Biomolecular Interaction Analytics using MicroScale Thermophoresis

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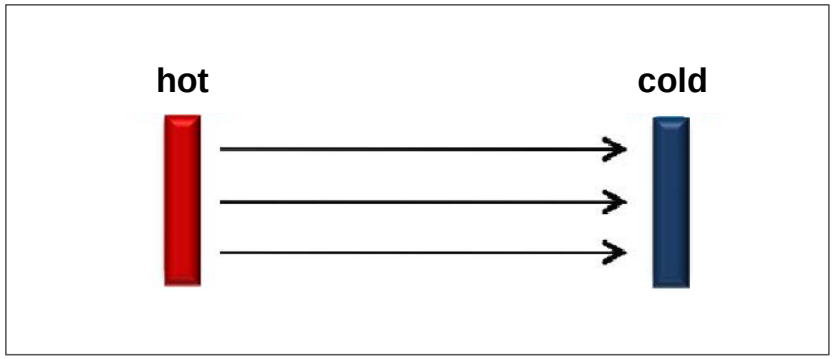
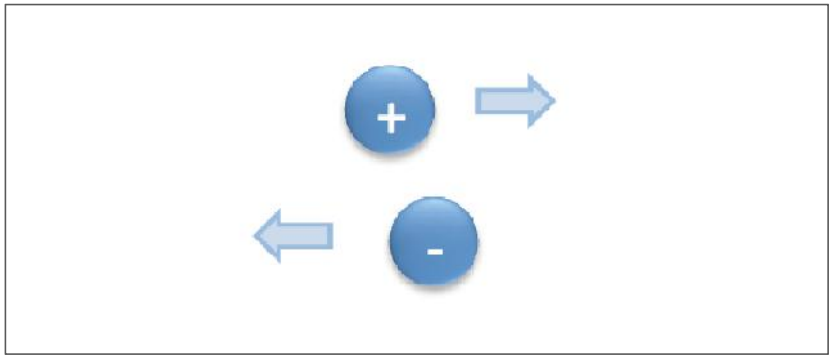
MicroScale Thermophoresis (MST)

Electrophoresis: Electric Field

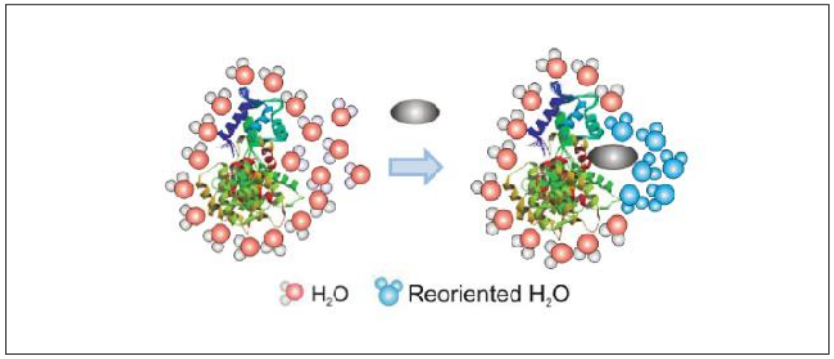
Thermophoresis: Temperature Gradient



Charge, (Size)

