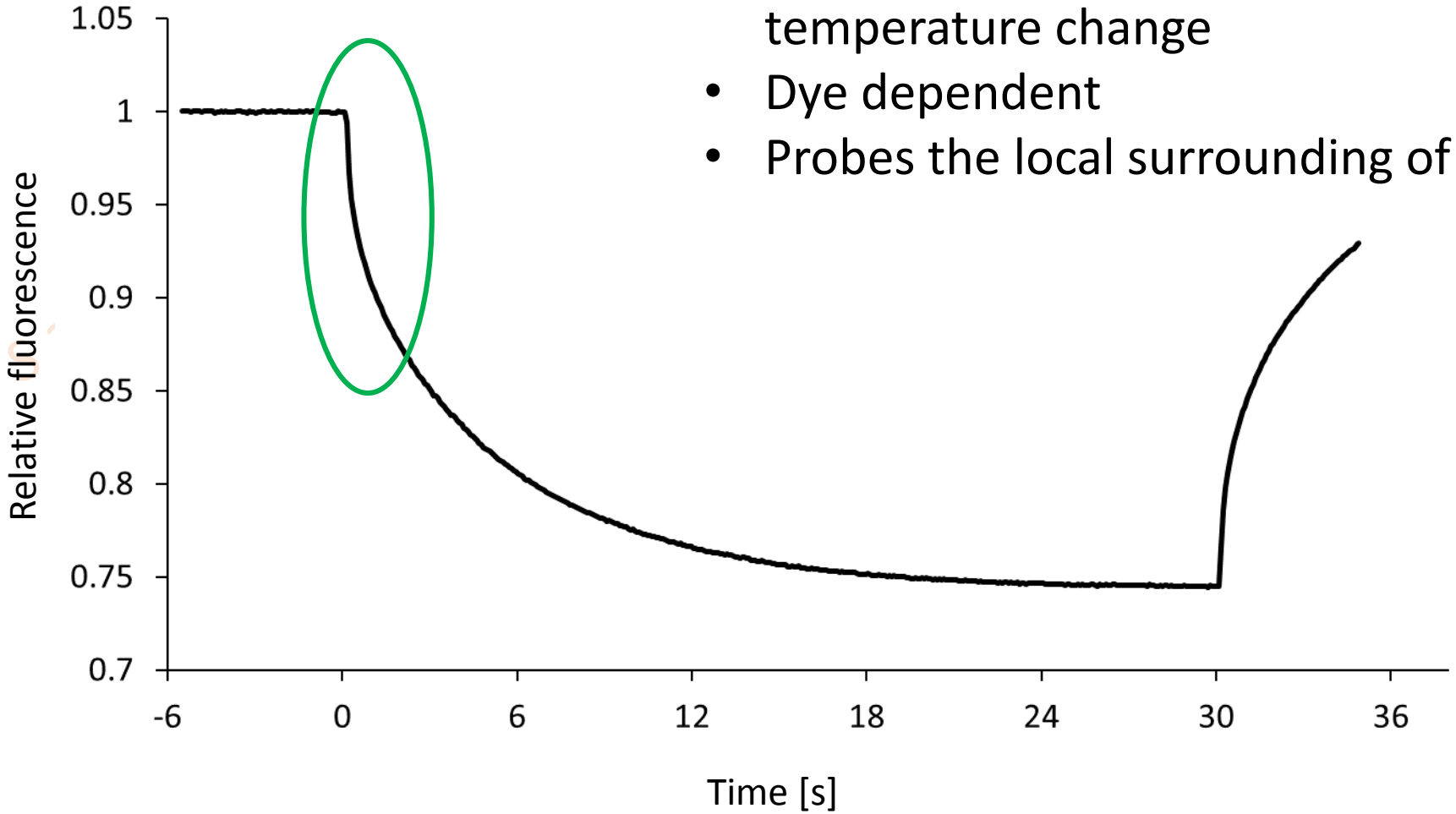
- Feature of the dye
- Can be induced/influenced by ligand



Dissecting the MST timetrace

T-jump

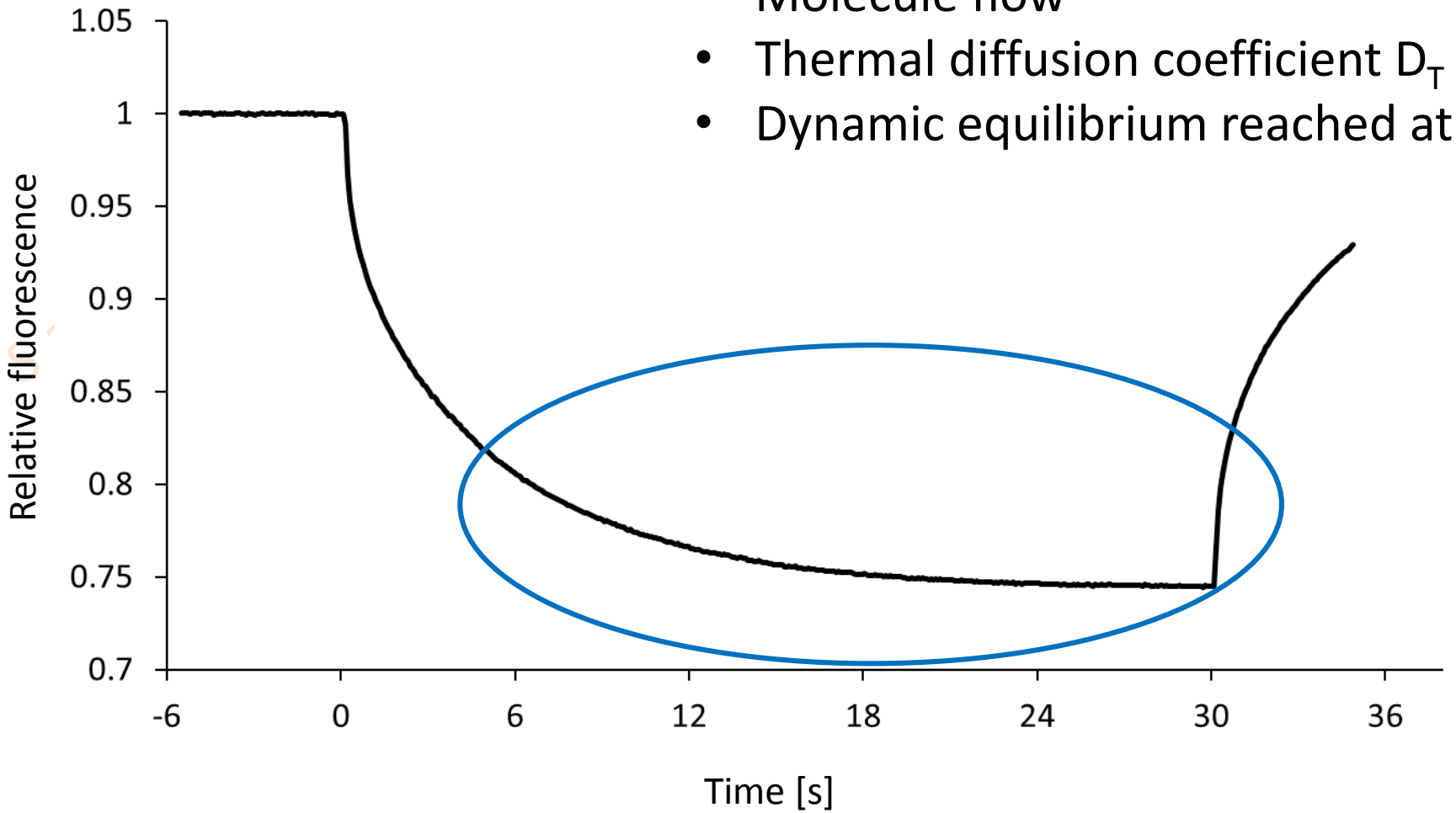
- Rapid decrease in fluorescence signal caused by temperature change
- Dye dependent
- Probes the local surrounding of the dye



Dissecting the MST timetrace

Thermophoresis

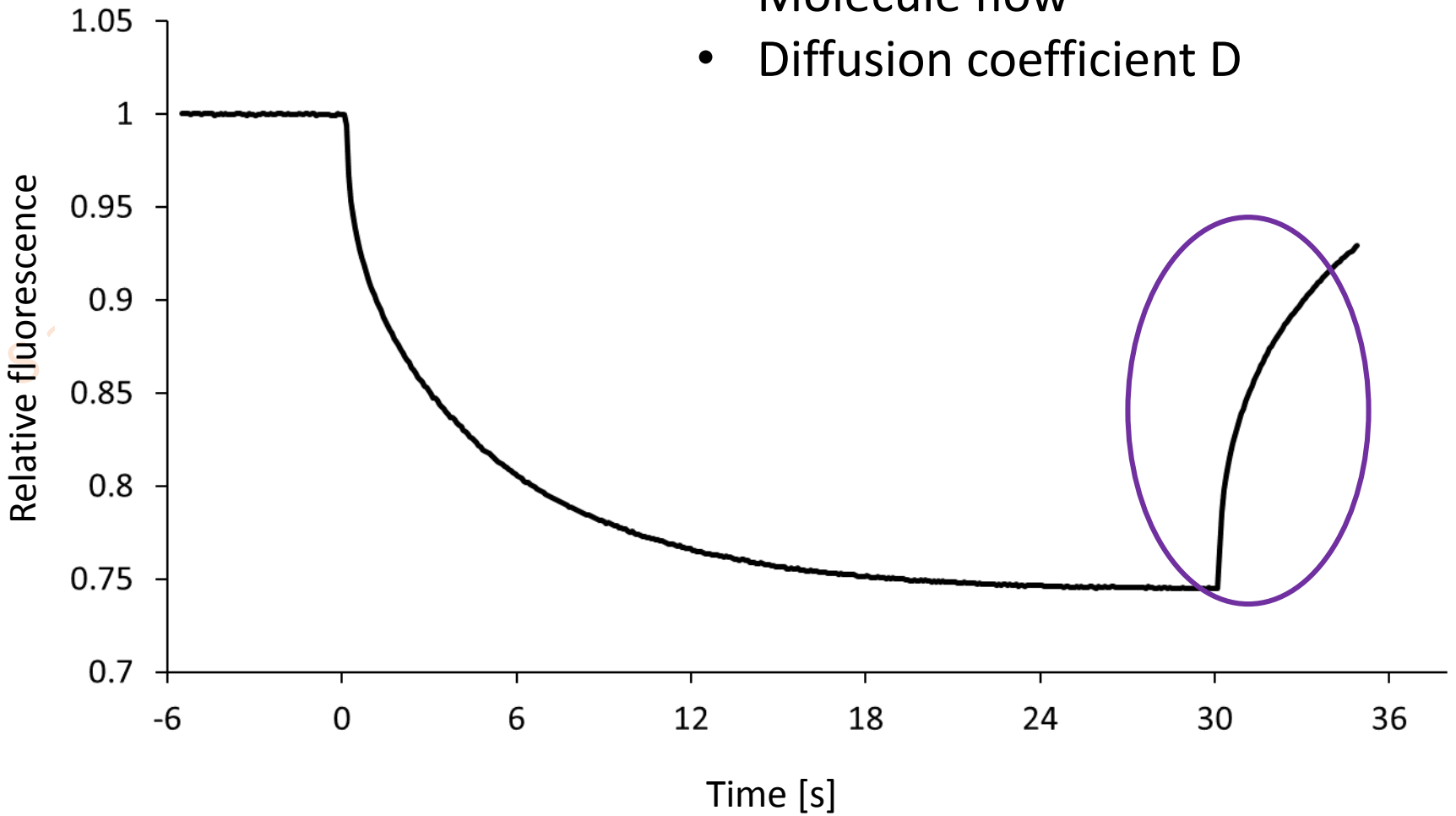
- Molecule flow
- Thermal diffusion coefficient D_T
- Dynamic equilibrium reached at steady state



