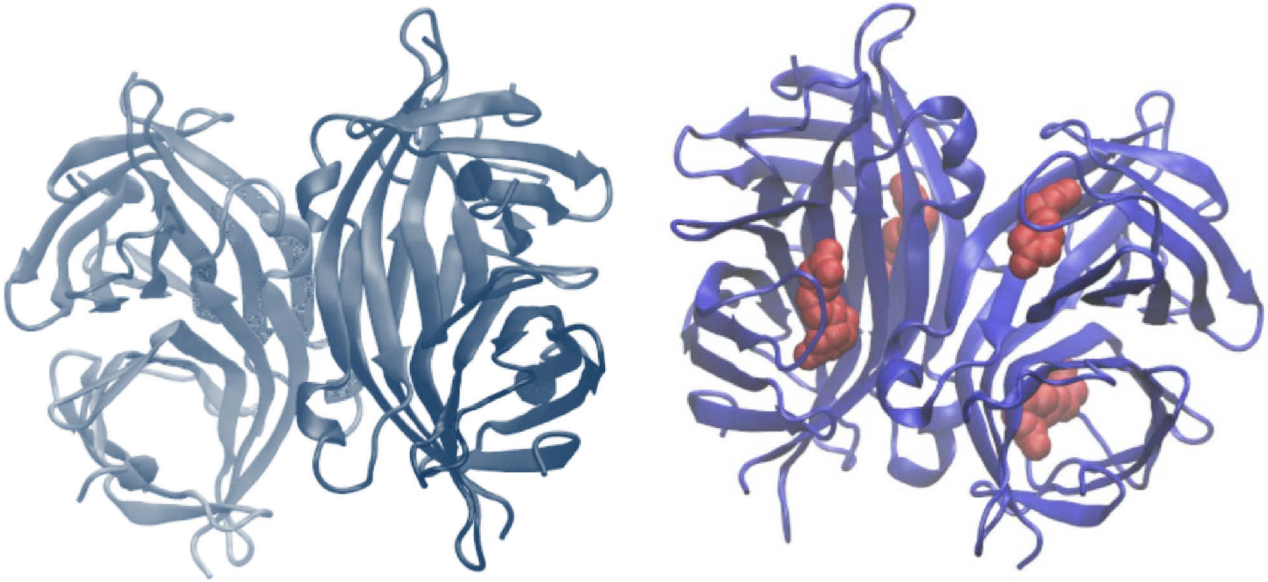


THERMODIFFUSION AS A PROBE FOR PROTEIN HYDRATION

11.09.2018 I DOREEN NIETHER, MONA SARTER, ANDREAS STADLER AND SIMONE WIEGAND

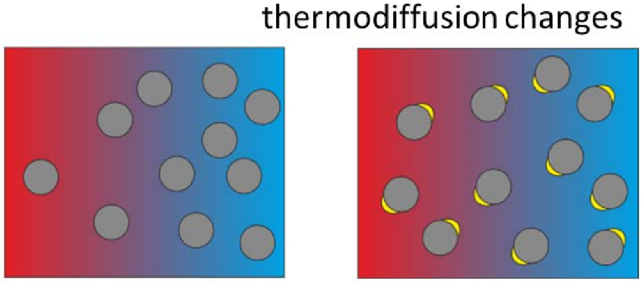
13th International Meeting on Thermodiffusion

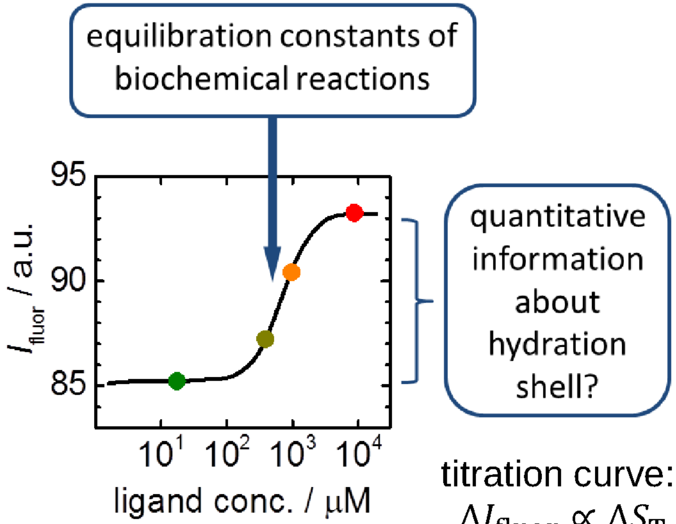
JÜLICH
Forschungszentrum
Mitglied der Helmholtz-Gemeinschaft

MOTIVATION

Microscale thermophoresis (MST)

- method to determine kinetic constant of binding reactions
  - protein’s response to thermal gradient changes when ligand binds
  - detected through change in fluorescence intensity during titration
- change due to modification of hydration shell