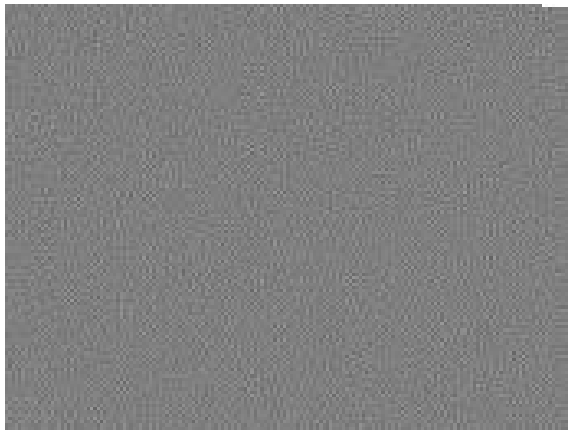


Charge, Size and Hydration Shell

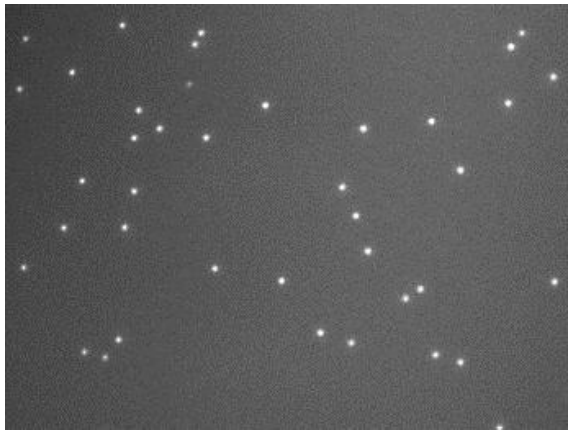


Thermophoresis in Real Time

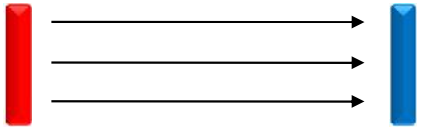
DNA



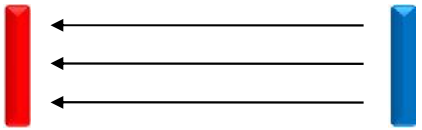
Microbeads



„Positive“ Thermophoresis



„Negative“ Thermophoresis

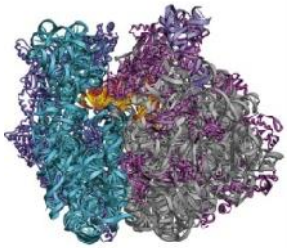


Theory of Thermophoresis

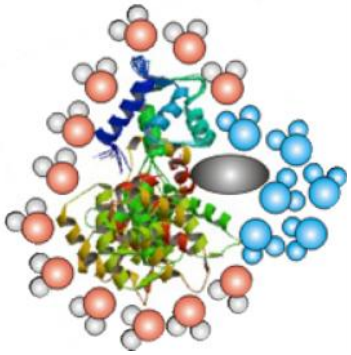
$c_{hot}/c_{cold} = \exp(-S_T \Delta T)$

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

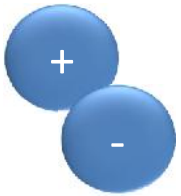
size



hydration shell

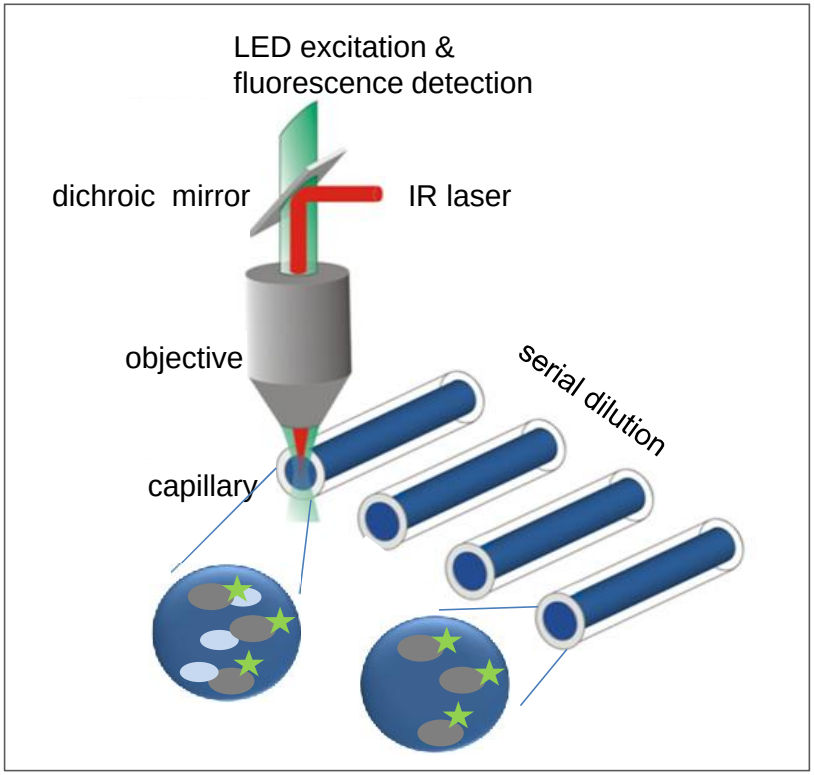


charge ²



Duhr and Braun PNAS 103, 19678–19682 (2006)
Duhr and Braun PRL 96, 168301 (2006)

