# When binding affinity matters.

Jakub Nowak| PhD

19.05.2018 8th Regional Biophysics Conference RBC 2018, Zrece, Slovenia

NANOTEMPER

## 1856 / 1879

Carl Ludwig

Charles Soret

## 2008

**Foundation: LMU Munich**

Fast thermo-optical particle characterisation
**PCT Patent 2008/061706**

## 2010

**Monolith NT.115**

**Red Dot Design Award**

**German Innovation Award**

## 2012

## 2014

**Monolith NT.Automated**

**Prometheus NT.48**

**German Founders Prize**

## 2016

**Solutions for full automation**

## 2017

**Growth Champion**

## 2018

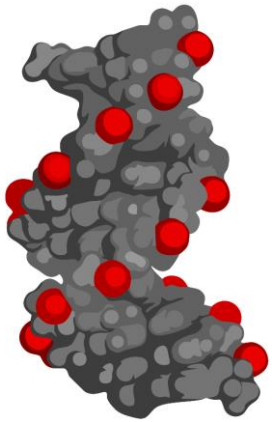
**Tycho NT.6**

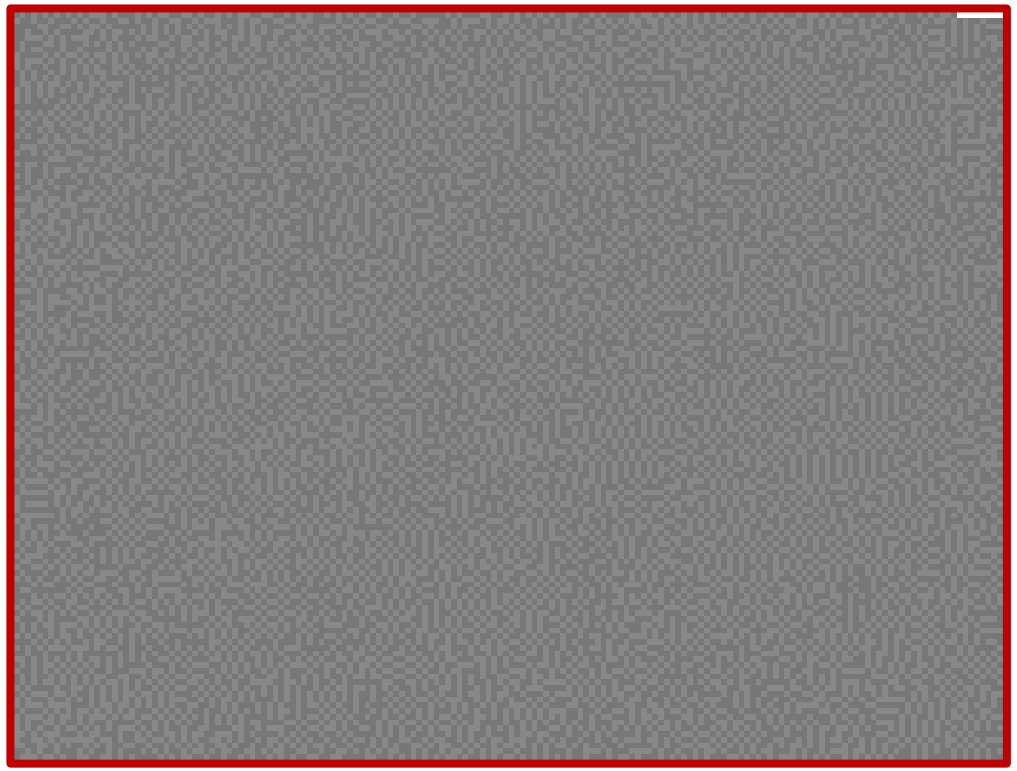


NANOTEMPER

# MicroScale Thermophoresis

DNA



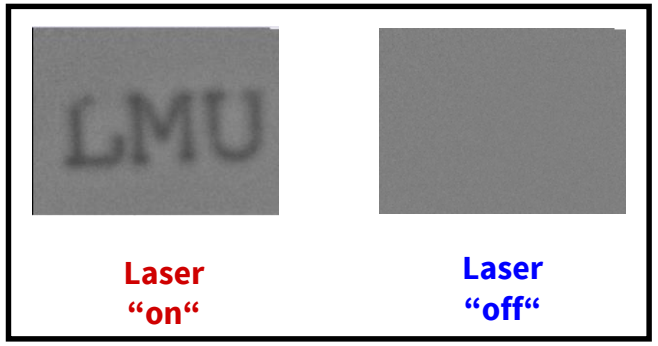


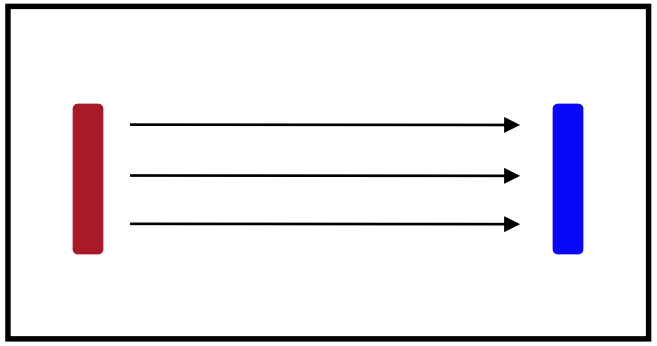
Duhr and Braun, PNAS, 2006
Duhr and Braun, PRL, 2006

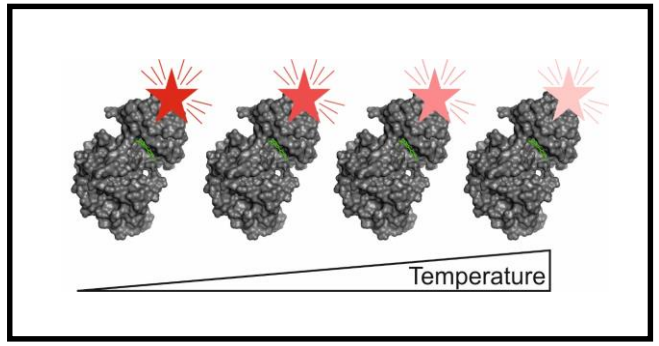
# Theory of MST: Thermophoresis and TRIC

$$\frac{\partial}{\partial T}(cF) = F\frac{\partial c}{\partial T} + c\frac{\partial F}{\partial T}$$

Change in overall fluorescence signal with temperature = Change in concentration with temperature (thermophoresis component) + Change in fluorescence with temperature (TRIC component)