Dissecting the MST timetrace

Mass diffusion

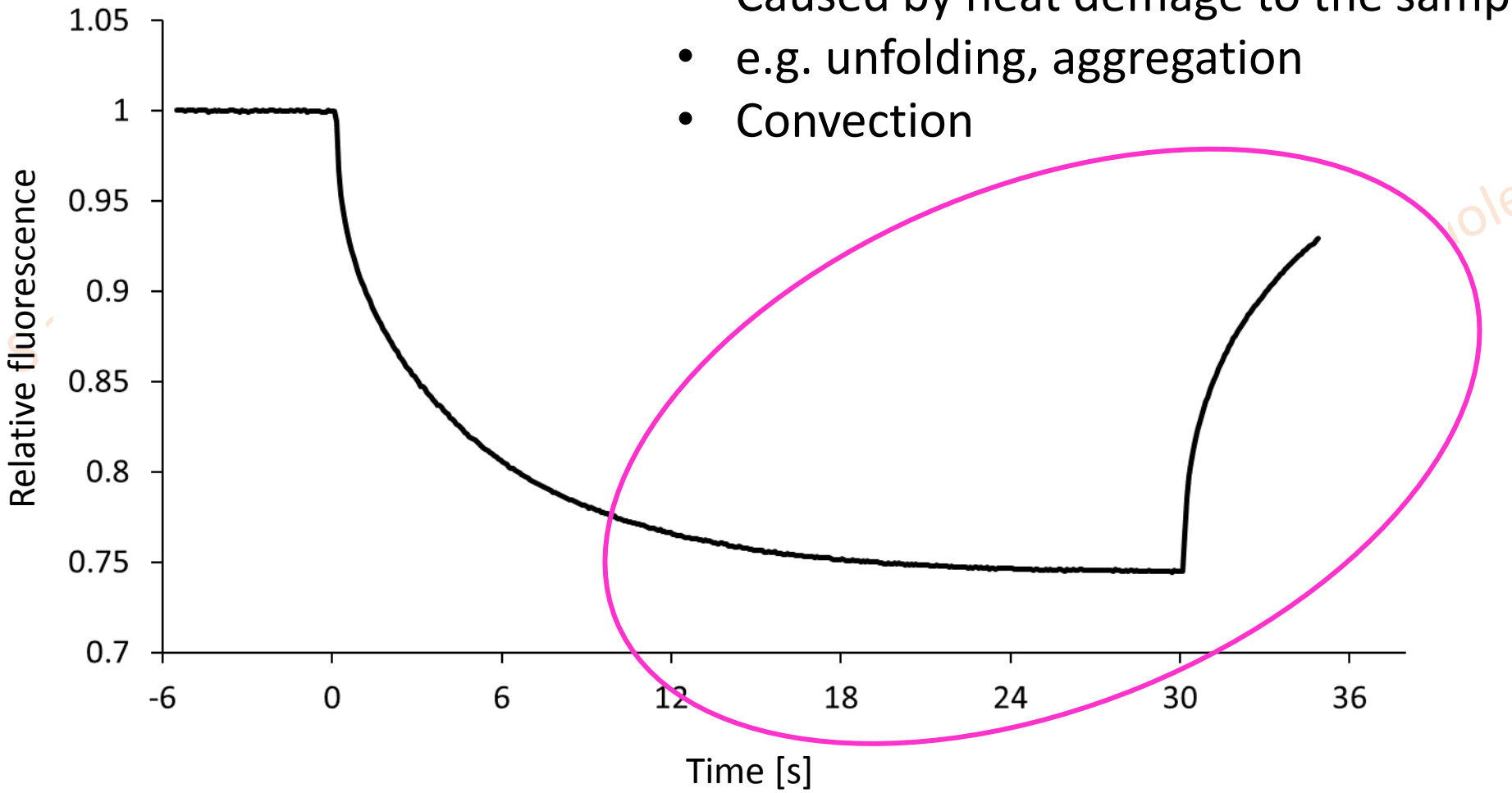
- Molecule flow
- Diffusion coefficient D



Dissecting the MST timetrace

Irreversible effects

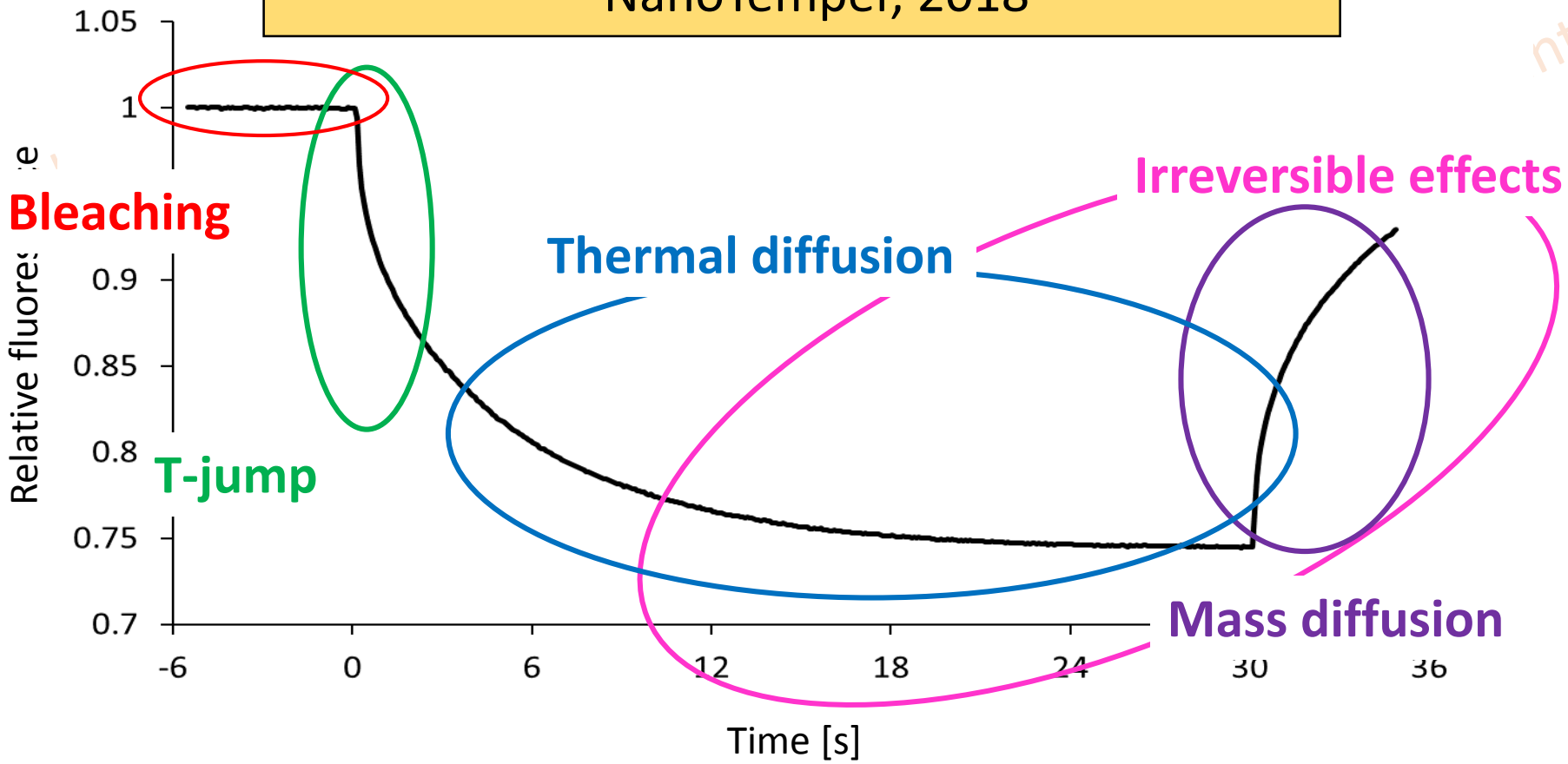
- Caused by heat demage to the sample
- e.g. unfolding, aggregation
- Convection