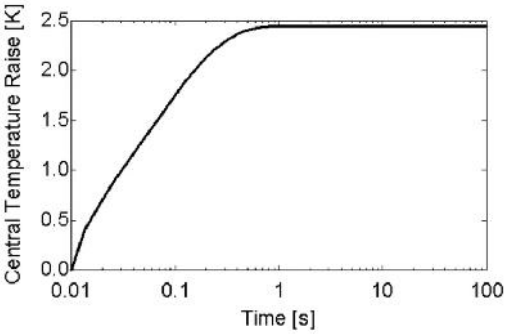
Basic Setup of the Instrument



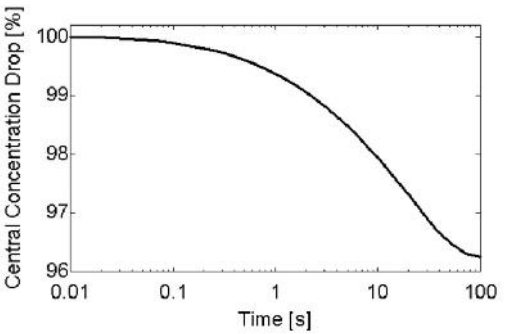
- **immobilization-free** in any buffer
- **4 µl** of sample per titration point
- **pM - nM concentrations** of fluorescent molecule
- **40 sec** measurement per titration point