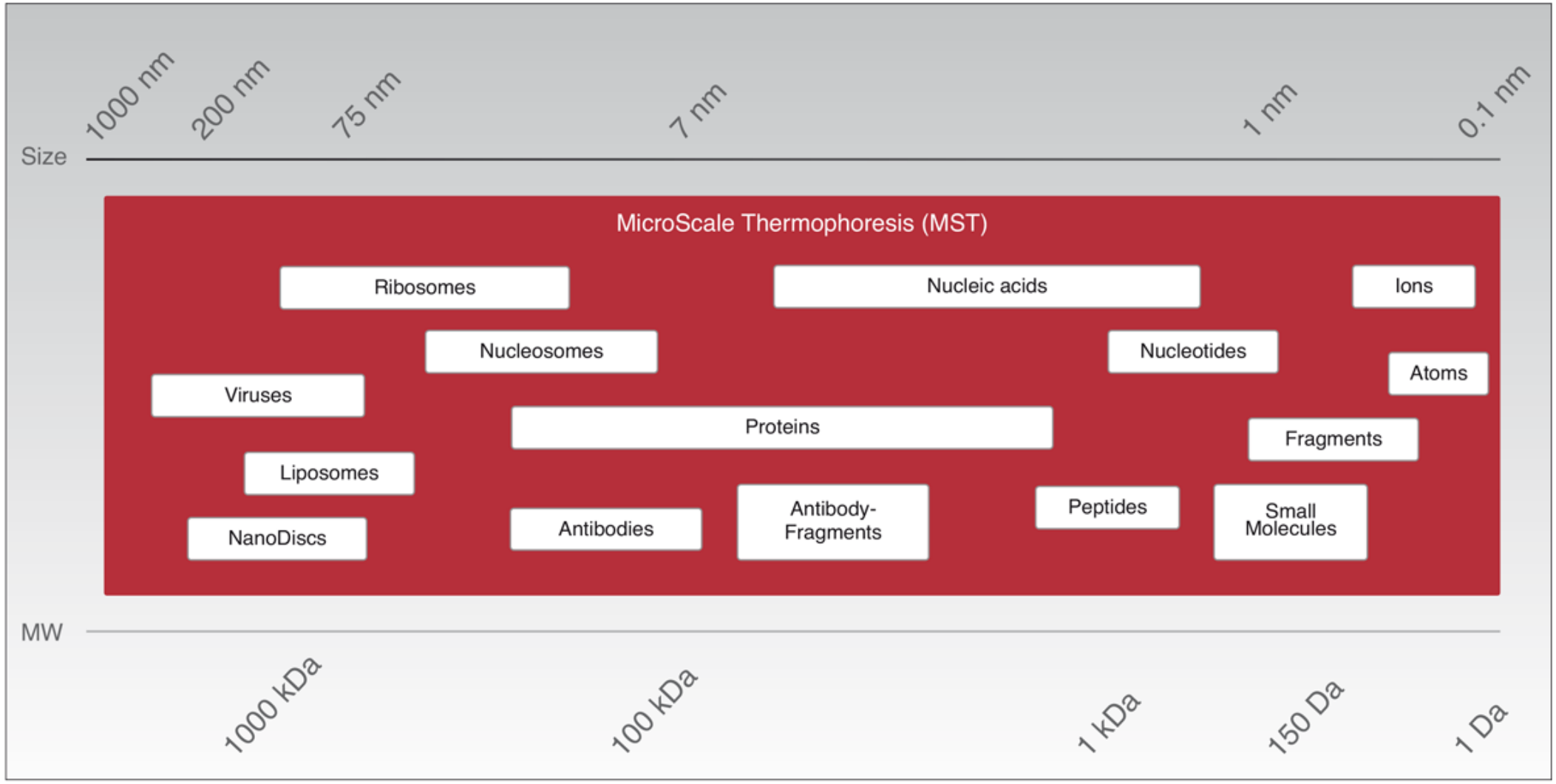





Baaske et al., ***Angew Chem Int Ed Engl***, *2010*

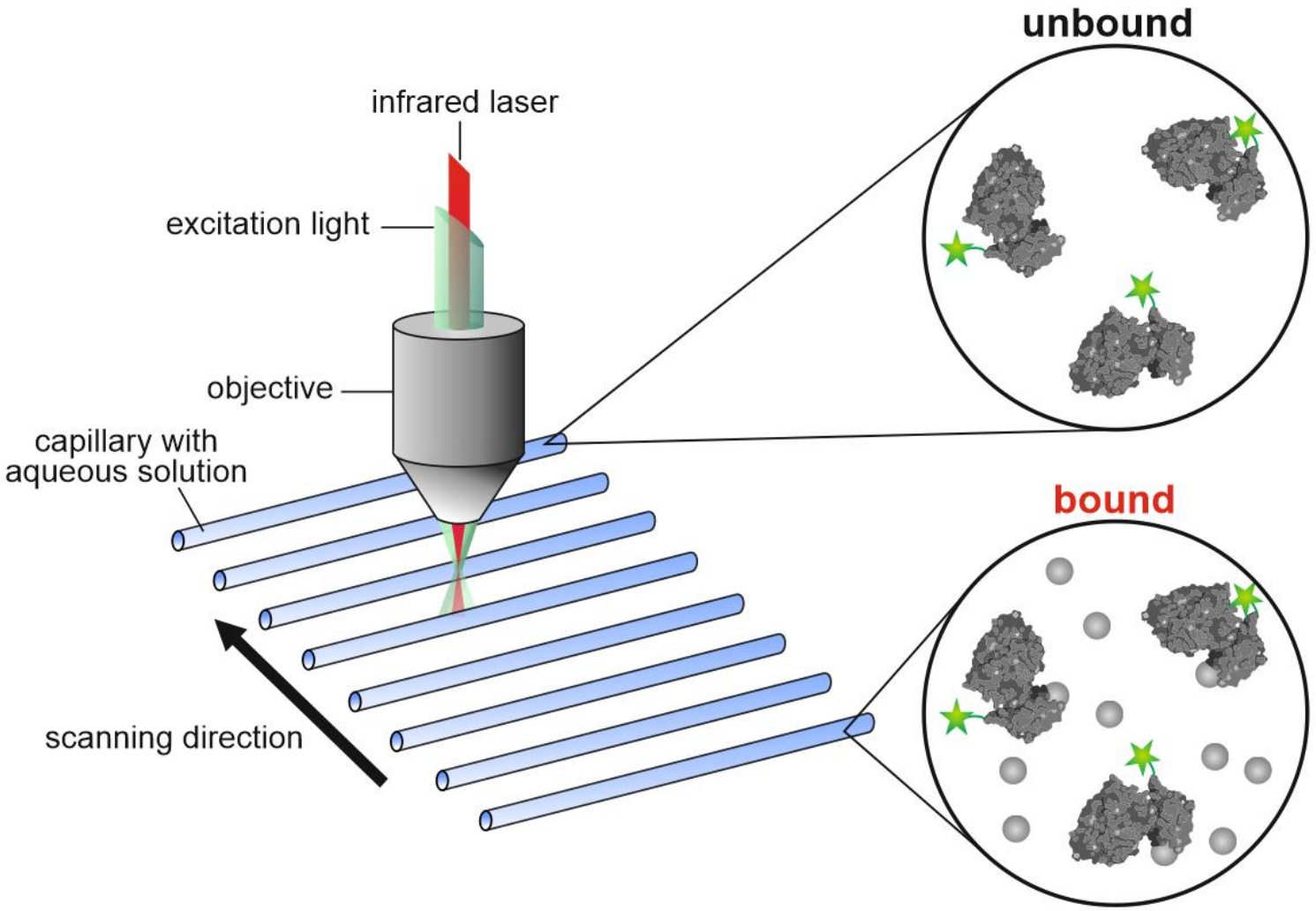
# Interactions accessible with MST



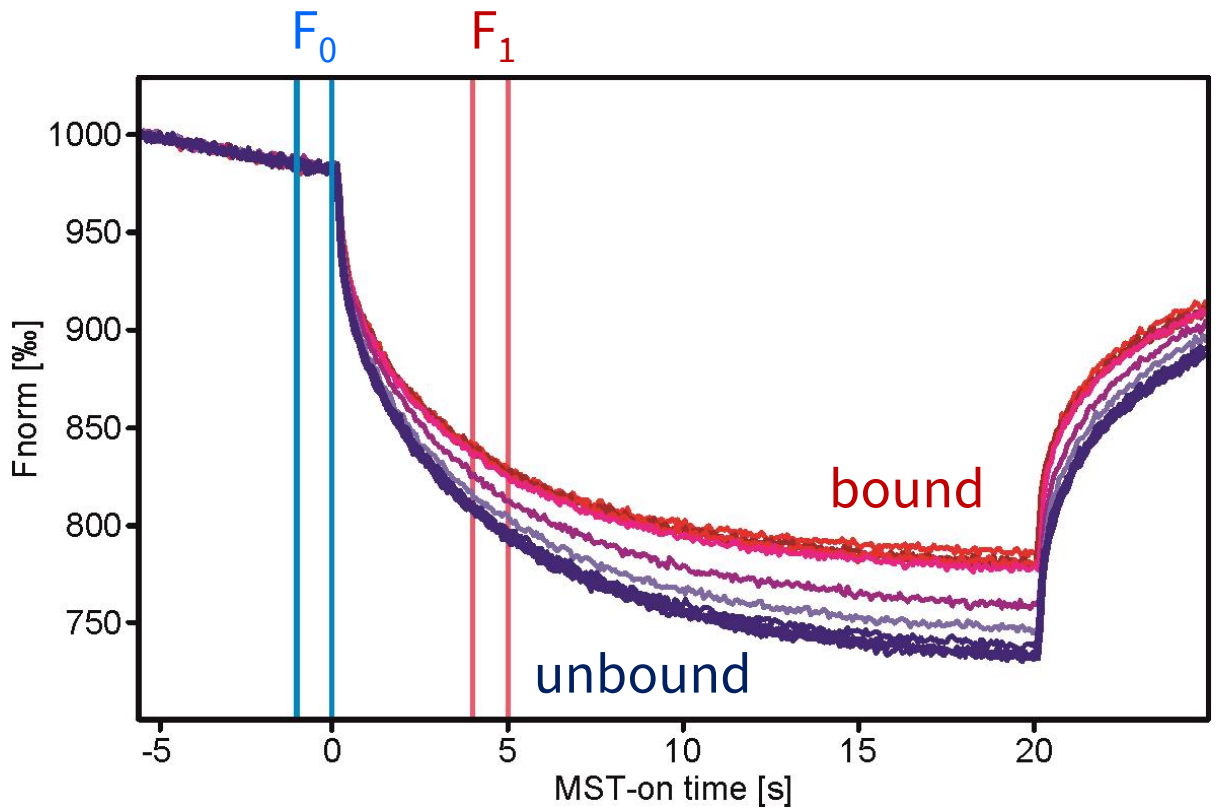


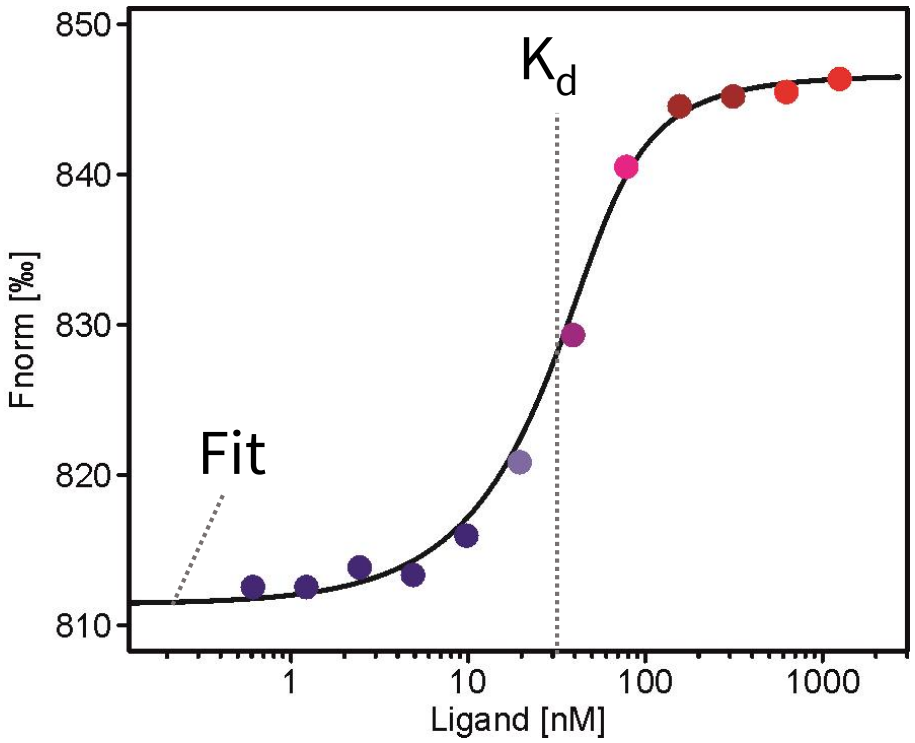

NANOTEMPER

# Optical MicroScale Thermophoresis



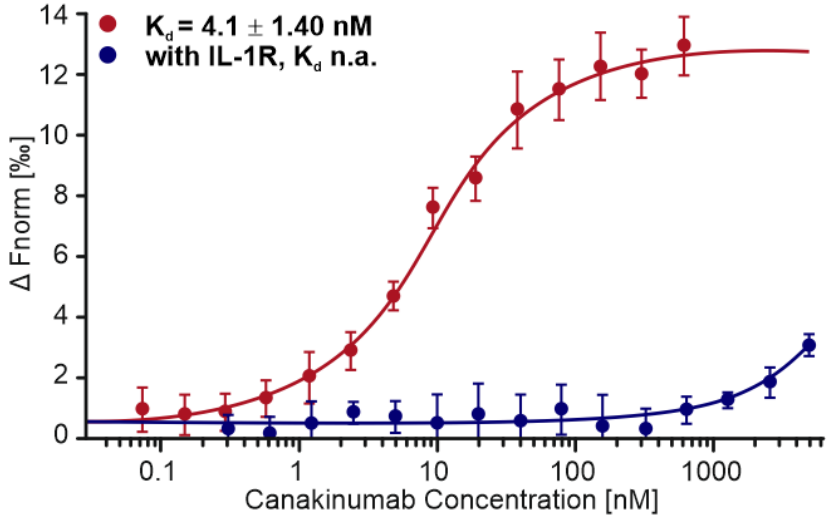