Dissecting the MST timetrace

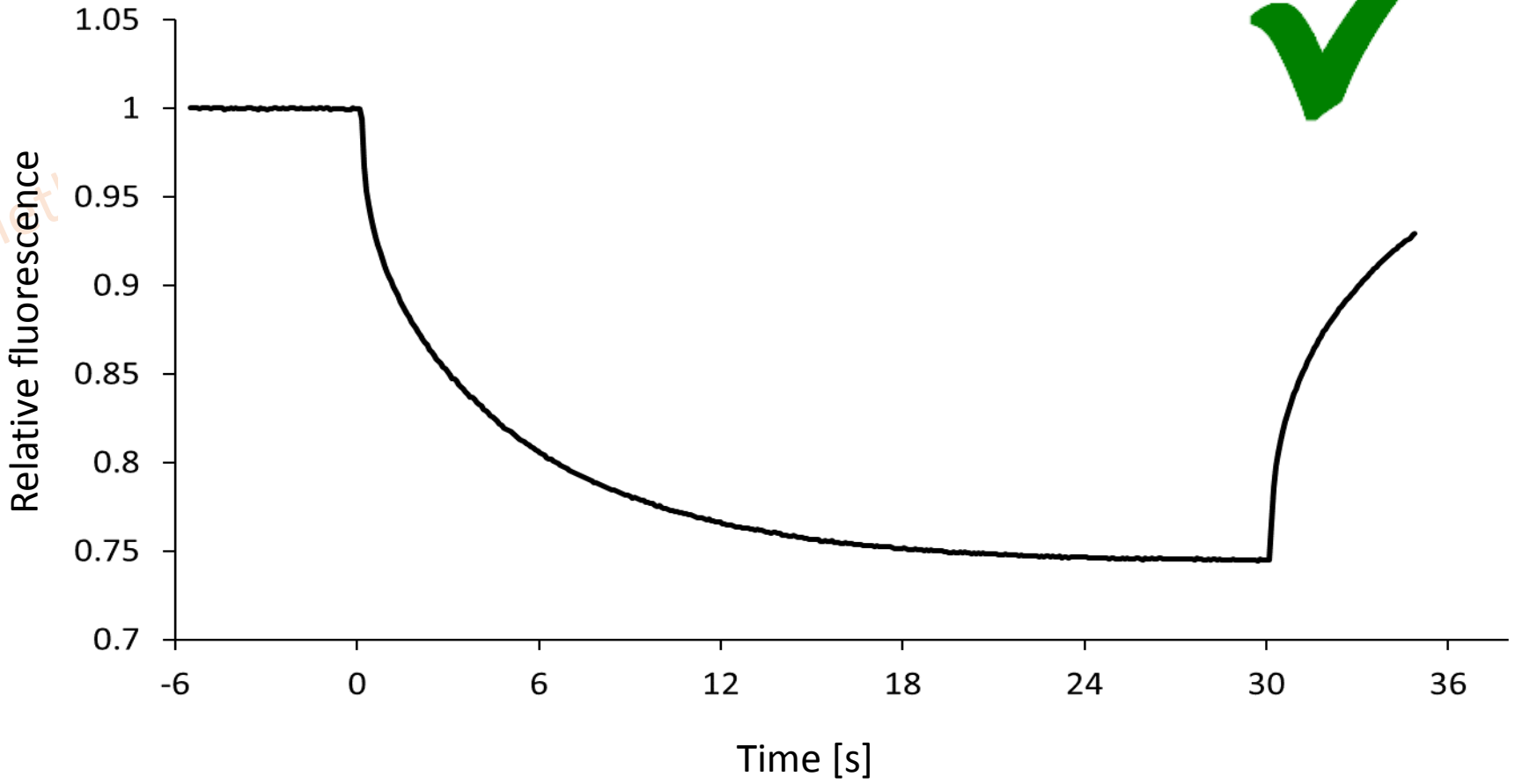
measures not only thermophoresis but „fluorescence under thermal perturbation“

TRIC – temperature related intensity change
NanoTemper, 2018

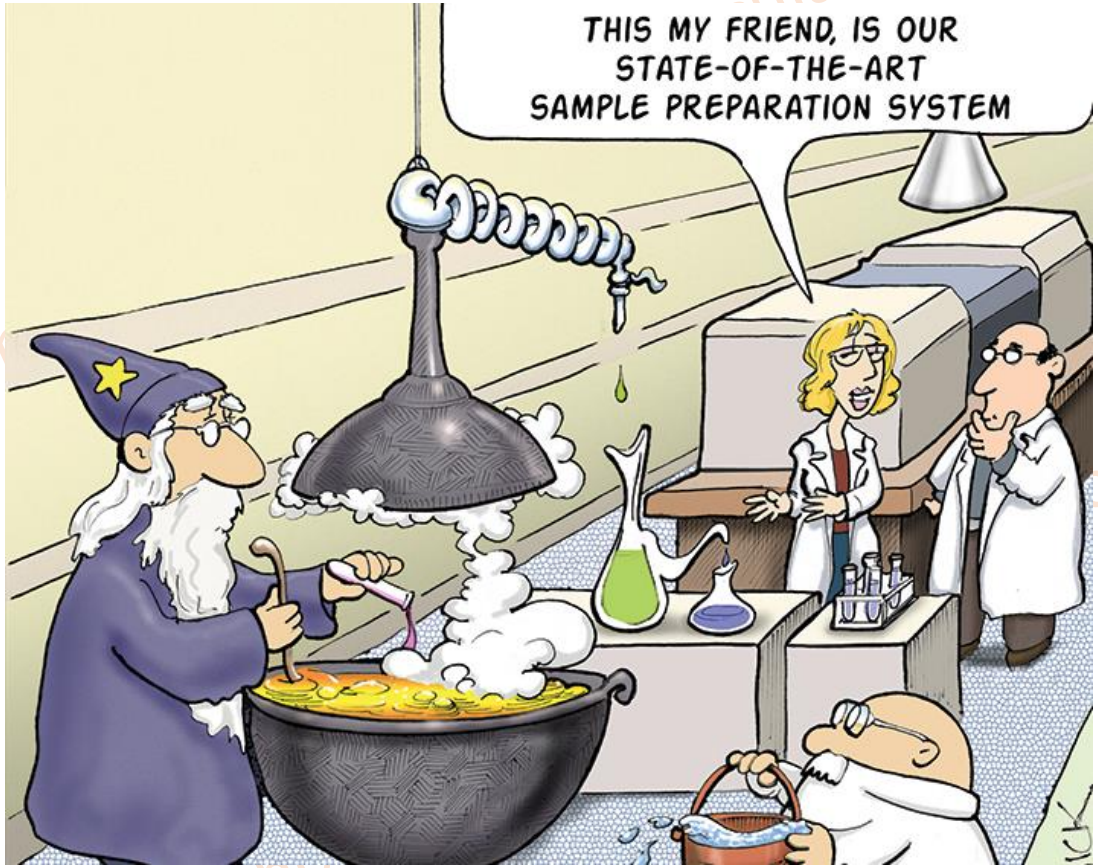


Dissecting the MST timetrace

Dissecting the TRIC timetrace

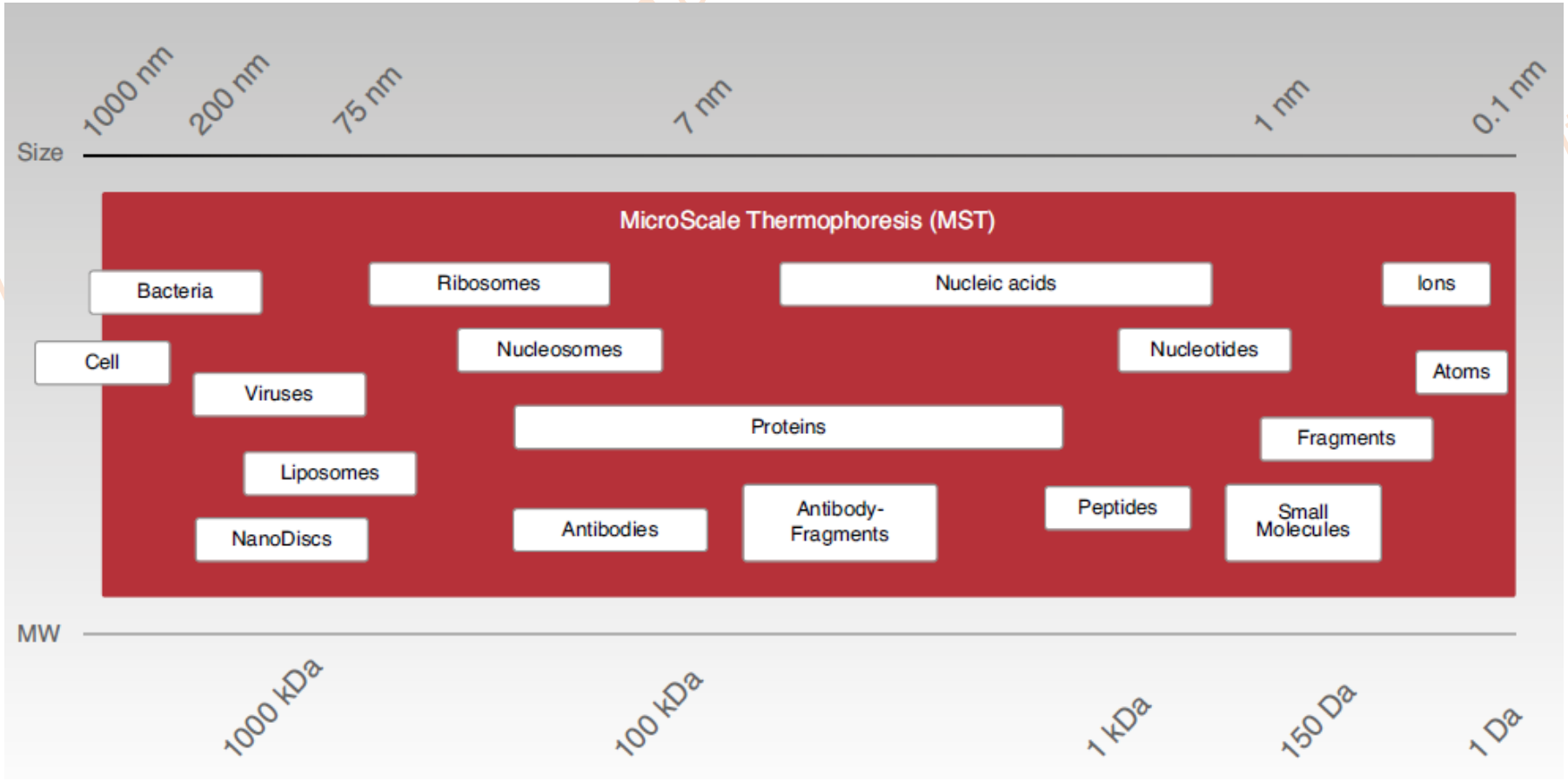


Sample



Sample

Size range



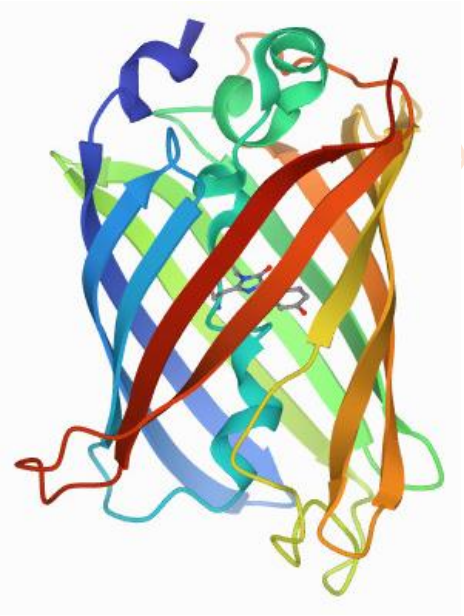
Sample

Labelling

- Monolith NT measures fluorescence signal

- Labelling is necessary unless
  - You work with fluorescent proteins
    - GFP (green)
    - YFP (yellow)