Simulation of Thermophoresis

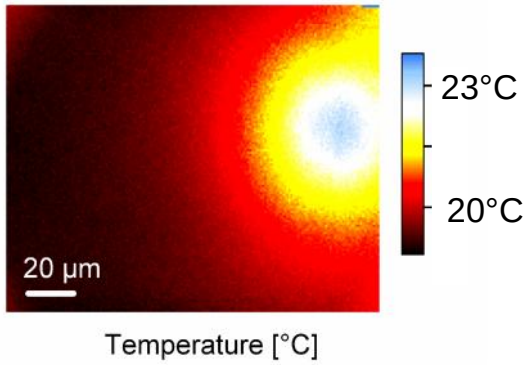
Sample Heating < 1 sec



Thermophoresis ~ 30 sec



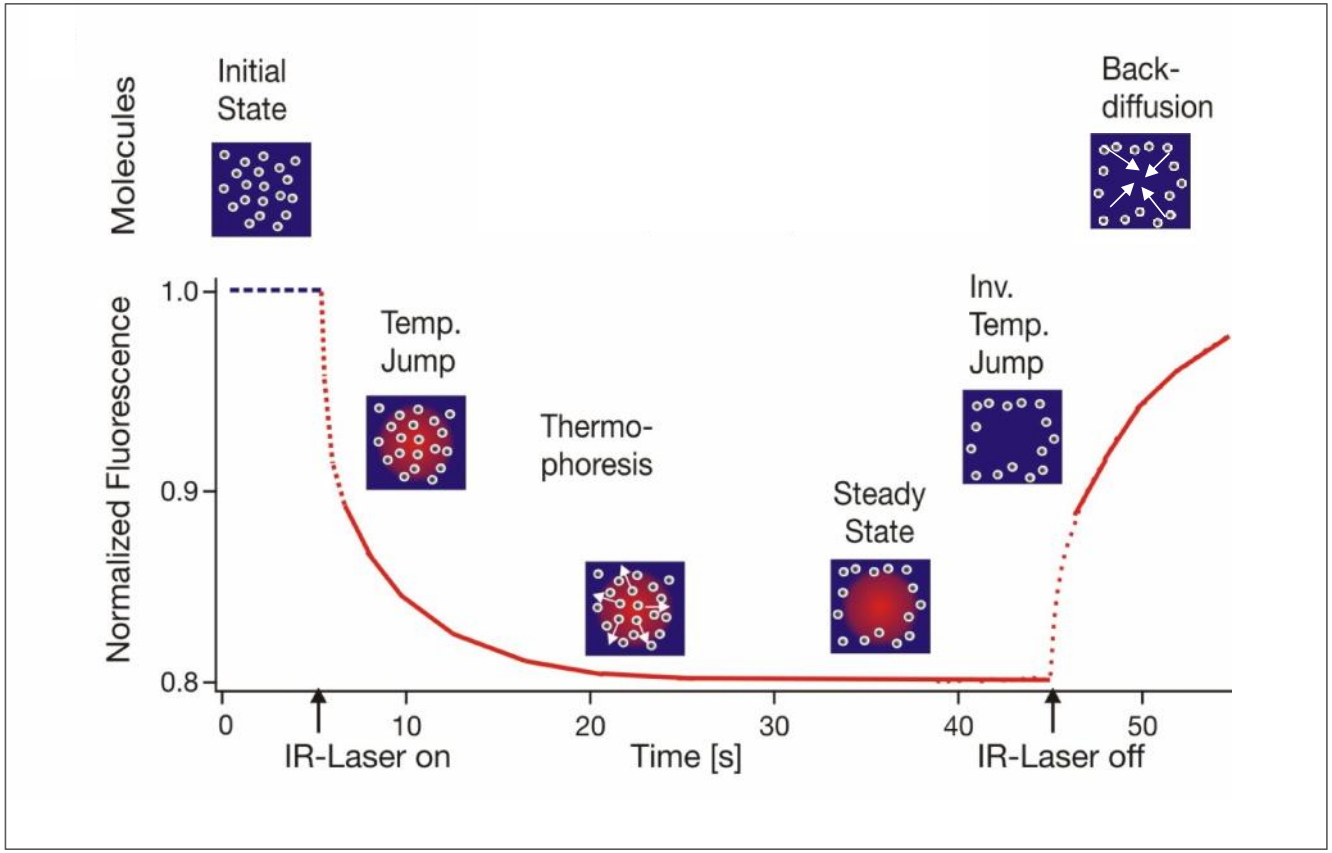
IR laser induced Temperature Field
(BCECF-dye in TRIS-buffer)