# MST binding curve

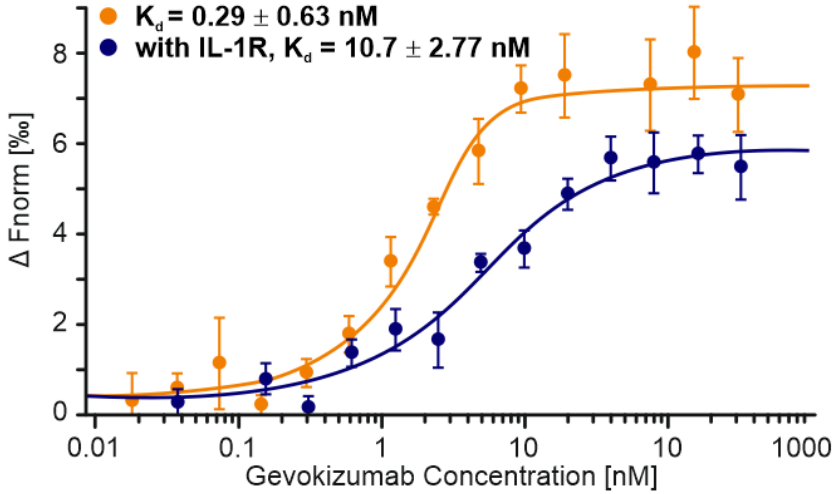


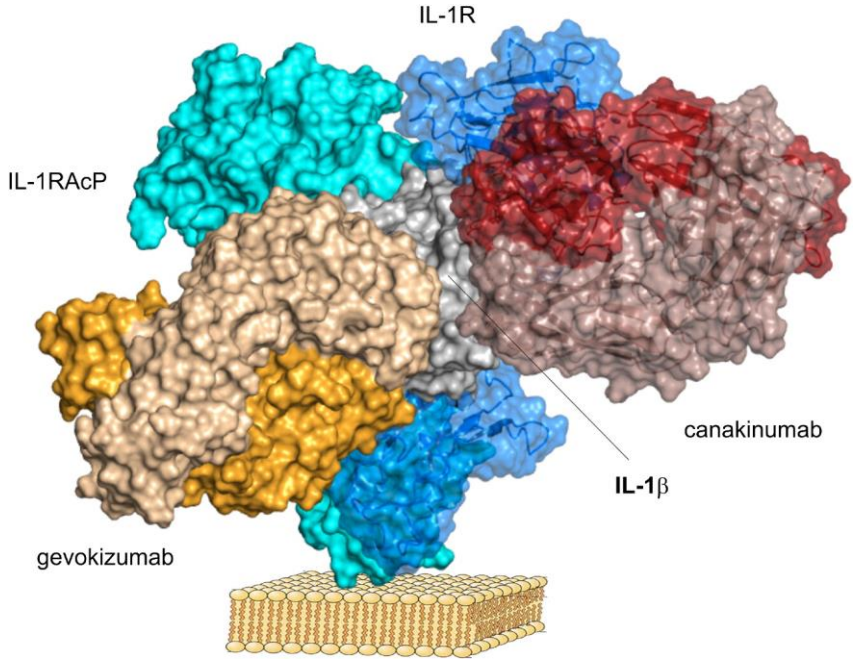
# MicroScale Thermophoresis (MST) application slides

# Therapeutic IL1β antibodies




- Analyze multi-protein interactions, e.g. of bispecific antibodies and new biological entities (NBEs) with minimal sample and time consumption