  - You have Monolith label free instrument
    - Uses intrinsic flourescence of tryptophanes



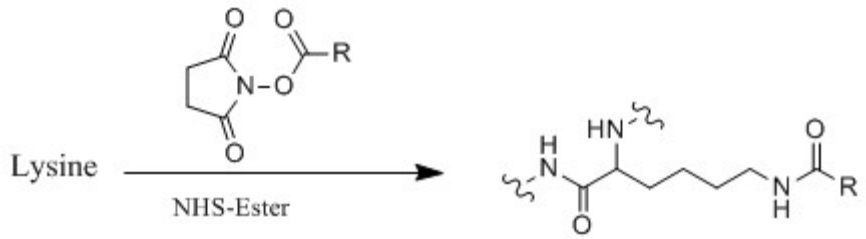
GFP, PDB: 4OGS

Sample

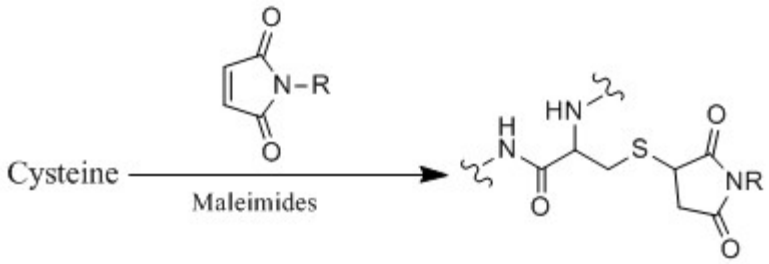
Labelling

Reactive groups availability:

Amino group – Lysine, N-terminus



Thiol (sulfhydryl) group – Cystein



His-tag

Sample

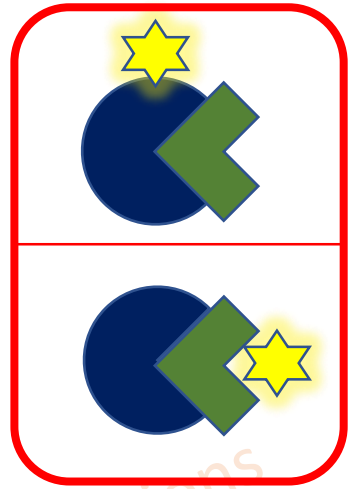
Labelling

- Dyes compatible with blue, green or red laser
- Commercial dyes or specialised dyes from NanoTemper

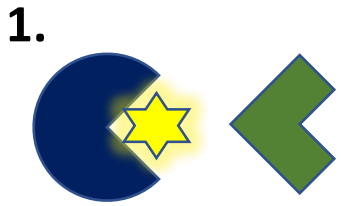
Monolith NT.115	LED 1 /nm	LED 2 /nm	Blue Dyes	Green Dyes	Red Dyes
NT.115 Blue/Green	Ex:470 Em:520	Ex:550 Em:600	FITC/FAM/GFP/YFP	Cy3/RFP/mCherry	no detection
NT.115 Blue/Red	Ex:470 Em:520	Ex:625 Em:680	FITC/FAM/GFP/YFP	no detection	Cy5/Alexa647
NT.115 Green/Red	Ex:520 Em:570	Ex:625 Em:680	YFP	Cy3/RFP	Cy5/Alexa647

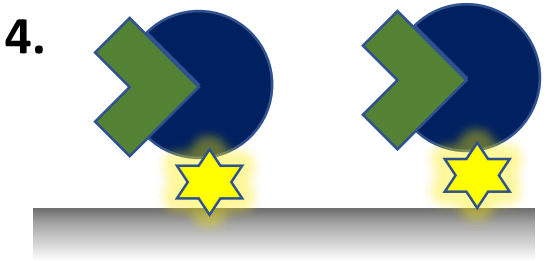
Which binding partner to label?

Interference with interaction

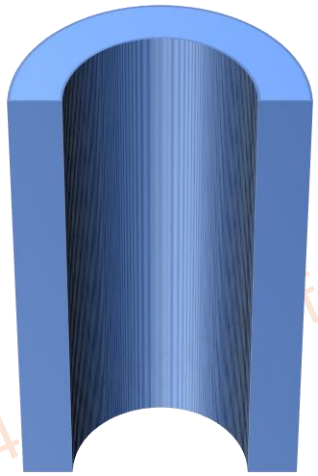


1. Sterical hindrance
2. Conformation changes
3. Non-specific interaction
4. Adhesion to labware
5. Solubility change, aggregation

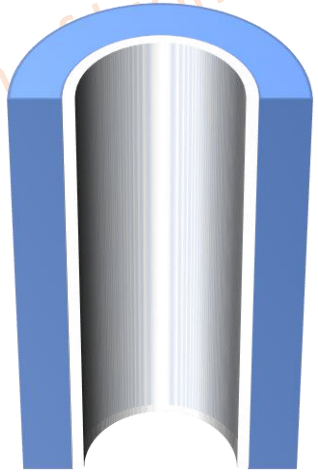




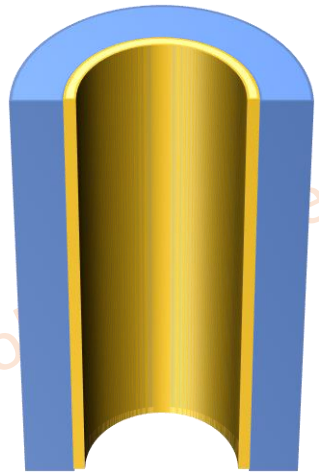
Capillaries



- Standard



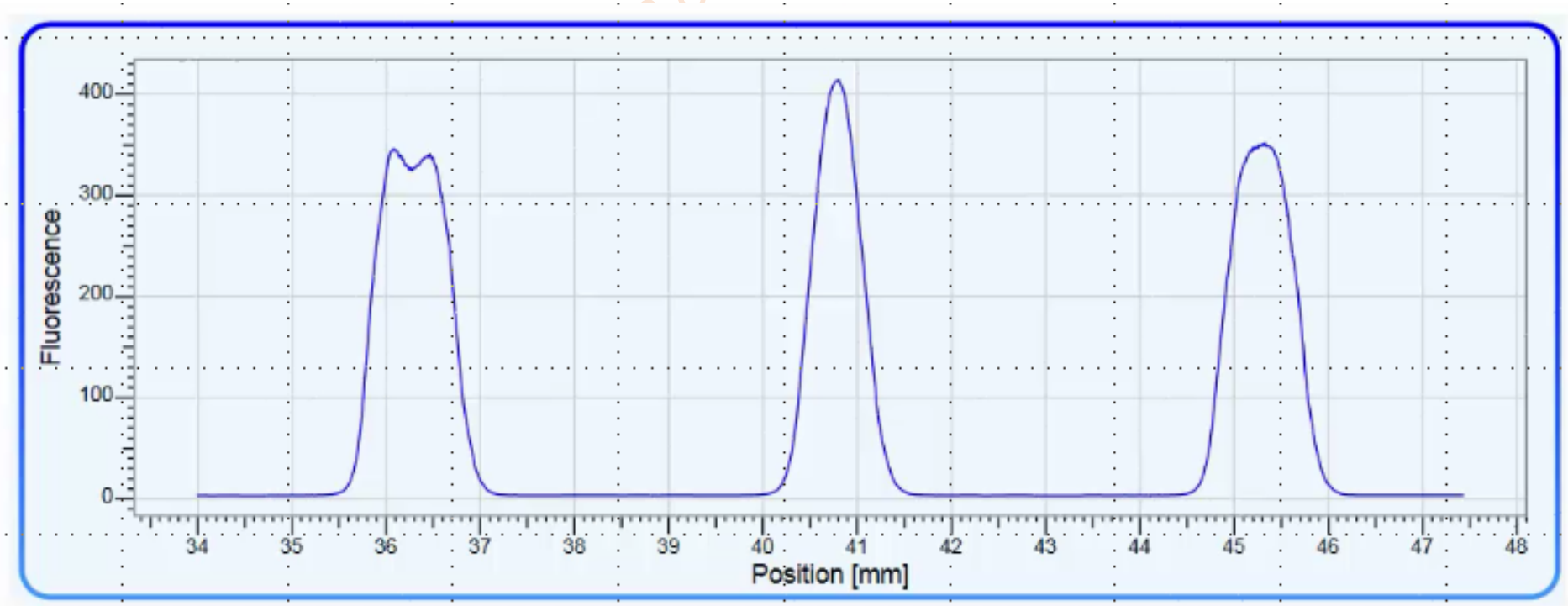
- Coated (premium)



- Hydrophobic

Which capillary to use?

Sample sticking to the capillary wall



A lot of sticking

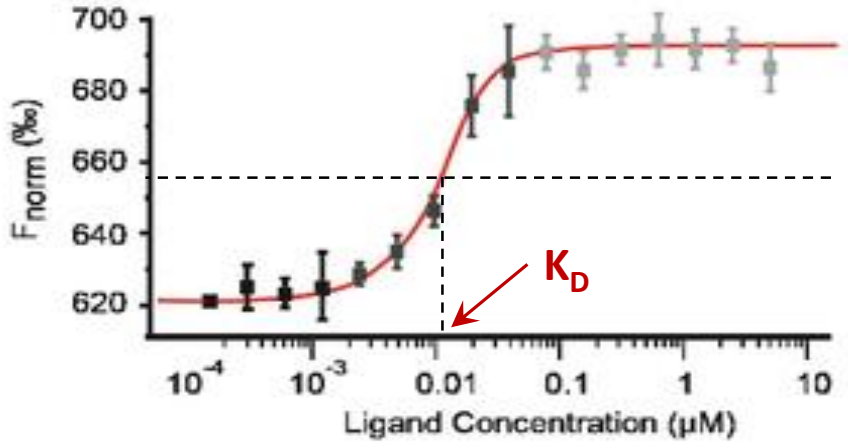
No sticking

A bit of sticking

What can be measured by MST?