1K/10 µm corresponds to 1000K/cm

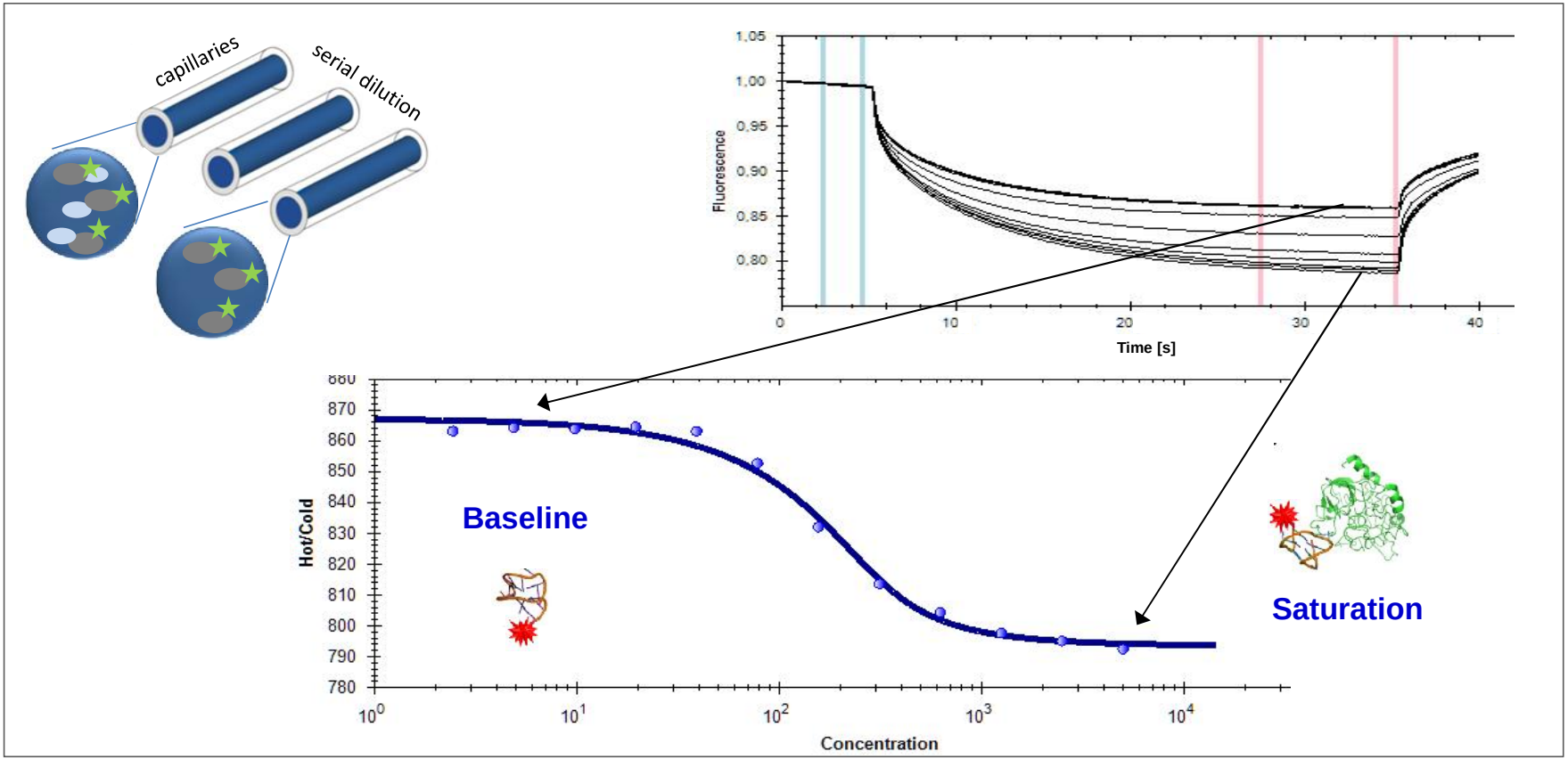
Jerabek-Willemsen et al, ADDT, 2011

Phases of a MST Signal



Jerabek-Willemsen et al, ADDT, 2011

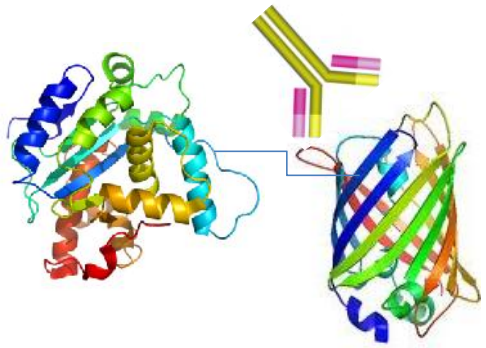
MST Binding Curve



Baaske et al., Angewandte Chemie, 2010

Interactions accessible with MST

- protein - **protein**
- protein - **DNA/RNA**
- nucleic acid - nucleic acid
- protein - **small molecule**, **peptides**, **ions**
- protein or small molecule - **HMW complexes** (e.g. ribosome)
- protein or peptide - **liposome/vesicle**
- ligand binding to **membrane receptors**
- …



Instruments for MicroScale Thermophoresis

Monolith NT.115

- **broad application range**
  from ions to ribosomes
- **buffer independency**
  even serum or cell lysate
- **purification free**
  fluorescent fusion proteins
- dynamic range
  1 nM to mM

Monolith NT.115^{Pico}

- **high sensitivity**
  limit of detection at 50 pM
- **low sample consumption**
  pM concentrations
- **better resolution**
  for high affinity interactions
- dynamic range
  10 pM to mM