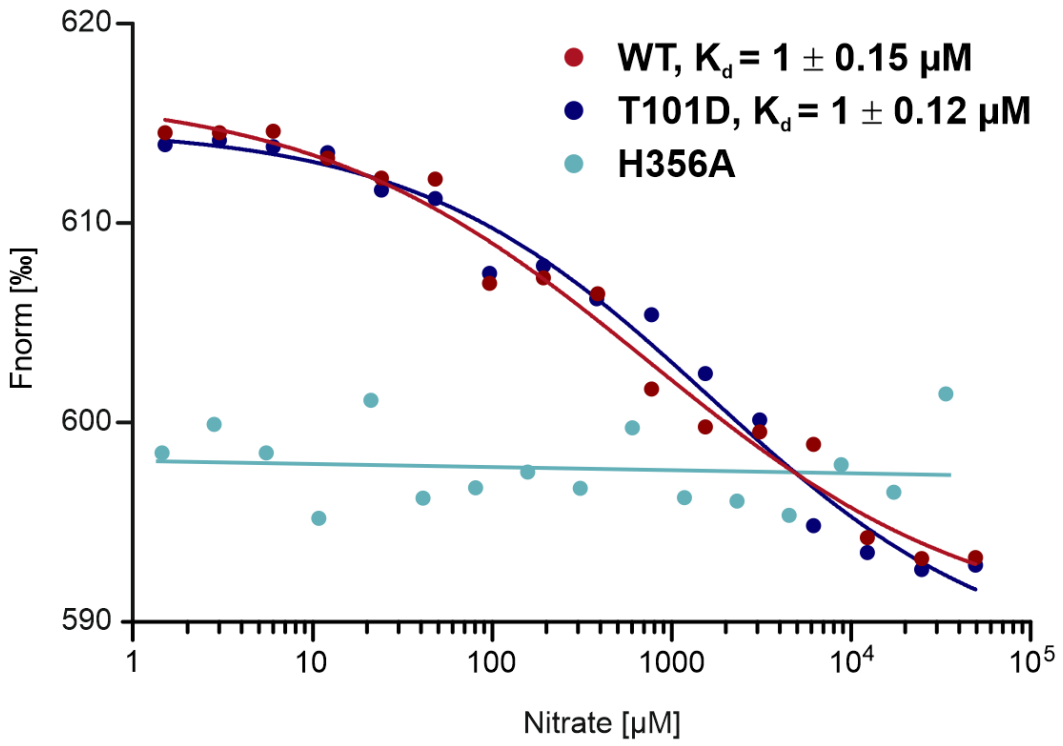

Adapted from Blech et al., Boehringer Ingelheim
J Mol Biol, 2013

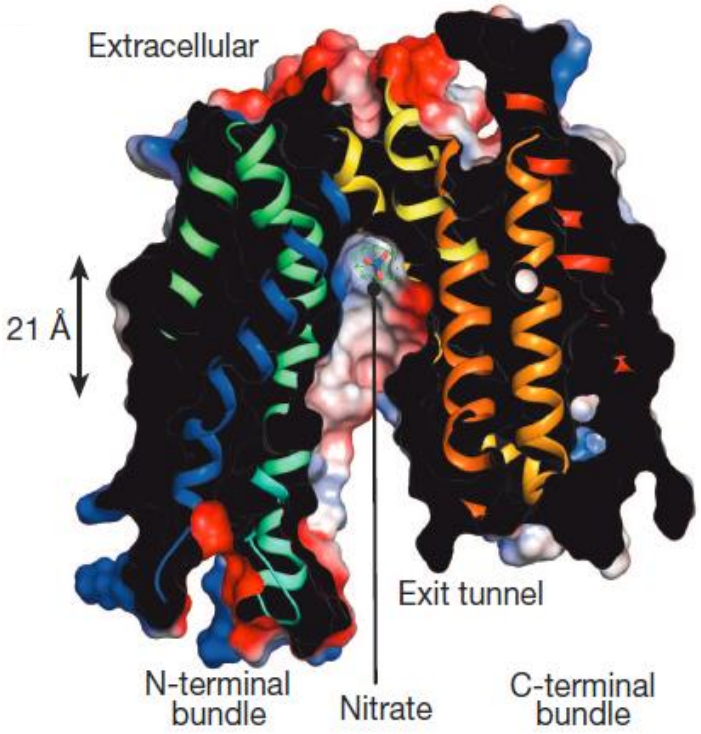
Boehringer Ingelheim

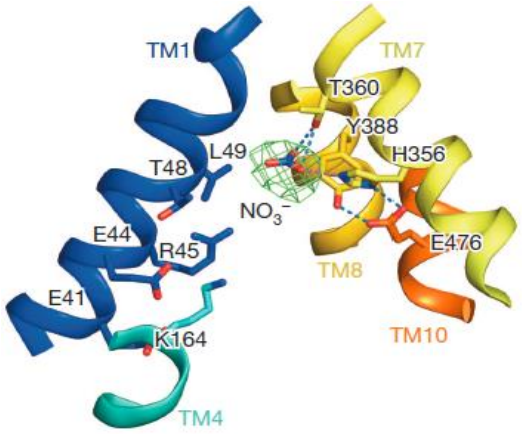


NANOTEMPER

# Nitrate binding to a 12 transmembrane-helices transporter



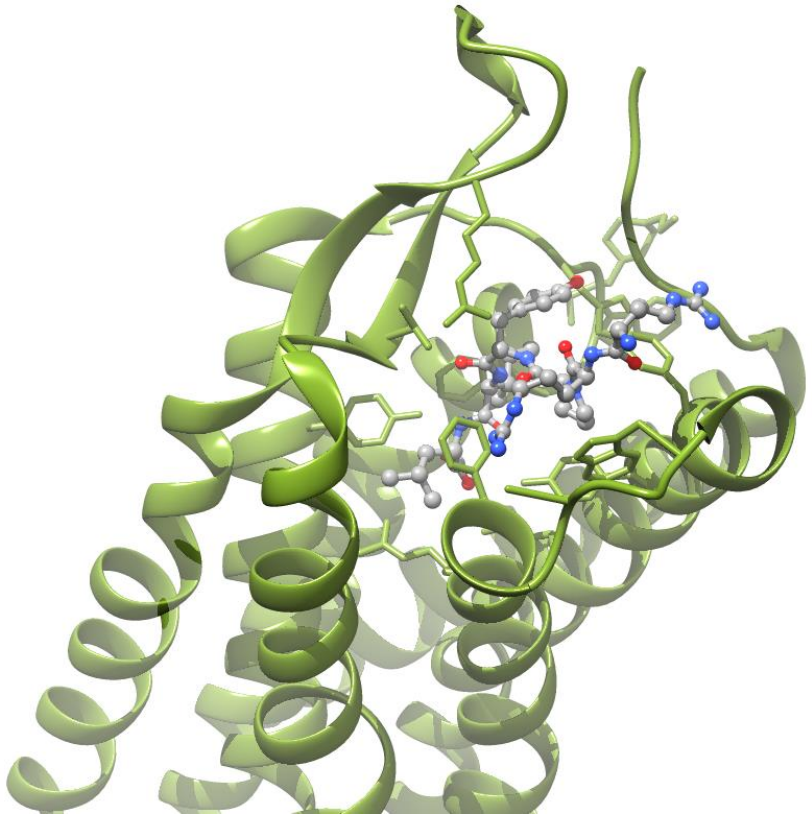



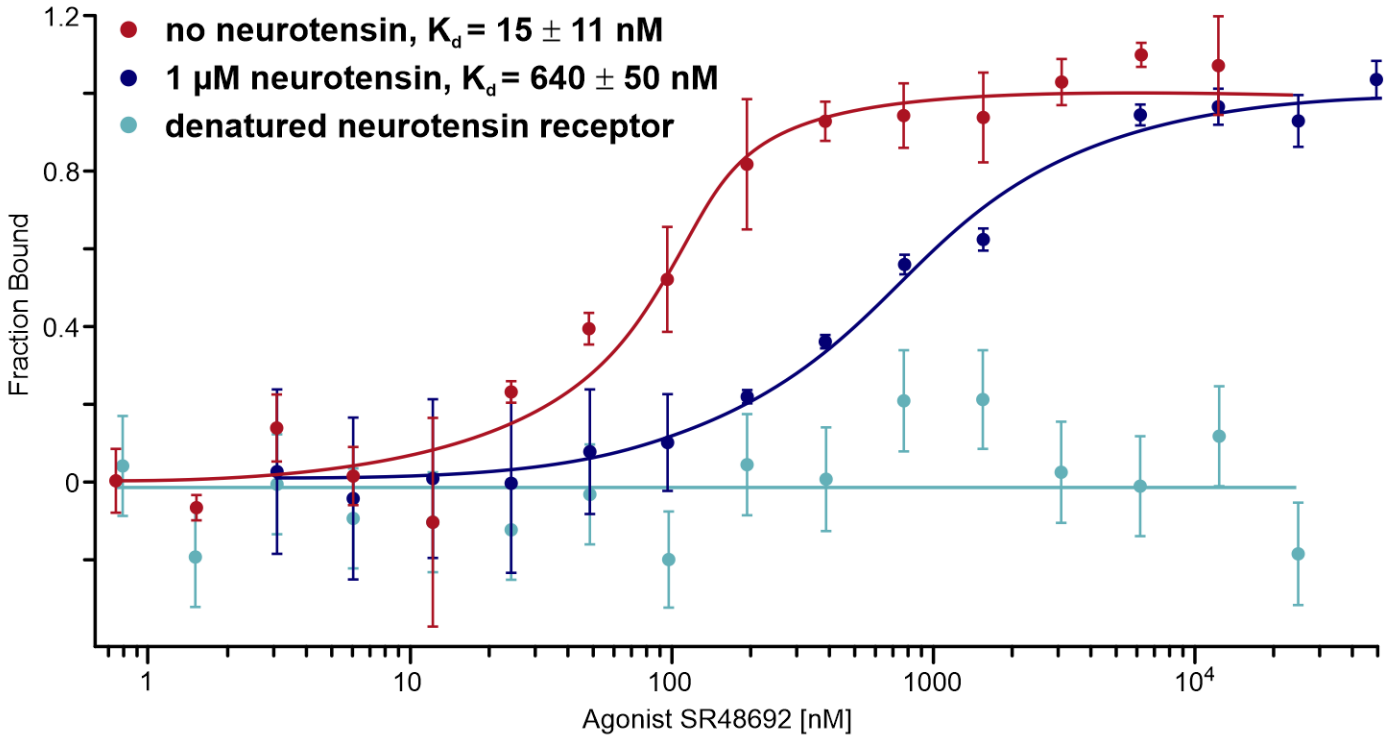




- Measure the unmeasurable: Detect smallest binding induced changes – even for difficult targets.

Adapted from Parker & Newstead, University of Oxford, UK
Nature, 2014

# GPCR-small molecule interactions






- Fast and label-free binding experiments with fragile GPCRs

Anthony Watts, University of Oxford, UK
adapted from Seidel et al., Methods, 2013

# The added value of MST

- **Get results quickly**
  Measure $K_d$s in 10 minutes. Software with intelligent data analysis and guided assay development

- **Measure previously unmeasurable targets**
  Work with very small amounts and sensitive samples

- **Benefit from close-to-native conditions**
  Immobilization-free interactions in all buffers and bioliquids (cell lysate, serum)

- **Do your research efficiently**
  Enjoy perfect ease-of-use, purification-free measurements and get rid of maintenance downtime

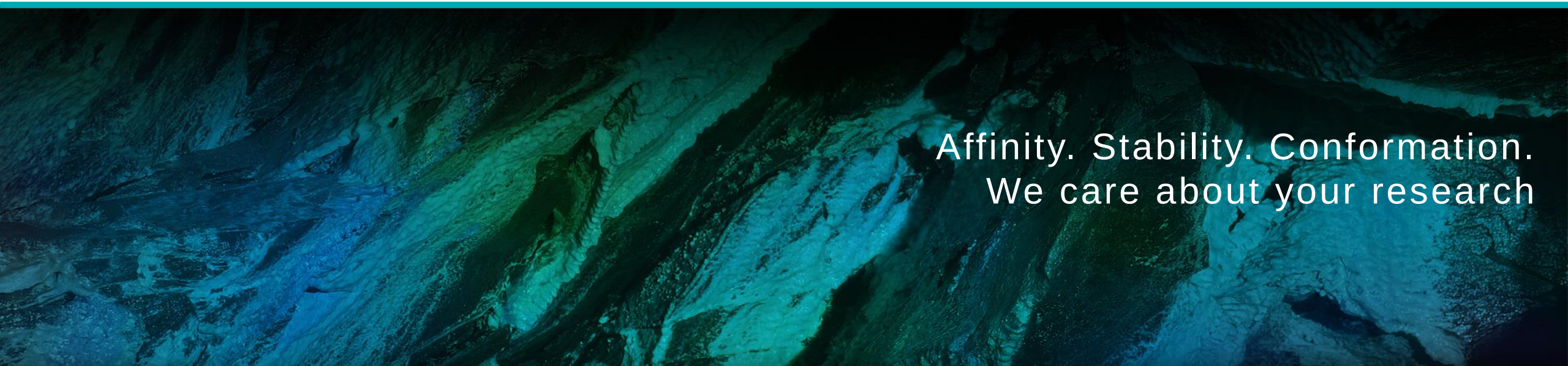
- **Work flexibly**
  $K_d$s for all molecular weights from ions to ribosomes and for pM to mM binding affinities