Affinity

- What is the strength of interaction?
- Labelled partner (target) at constant $c \leq K_D$
- Serial dilution of second partner (ligand) in range of expected K_D



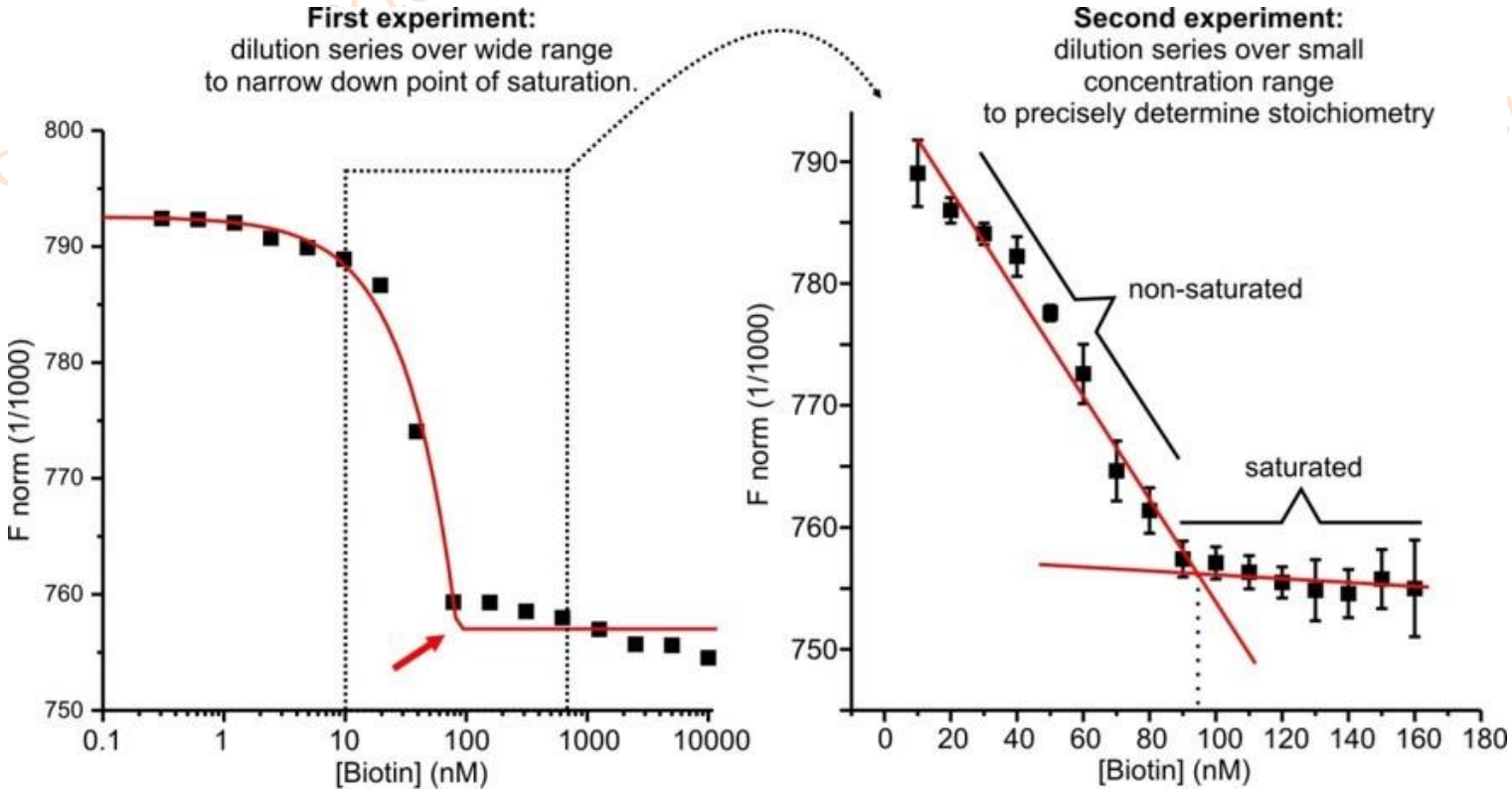
More than affinity (special cases)

- **Stoichiometry** determination
- **Multiple binding events** within one experiment
- **Inhibition assay**
- **Thermodynamics** measured by MST
- Interaction with **liposomes**
- Measurement in **crowdy samples**
  (blood, cell lysate)

S 2004 Methods for characterization of biomolecular interactions
S 2004 Methods for characterization of biomolecular interactions

Stochiometry

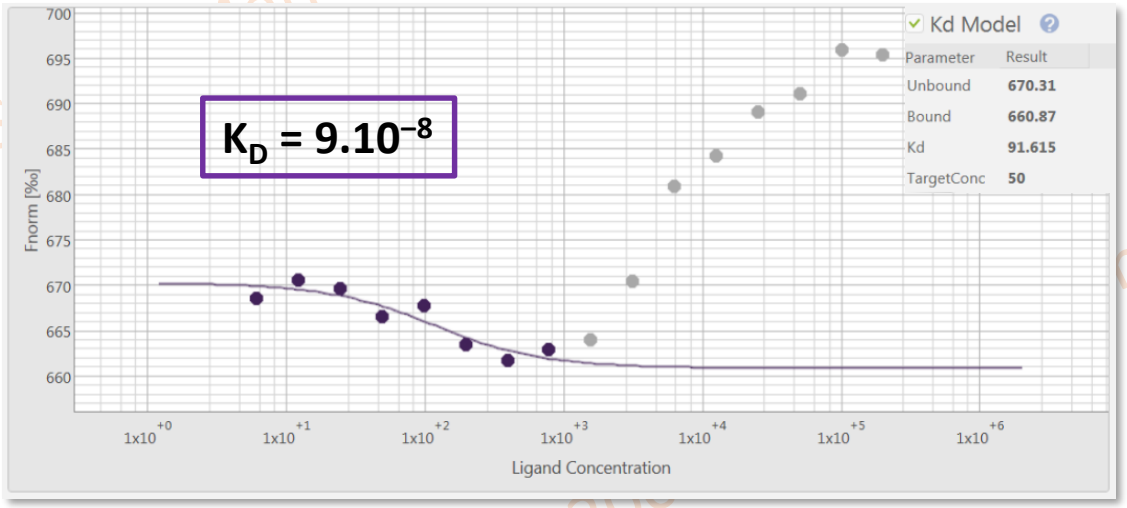
- Labeled partner at constant c > K_D
- Several dilution of second partner in range of expected molecular ratio

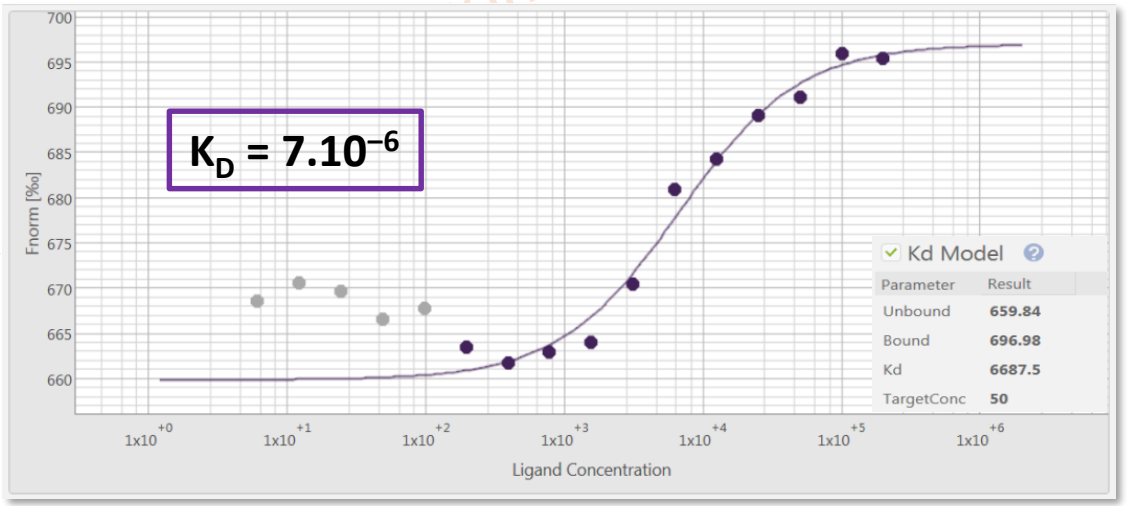


Multiple binding events

Two independent binding events in one measurement

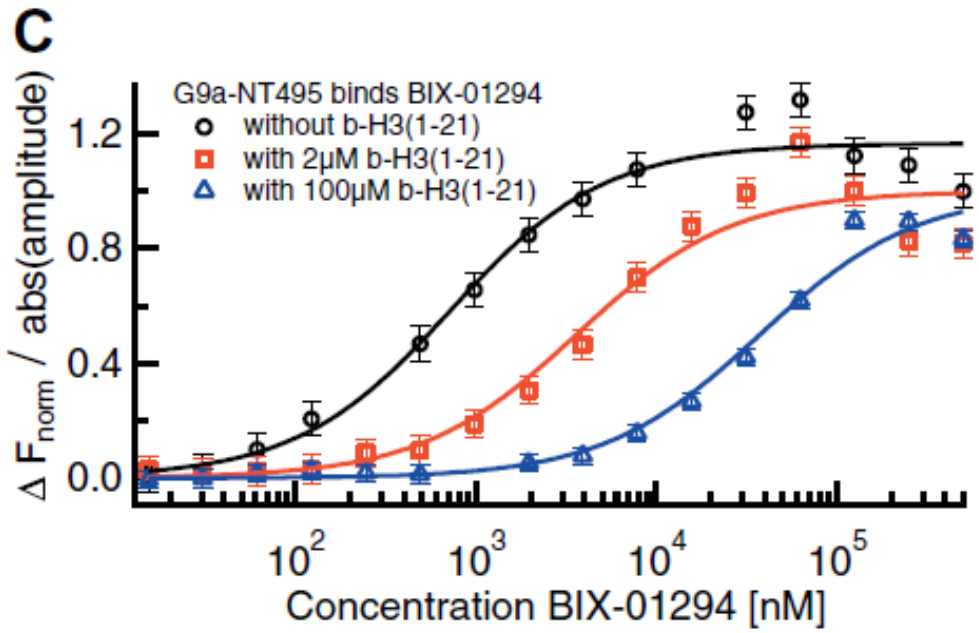
- Labeled partner at constant $c \leq K_{D,(stronger)}$
- Both K_D's far enough to be distinguishable but close enough to be covered within one dilution row