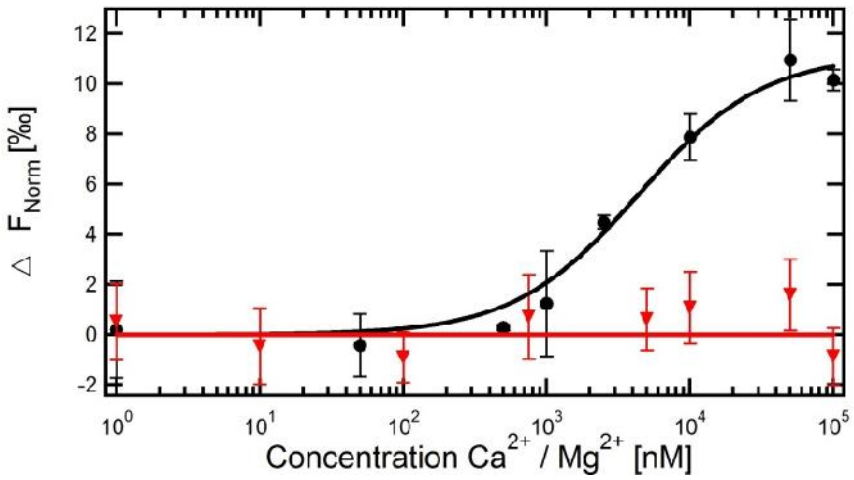
Monolith NT.LabelFree

- **TRULY label-free**
  intrinsic fluorescence
- **buffer independency**
  except complex bioliquids
- dynamic range
  10 nM to mM

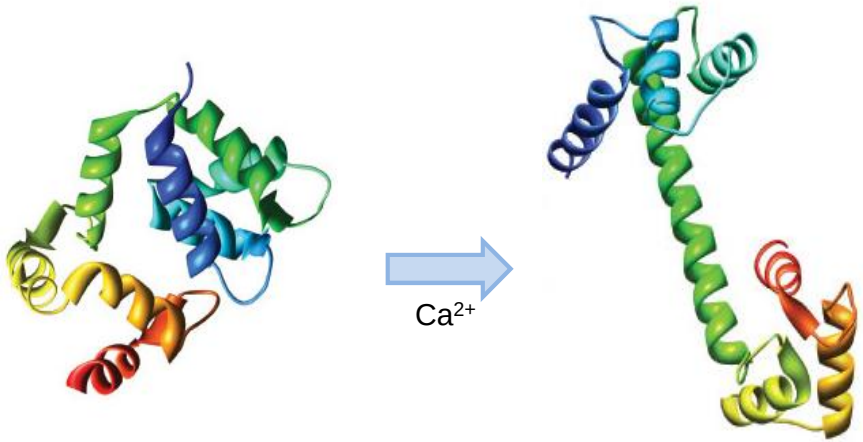
Interaction of fluorescently labeled Calmodulin with Ca^{2+} - ions

2

3



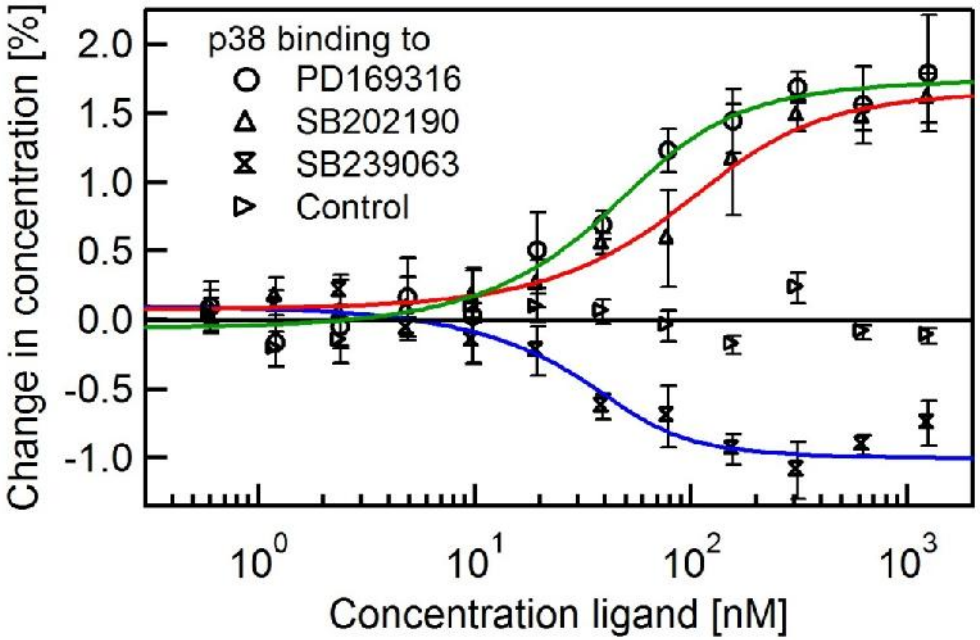
Conformational change of Calmodulin upon Ca^{2+}-binding