# When protein stability matters.

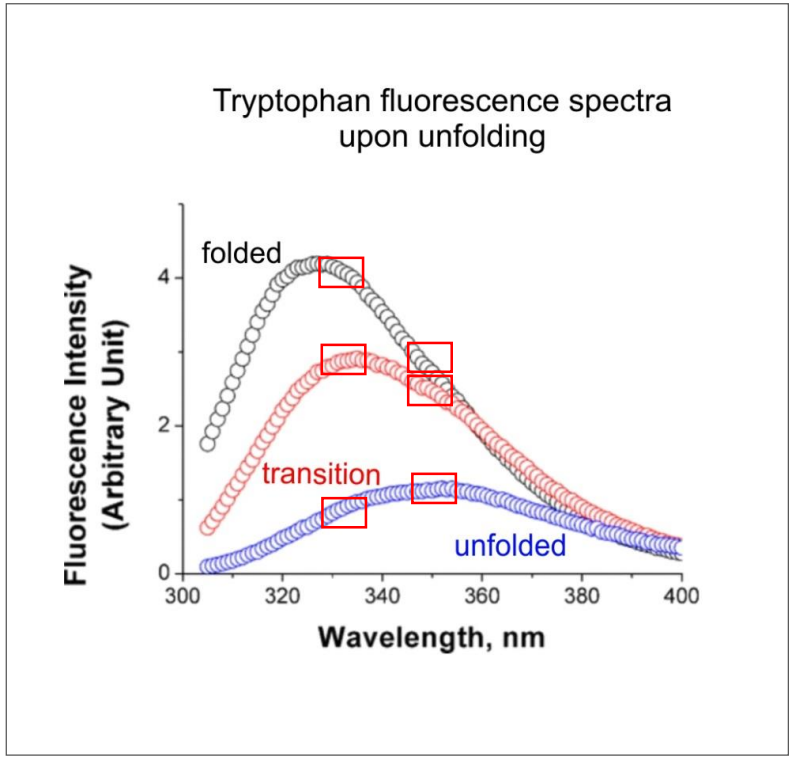
Jakub Nowak| PhD

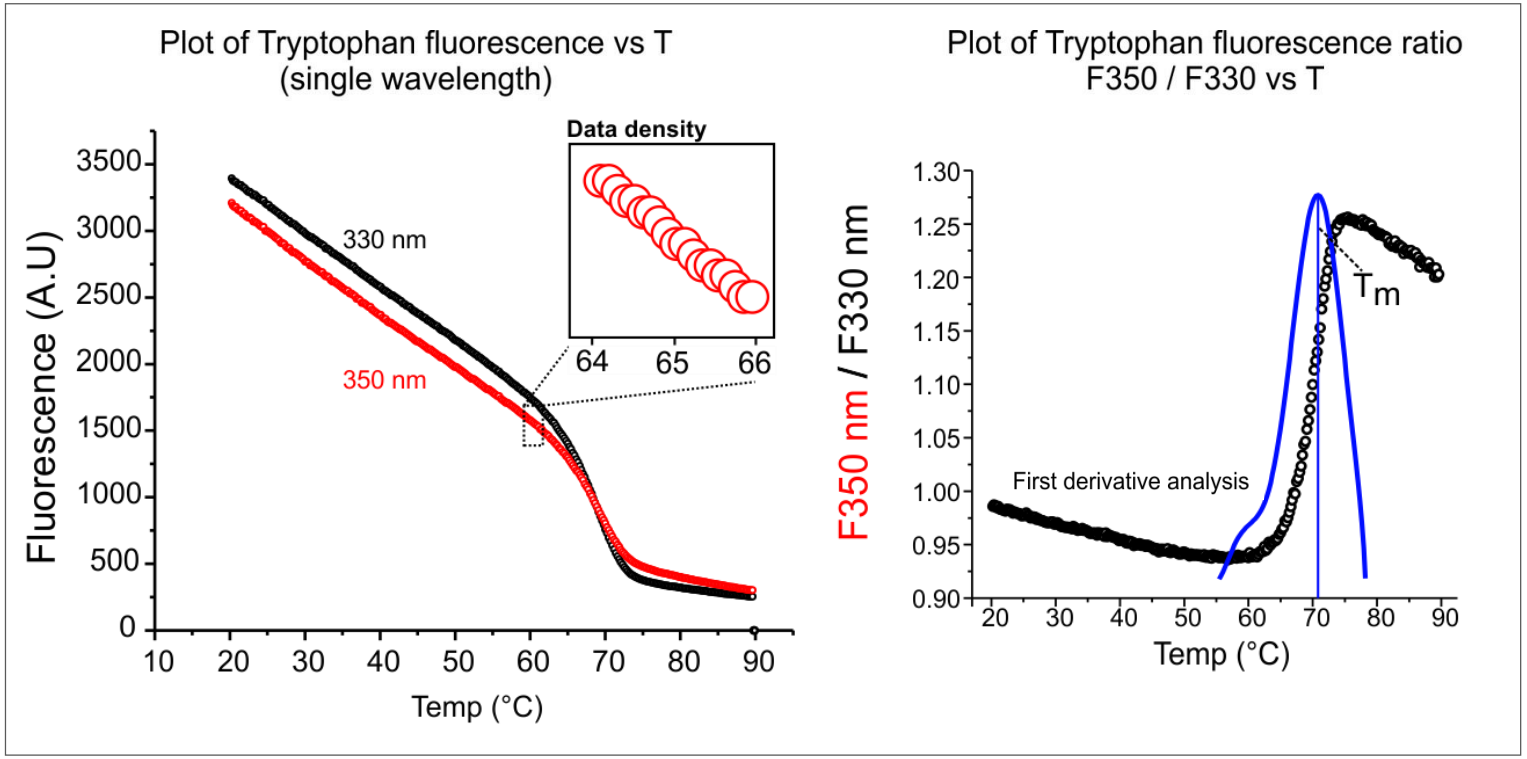
19.05.2018 8th Regional Biophysics Conference RBC 2018, Zrece, Slovenia