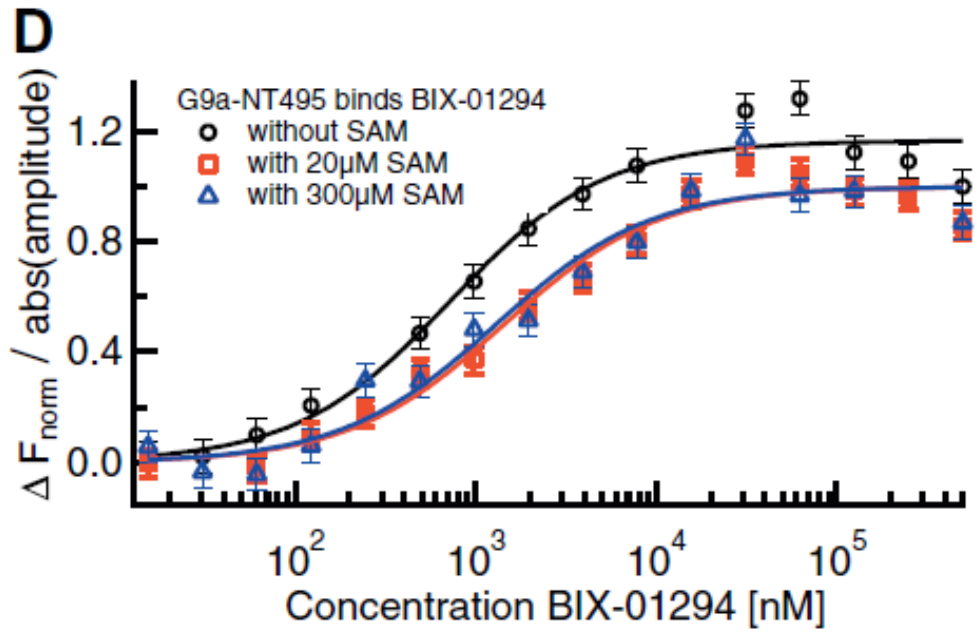




Inhibition assay

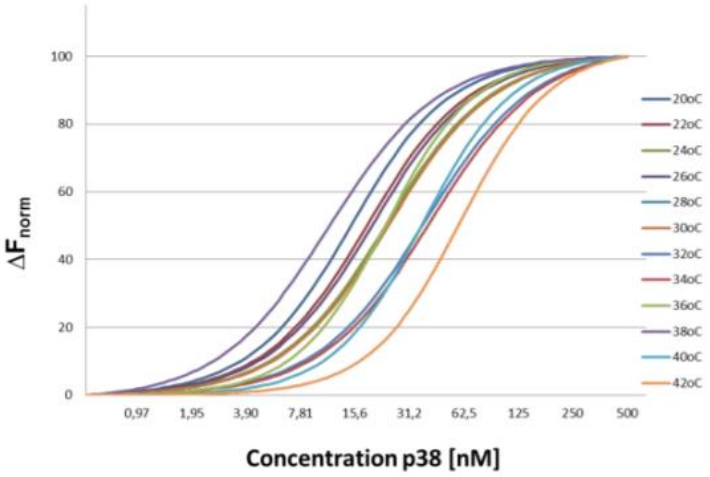
- Standard affinity measurement in **presence** and **absence** of inhibitor
- Comparison of curves / calculated K_D



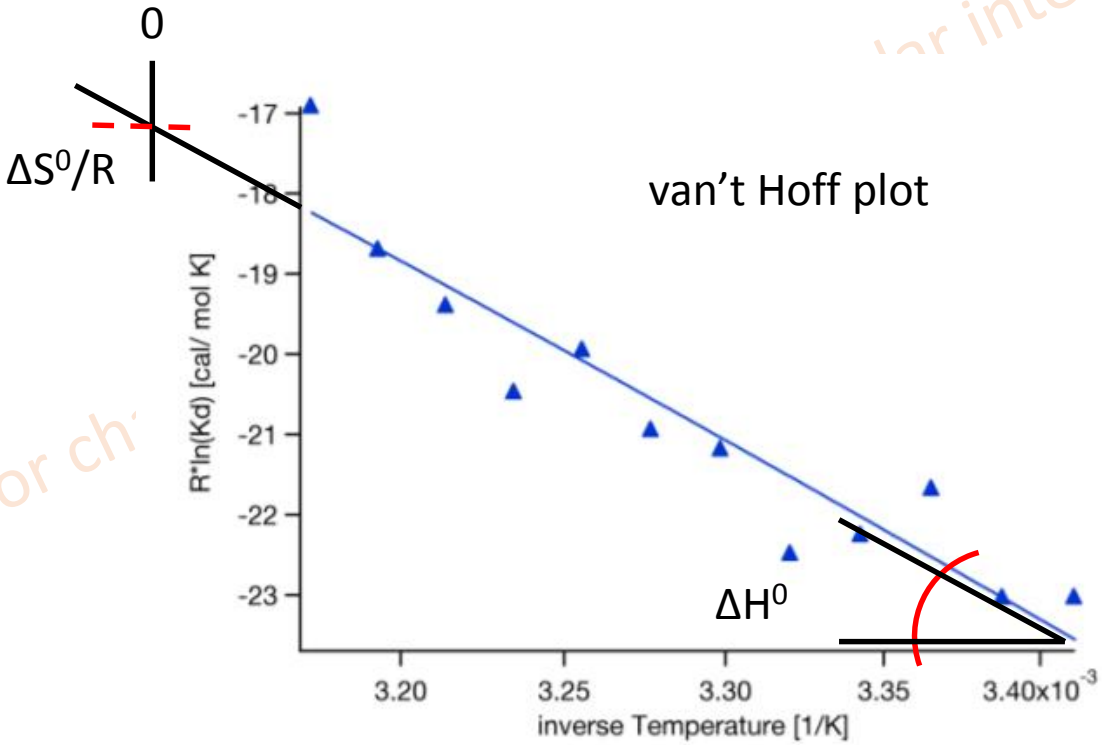


Thermodynamics

- K_D determination at various temperatures
- Calculation of thermodynamic parameters