5 *Wienken et al, Nature Communications, 2010*

4 ► **Interactions that do not alter the MW of a protein, only charge and the conformation (hydration shell)**

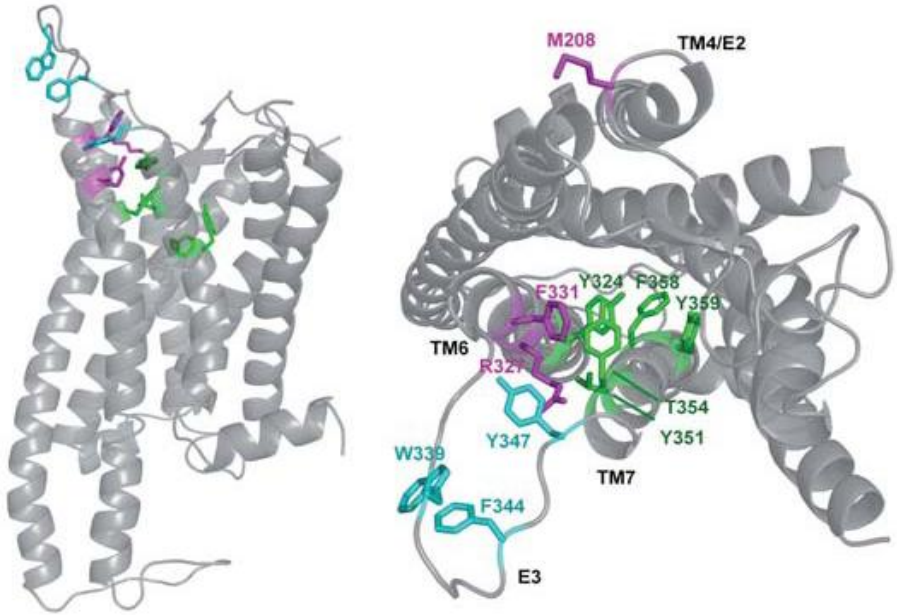
p38 Interactions with different Compounds

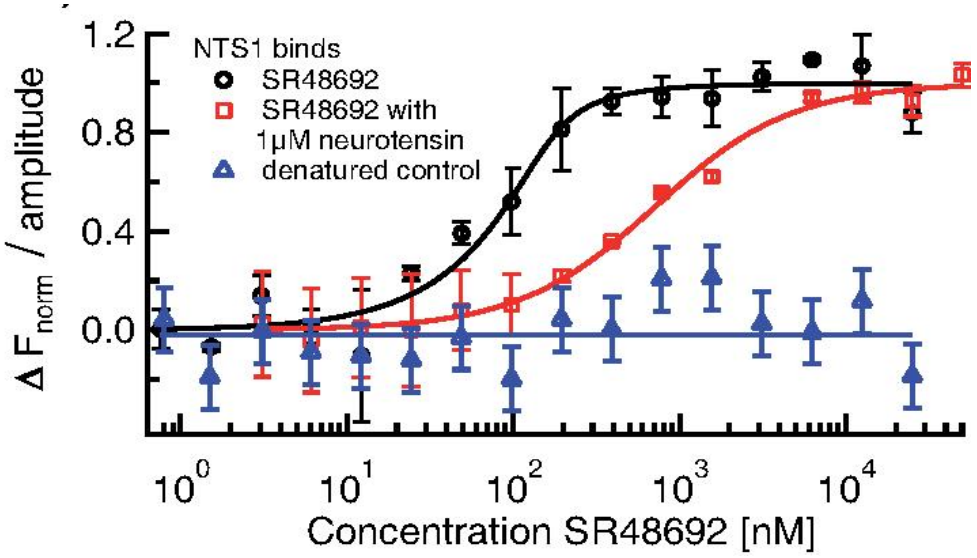


Seidel et al, Angwandte Chemie, 2012

- **Allows fast and label-free screening of various compounds binding to different types of proteins**

Neurotensin Receptor (GPCR) Interactions with different Molecules





Prof. Anthony Watts, University of Oxford, Biochemistry, UK
Seidel et al, Methods, 2012

- **Allows fast and label-free binding experiments with sensitive GPCRs**

Thank You For Your Attention!

Please feel free to contact us:

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