NANOTEMPER



# nanoDSF technology

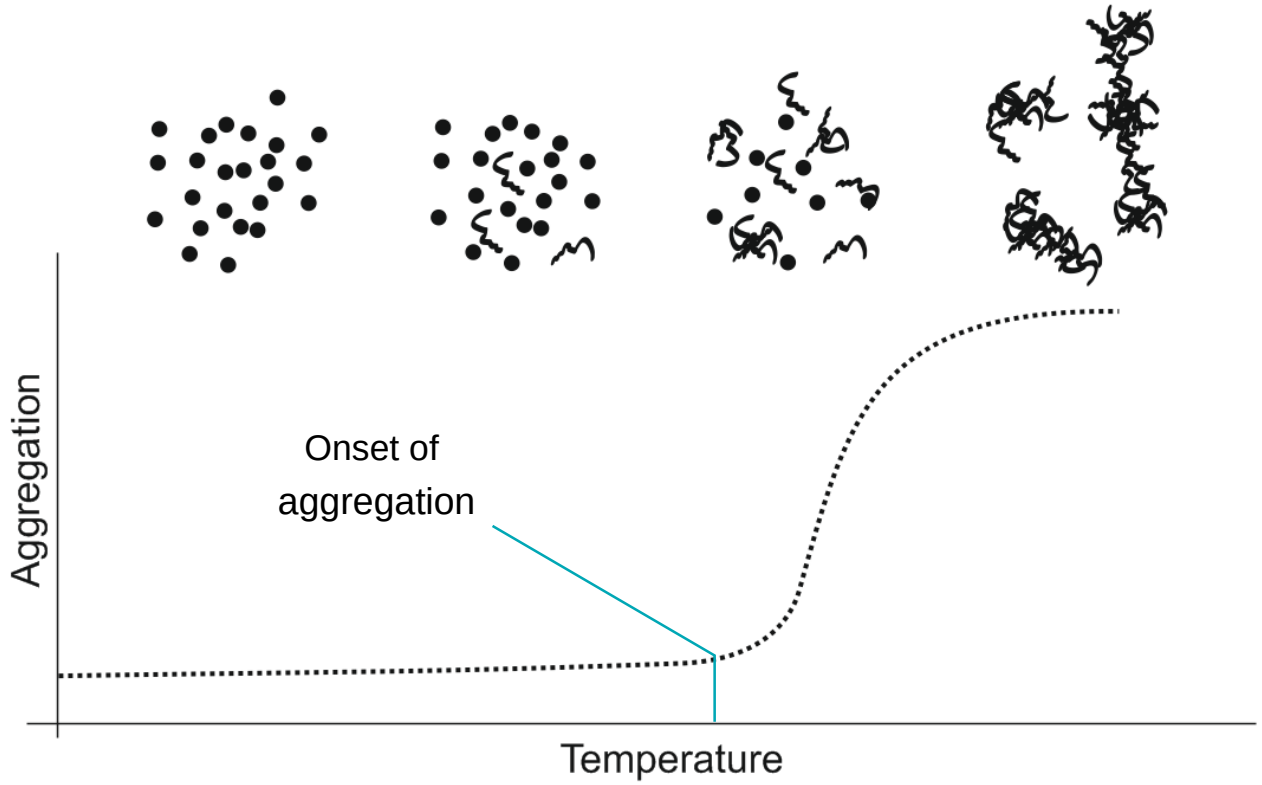


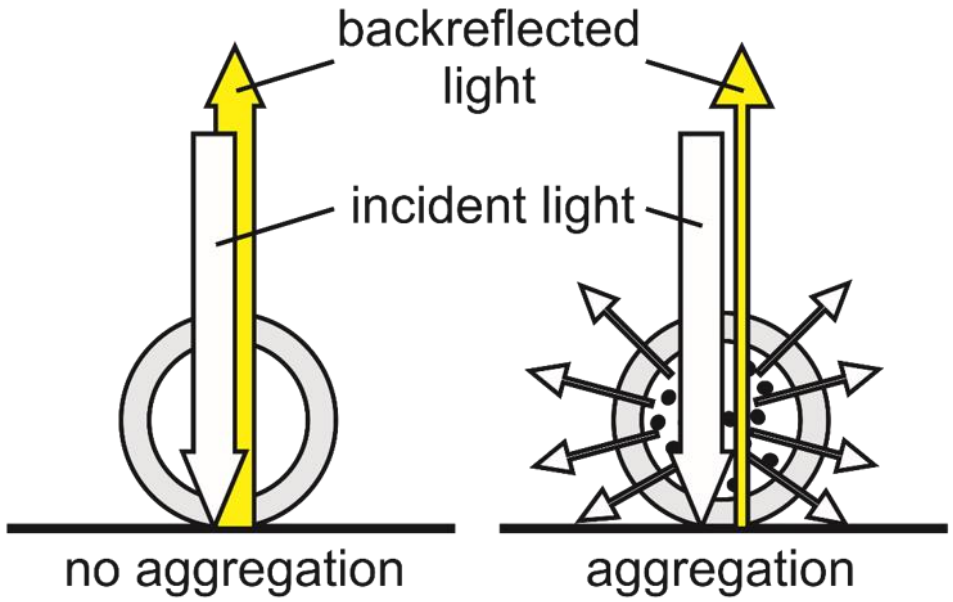




**Dual UV-detection**

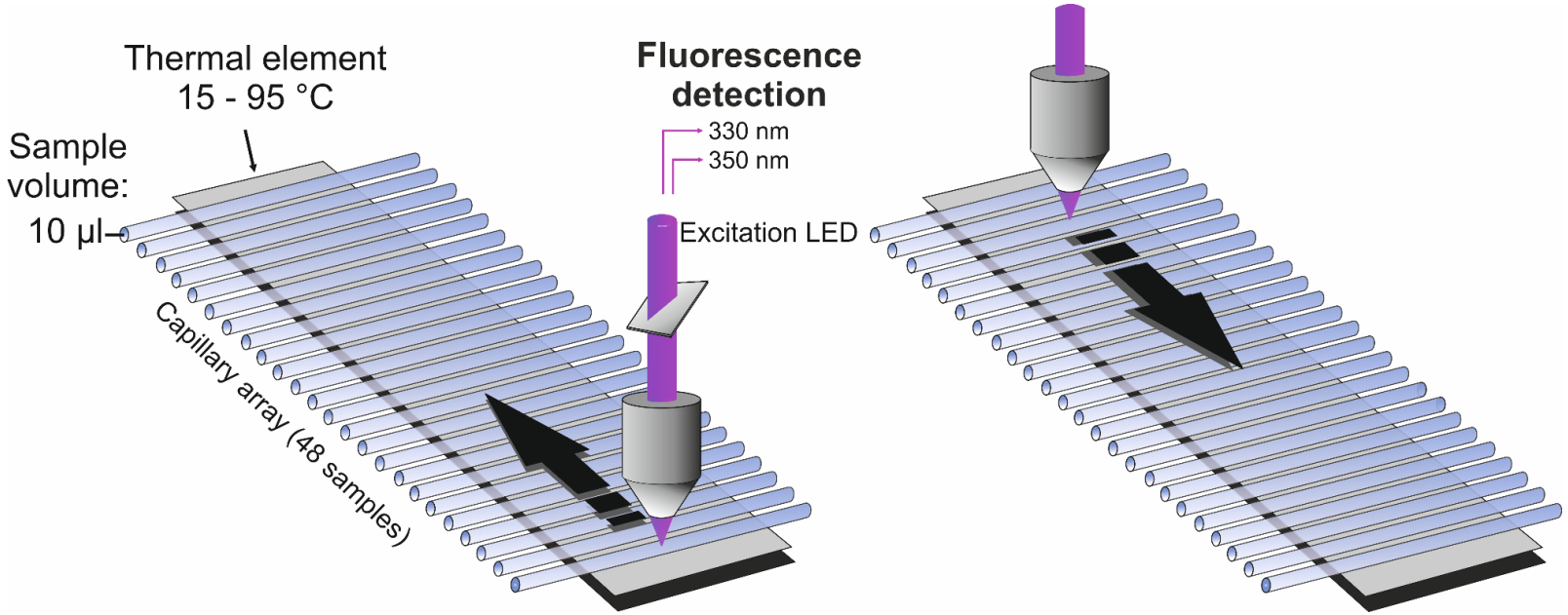
- Rapid detection of trytophan fluorescence without losing unfolding information

# Aggregation detection with the backreflection technology



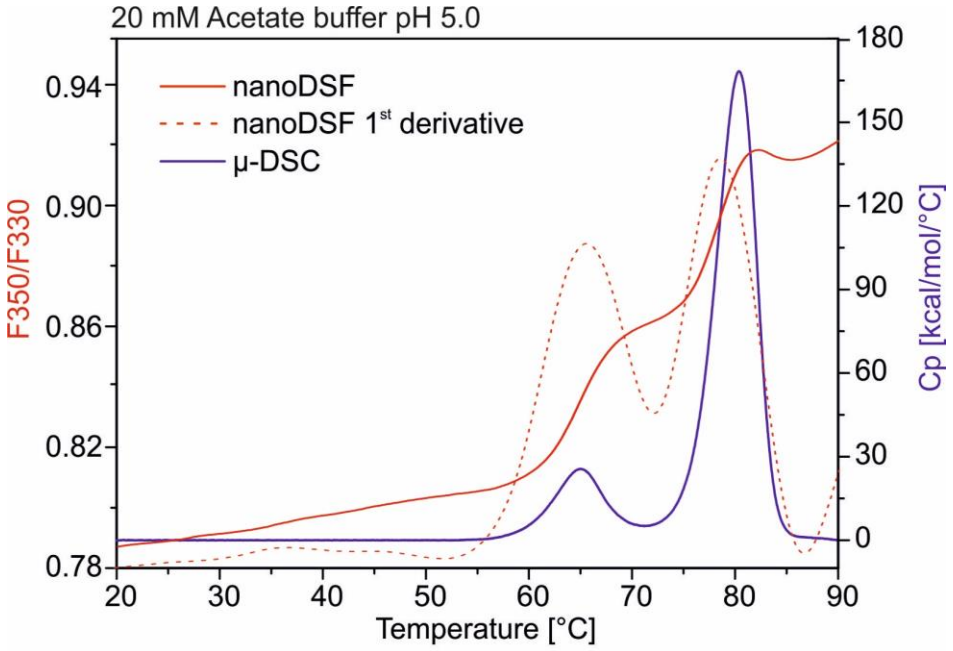
# How does the Prometheus NT.48 work?

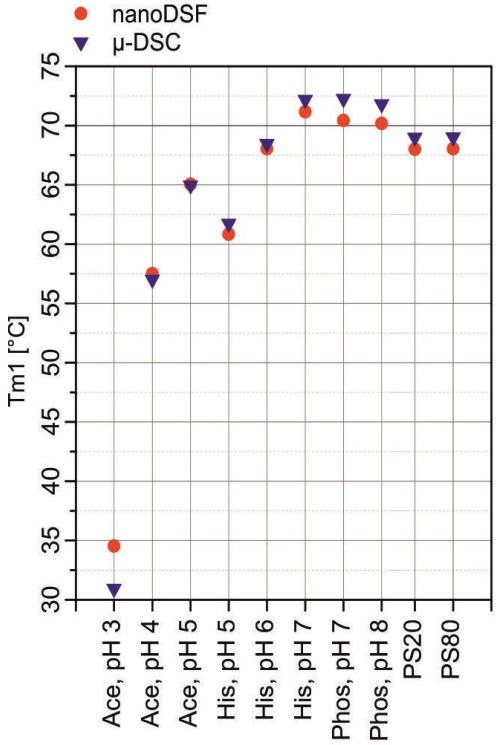


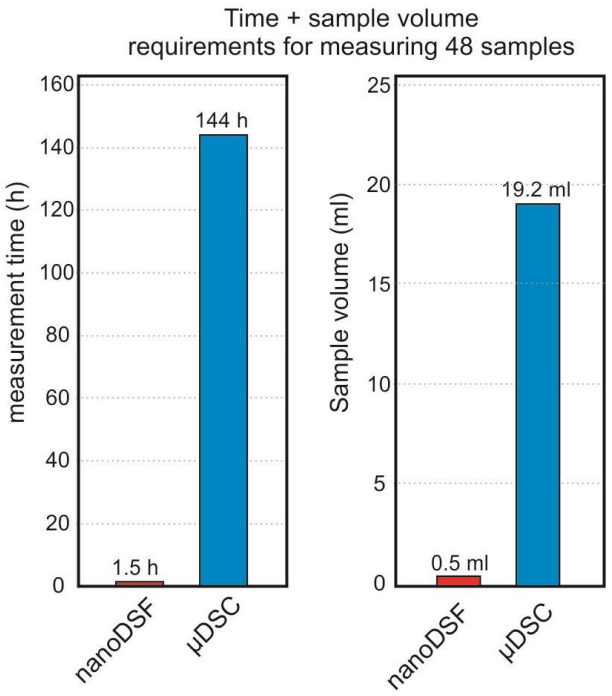

- **Heat and read**
  From 15 °C to 95 °C
  Heating rate: 0.1-7 °C/min
- **Measure 48 capillaries „on-the-fly“**
  Within 3 seconds