K_D's

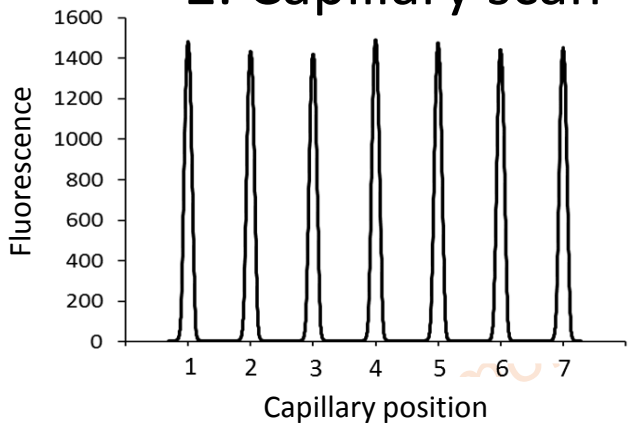


Enougth theory lets put it into practice

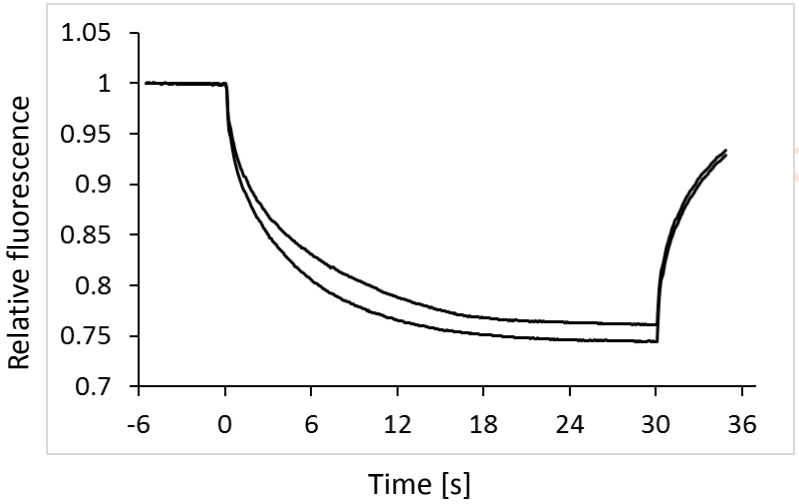
Workflow

1. Loading capillaries

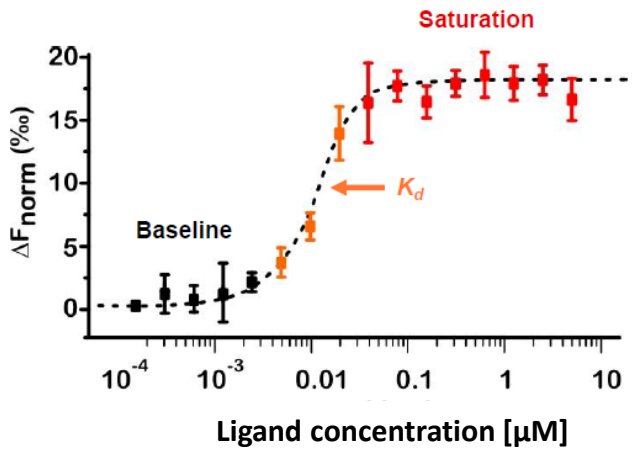
2. Capillary scan



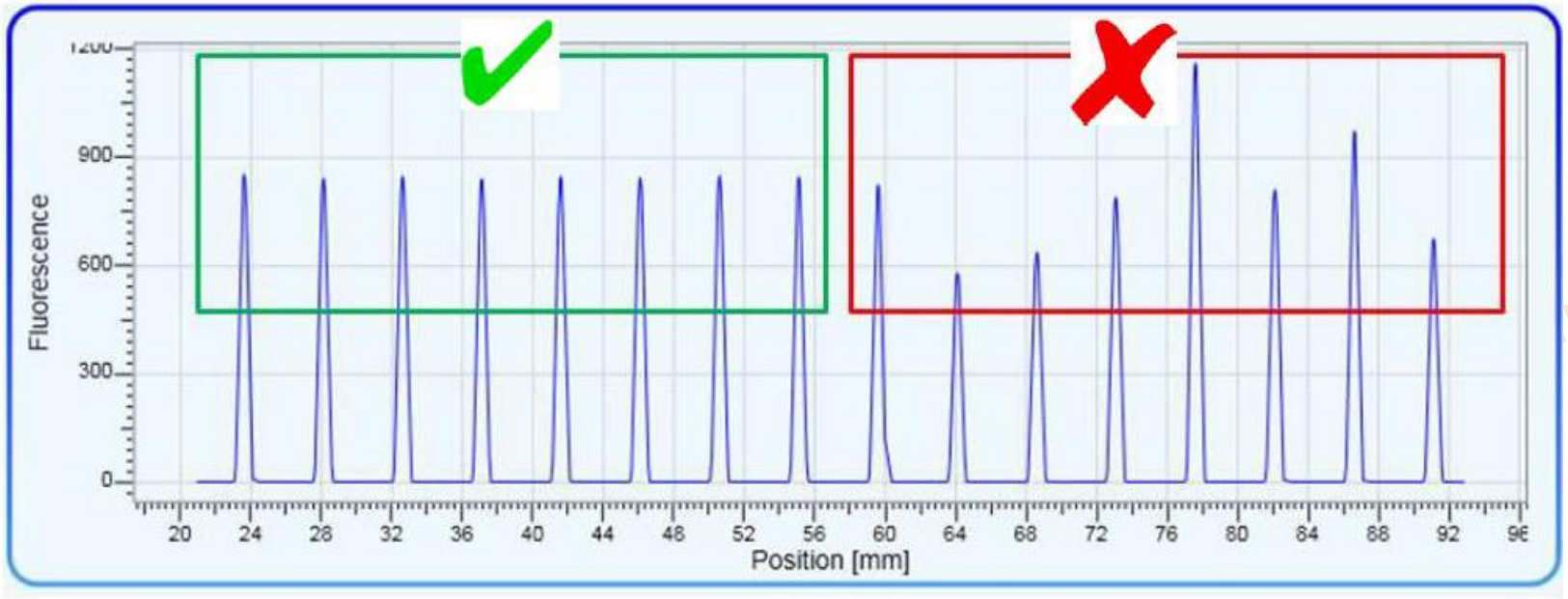
3. MST measurement



4. Data analyses

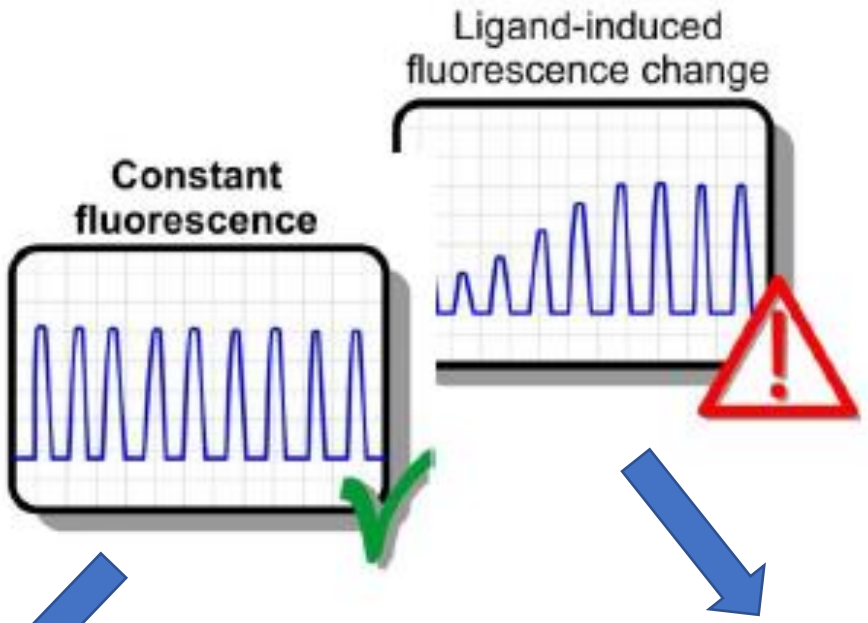


Troubleshooting: fluorescence



- Optimize sample quality
- Sample homogeneity
- Pipet more accurately
  - MST is super sensitive

Troubleshooting: fluorescence



Standard measurement

Direct analysis

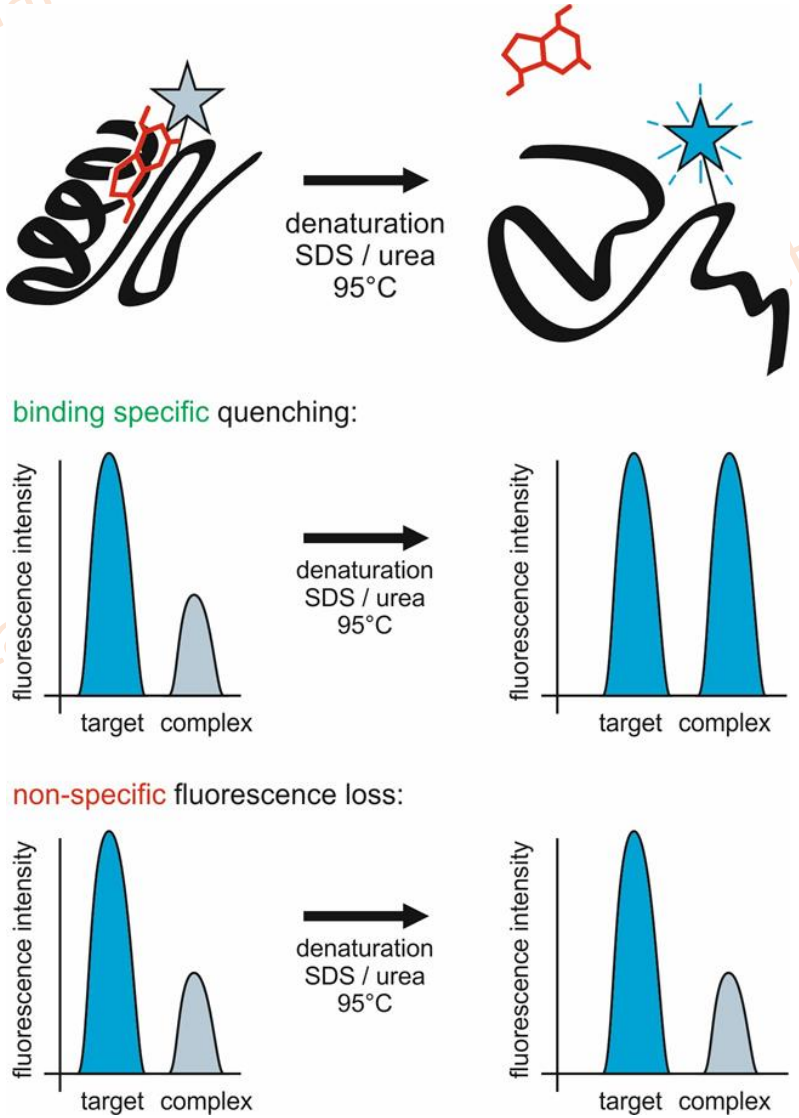
or

Optimization of conditions
(additives, buffer, labeling)

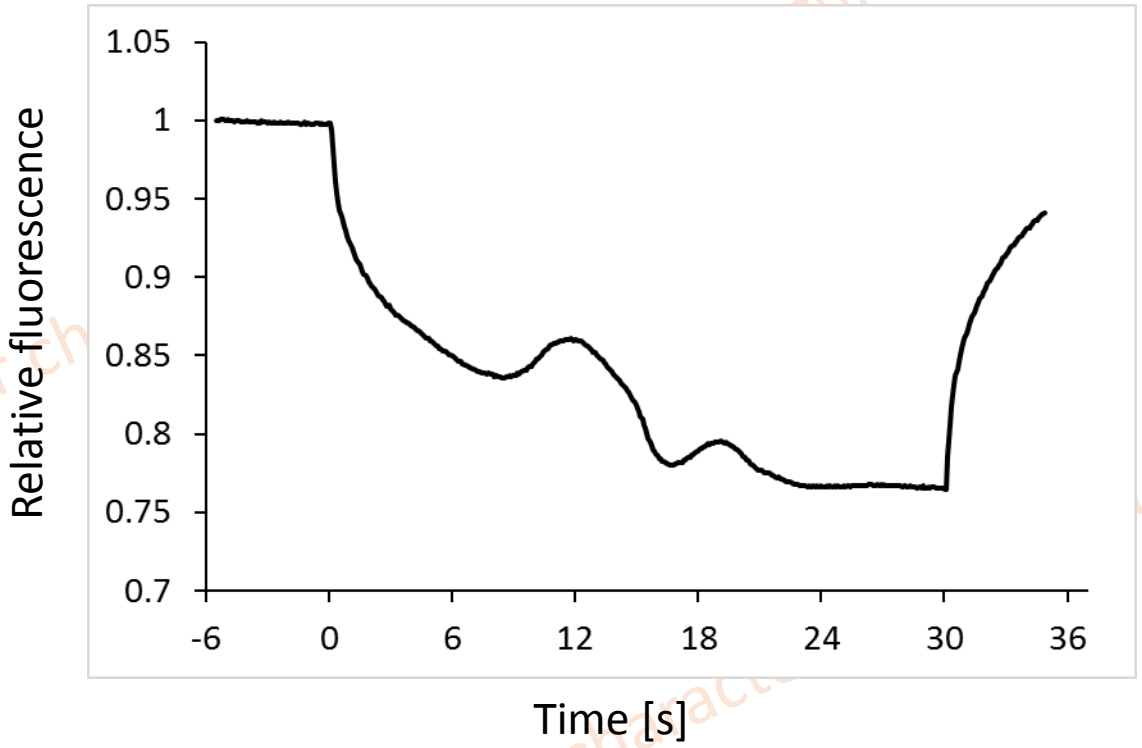
Troubleshooting: fluorescence

SD test

- add SDS + DTT mix to first and last sample
  (lowest and highest concentration)
- denature (95°C, 5 min)
- check fluorescence