# nanoDSF technology applications

# Comparison of technologies: nanoDSF - µDSC



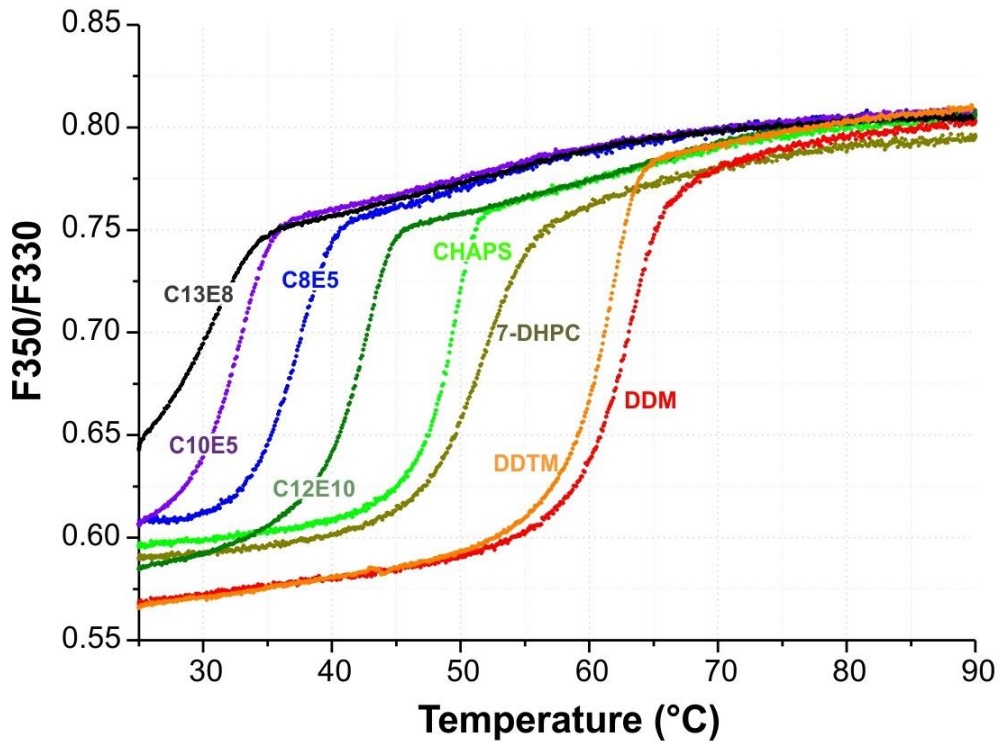



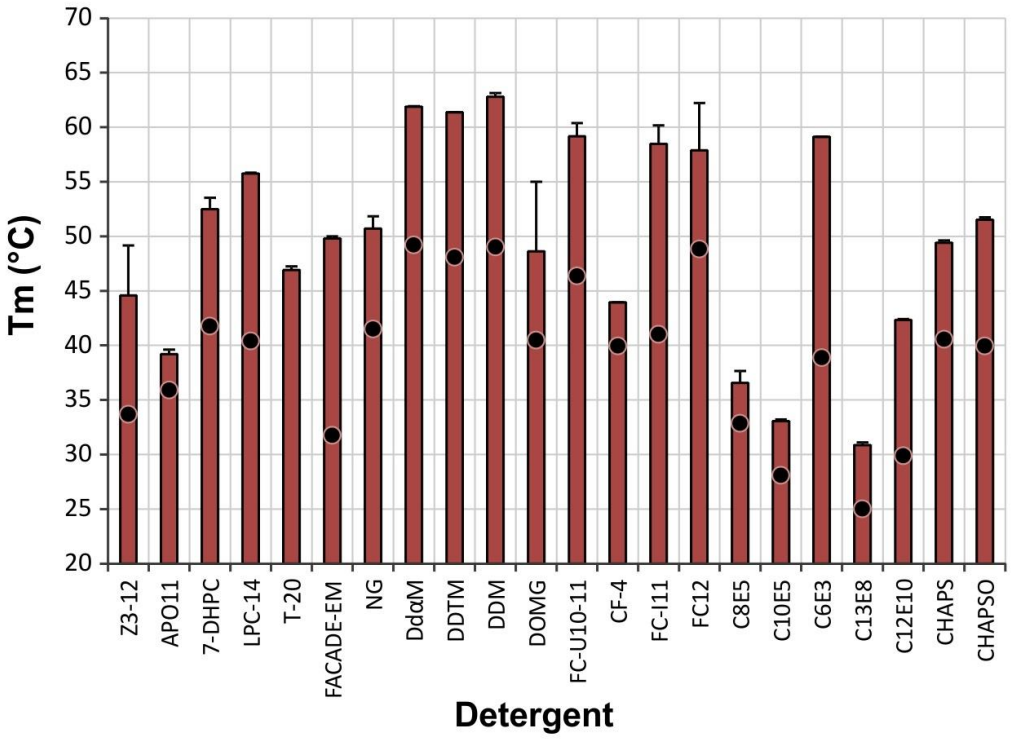




Measurements conducted in collaboration with Coriolis Pharma Research GmbH, Martinsried, Germany

- Both methods yield highly precise and consistent data
- nanoDSF overcomes several limitations of µDSC

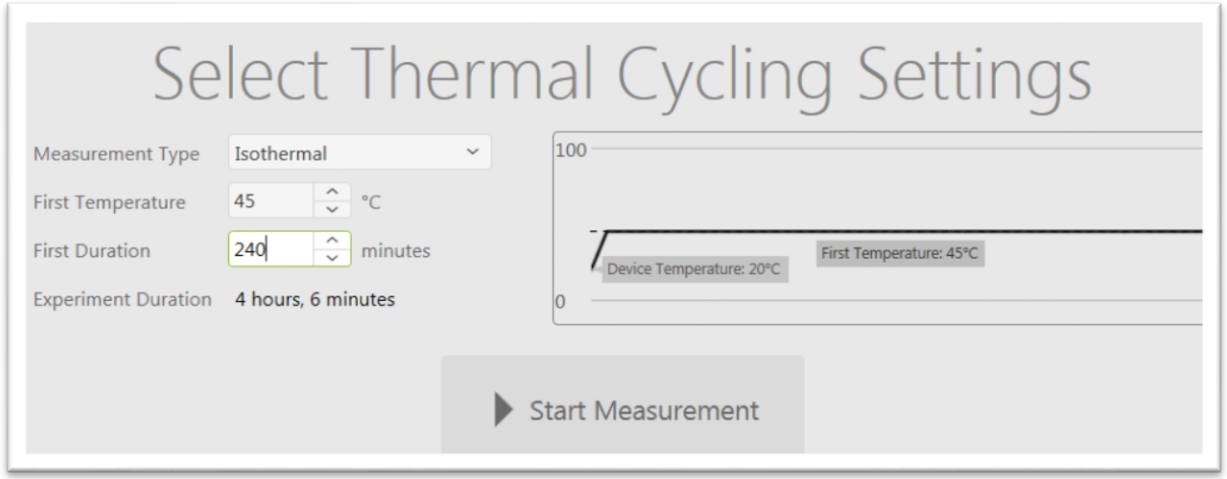
# Detergent screens for integral membrane proteins

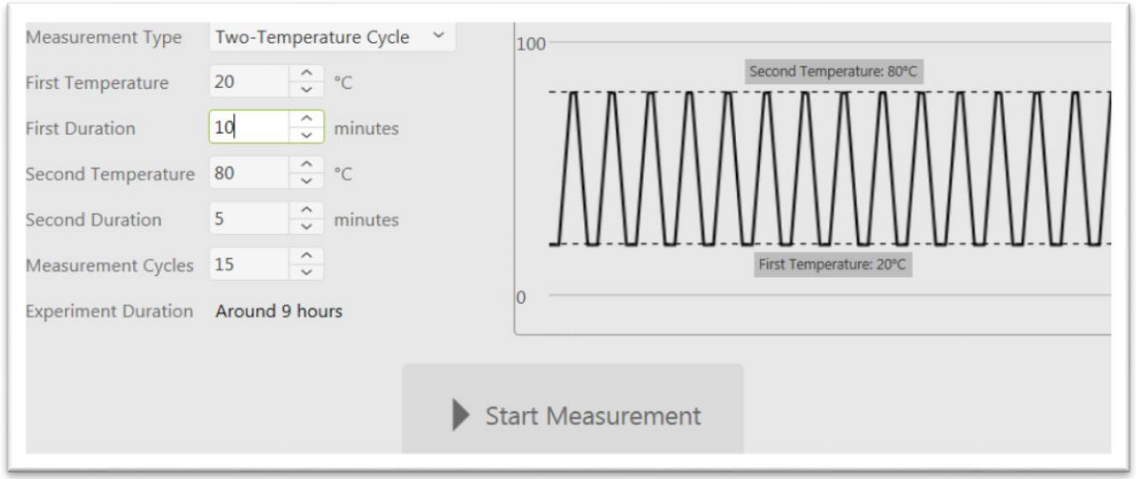


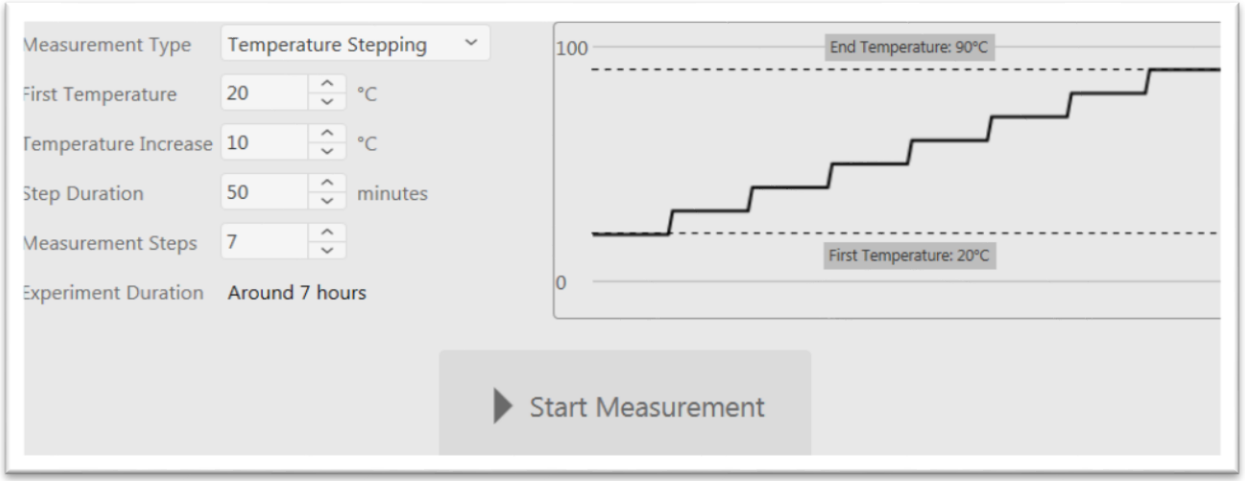



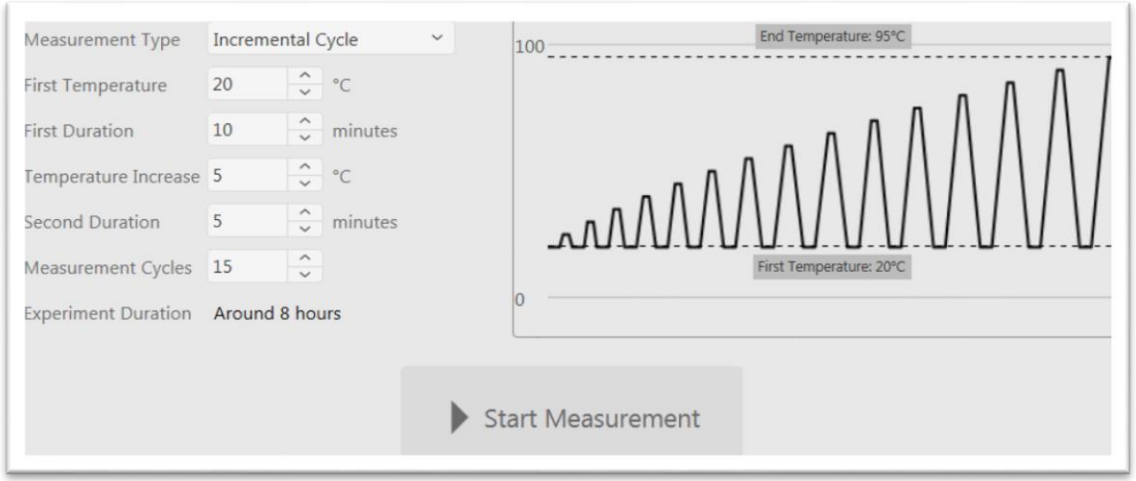

Detergent has strong effect on thermal stability with $T_m$ varying between 31°C and 63°C

# PR.TimeControl - protein refolding kinetics and efficiency

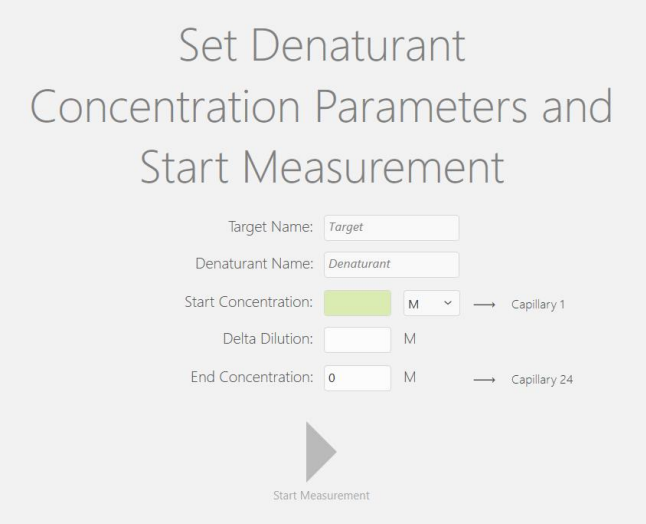


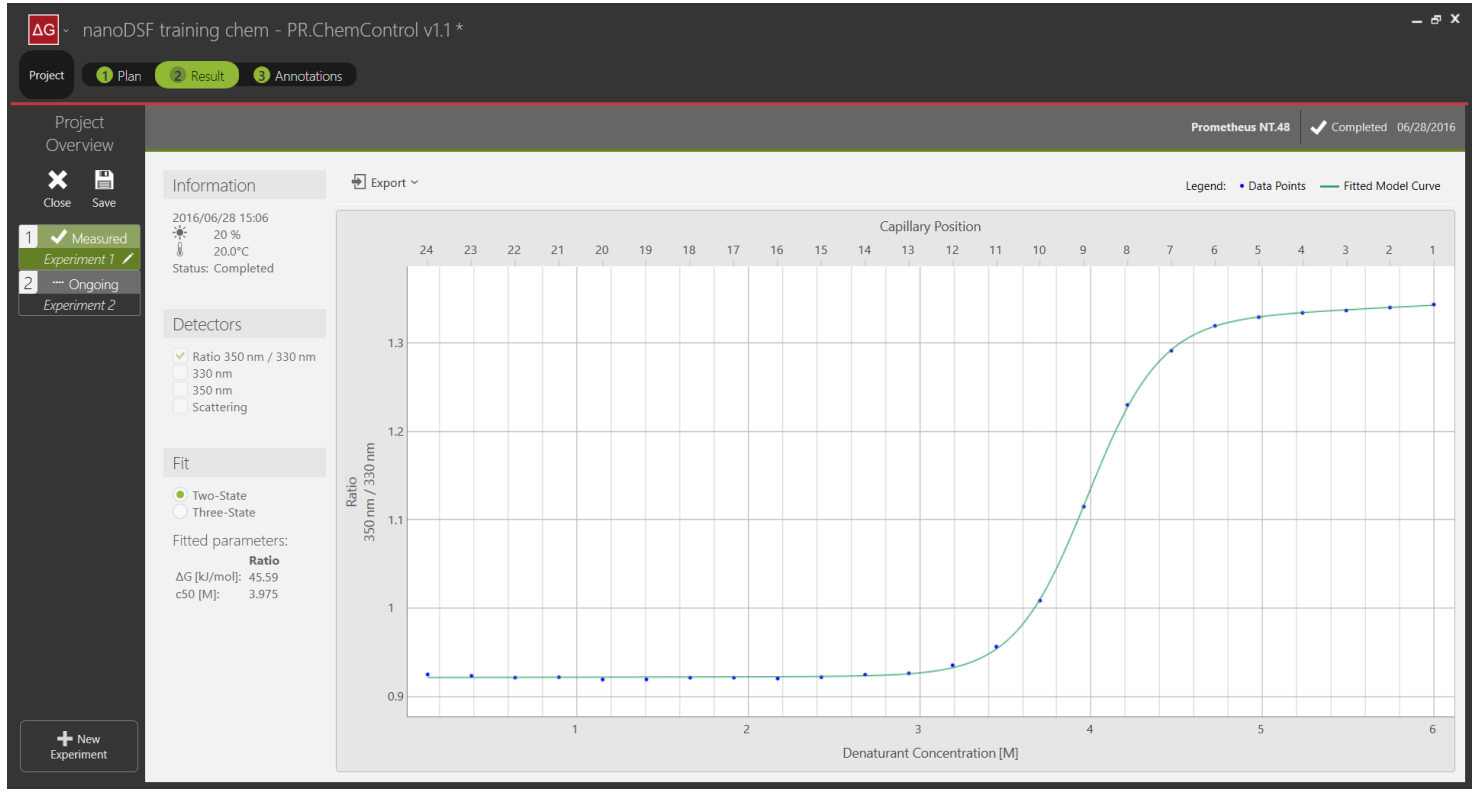



# PR.ChemControl




- One-button routine
- Enter denaturant concentration range and dilution conditions
- Measurement, automatic fit and result calculation finish within seconds

# Benefits of nanoDSF

- **Native conditions:**
  No fluorescent dyes, thus buffer independence, any detergent.

- **Ultra-high resolution:**
  Unmatched density of data points, more than 20 measurements per capillary and minute/°C.

- **Fast measurements:**
  Measure up to 48 capillaries „on-the-fly“ within 3 seconds.

- **Broad application range:**
  Detect protein concentrations from 5 µg/ml to > 250 mg/ml.

- **Address all your targets:**
  Soluble proteins, antibodies up to transmembrane or hydrophobic proteins.

- **Efficient research:**
  Easy to handle device, intuitive software with one-click-analysis and maintenance free instrument.

Come talk to us, enter a raffle and win Samsung Galaxy Tab

# Thank you for your attention.

nanotempertech.com | jakub.nowak@nanotempertech.com

NANOTEMPER