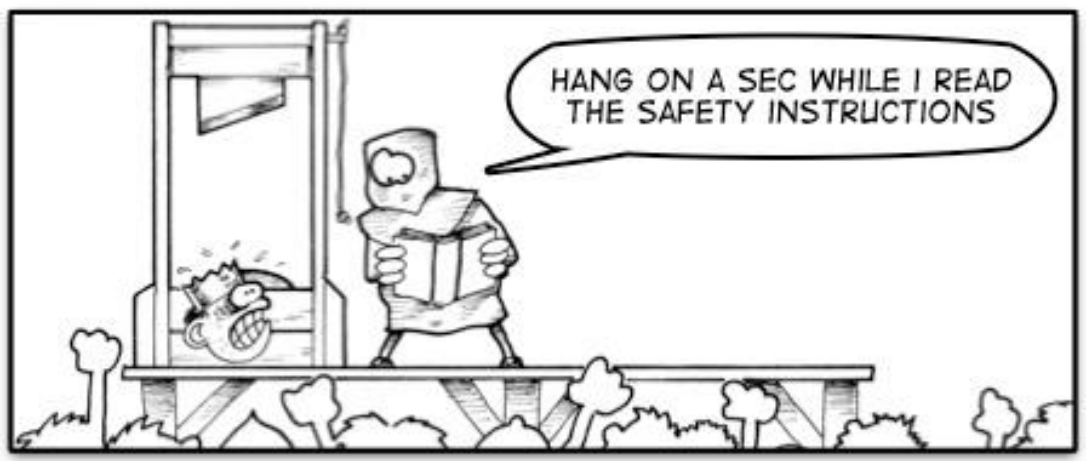
Troubleshooting: aggregation



Optimization is necessary:

- Centrifuge sample before loading capillary
- Add detergents (0.05% TWEEN20, pluronic F-12, BSA)
- Optimize buffer composition (pH, salt, additives)

Real hardware



MST machines

- Monolith NT.115
- Monolith NT.115^{Pico}
- Monolith LabelFree
- Monolith Automated

  – *all Nanotemper*

Monolith Automated

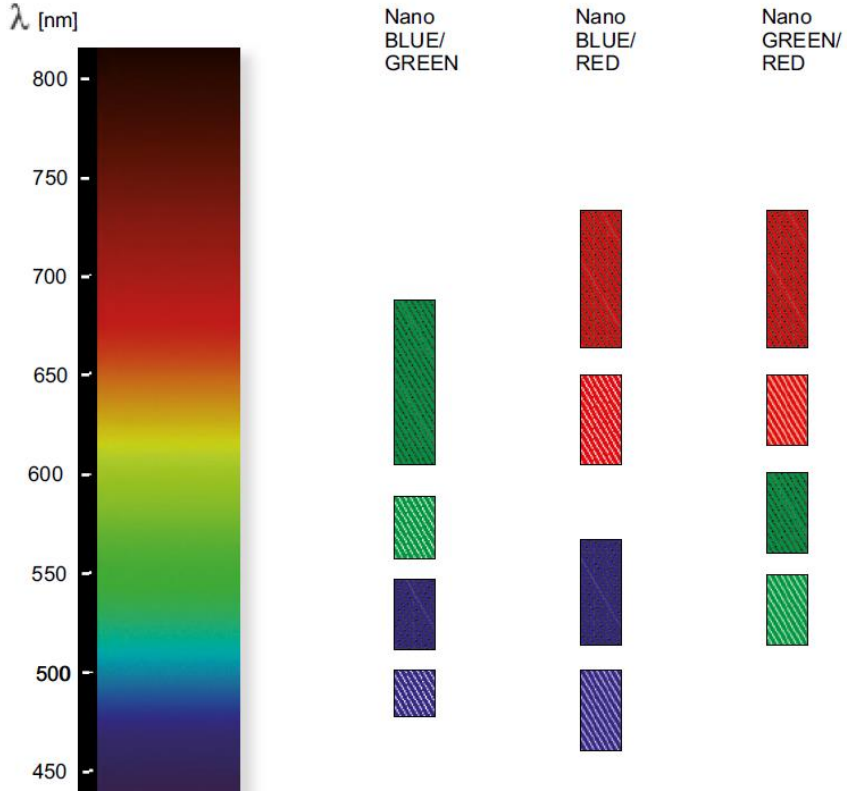
Monolith NT.115

Monolith LabelFree

Monolith NT.115^{Pico}

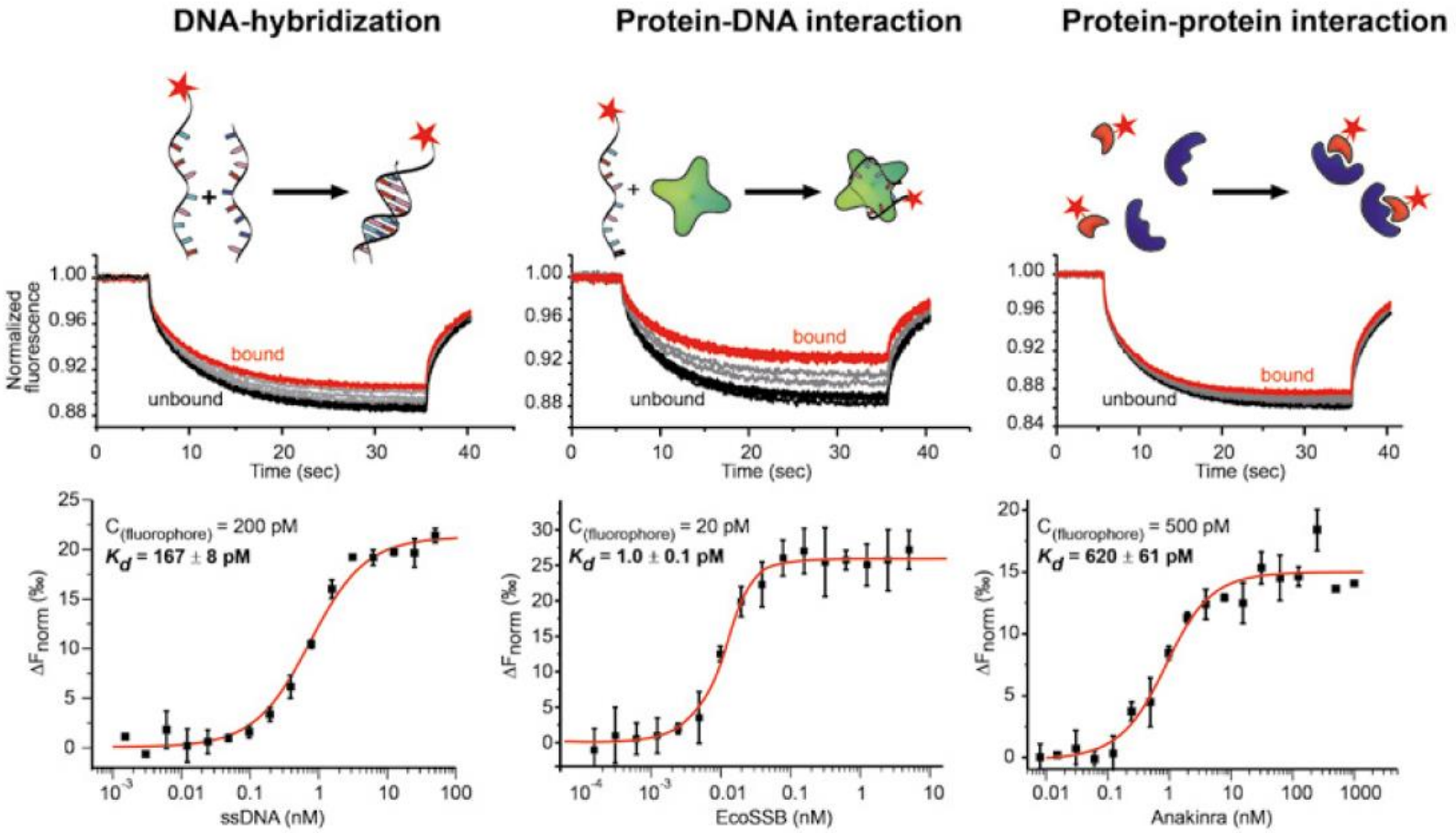
Monolith NT.115

- nM to mM K_D range
- 16 capillaries (24 in new version)
- Two fluorescence channels (BLUE, GREEN, RED)



Monolith NT.115^{Pico}

- pM to mM K_D range
- Only RED fluorescence channel



Monolith LabelFree

- One channel only
- Excitation wavelength: 280 nm
- Emission wavelength: 360 nm

Monolith NT.LabelFree	LED 1 /nm	Molecules (examples)
NT.Label Free	Ex:280 Em:360	Proteins containing Tryptophane 2-Aminopurin 8-vinyl-deoxyadenosine BIRB-796

Closest machines:

Prague – BIOCEV

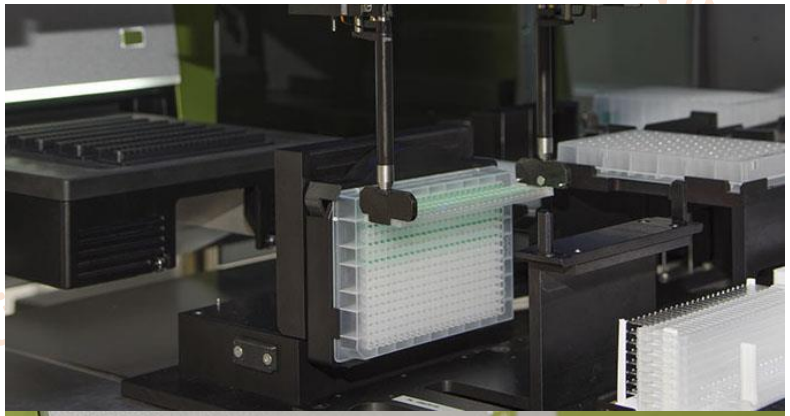
Vienna – VBCF (Vienna Biocenter)

Monolith Automated

Two channels possible

96 samples in a run

Fragment screening



Summary

- Thermophoresis is sensitive to subtle changes – almost every interaction will give a signal
- Almost every sample that goes in capillary can be measured: ions – cells
- Little sample consumption is used (compared to ITC)
- Raw data has to be carefully examined for additional effects

Summary

- Monolith NT measures not only thermophoresis but „fluorescence under thermal perturbation“

TRIC – temperature related intensity change
NanoTemper, 2018

- Evaluate MST curves ONLY IF the ligand DO NOT induce:
  - fluorescence change
  - bleaching

S 2004 Methods for characterization of biomolecular interactions
S 2004 Methods for characterization of biomolecular interactions

Materials for further study

- Ch.J.Wienken et al. (2010), Nature communication
  Protein-binding assays in biological liquids using microscale thermophoresis

- B.López-Méndez et al (2021), Eur. Biophys. J.

Microscale Thermophoresis and additional effects measured in NanoTempres Monolith instruments

- http://www.nanotempertech.com/
  basics, operation manuals, product sheets, explorer community

MST at BIC

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C rystallization Core Facility

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