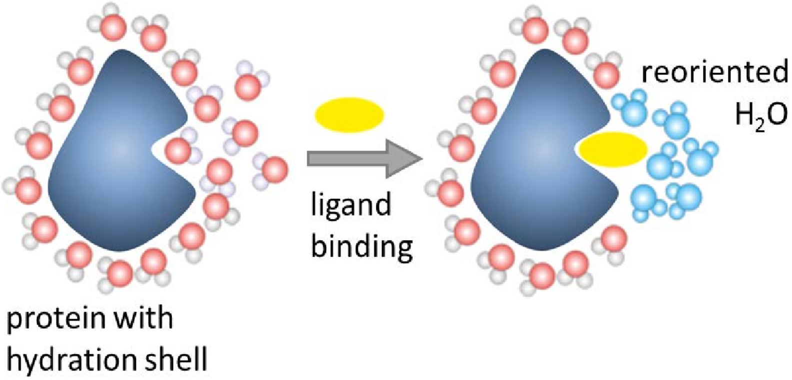




equilibration constants of biochemical reactions

quantitative information about hydration shell?

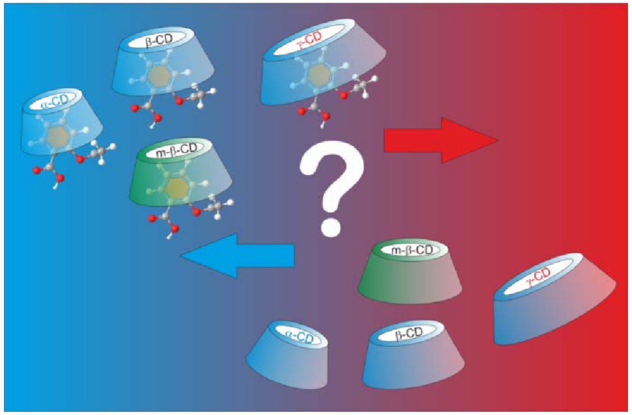
titration curve:
$\Delta I_{\text{fluor}} \propto \Delta S_{\text{T}}$



OPEN QUESTIONS

Hydration has strong influence on thermophoretic response.

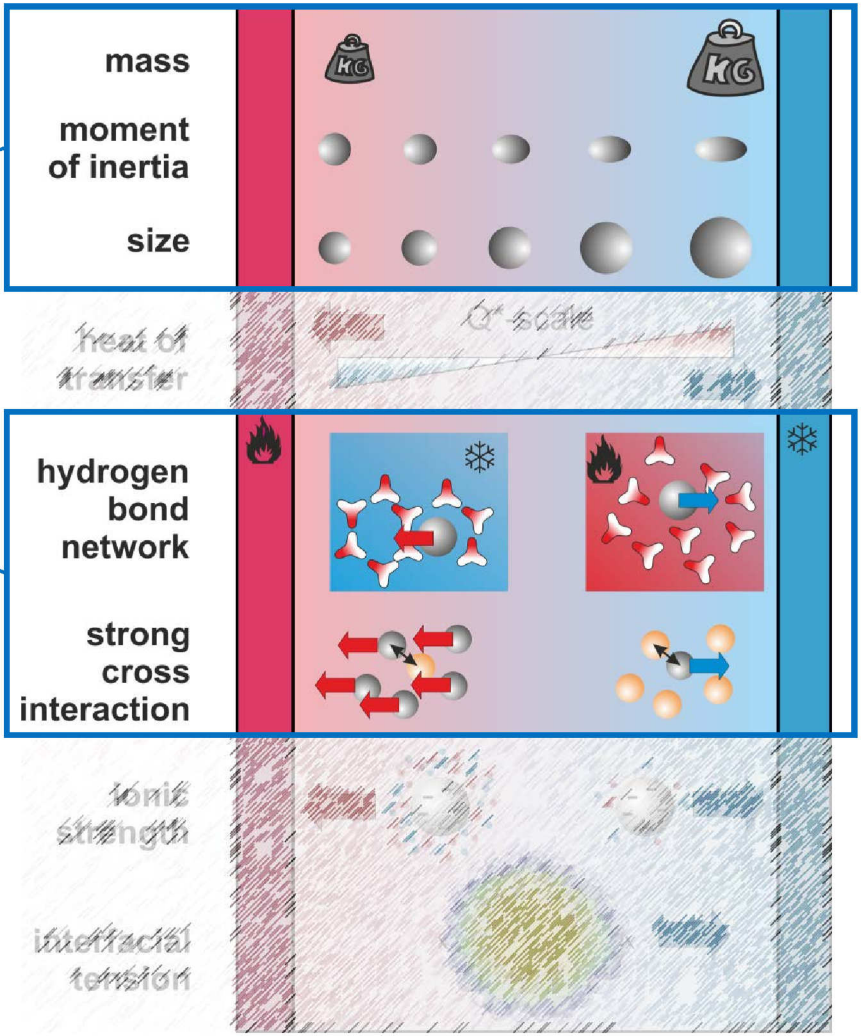
- Can this connection be quantified?
- Can it be used to gain information about change in hydration shell upon complex formation?



CONTRIBUTIONS

Aqueous systems

$$S_{\mathrm{T}} \approx S_{\mathrm{T}}^{\,i} + S_{\mathrm{T}}^{\,chem}$$

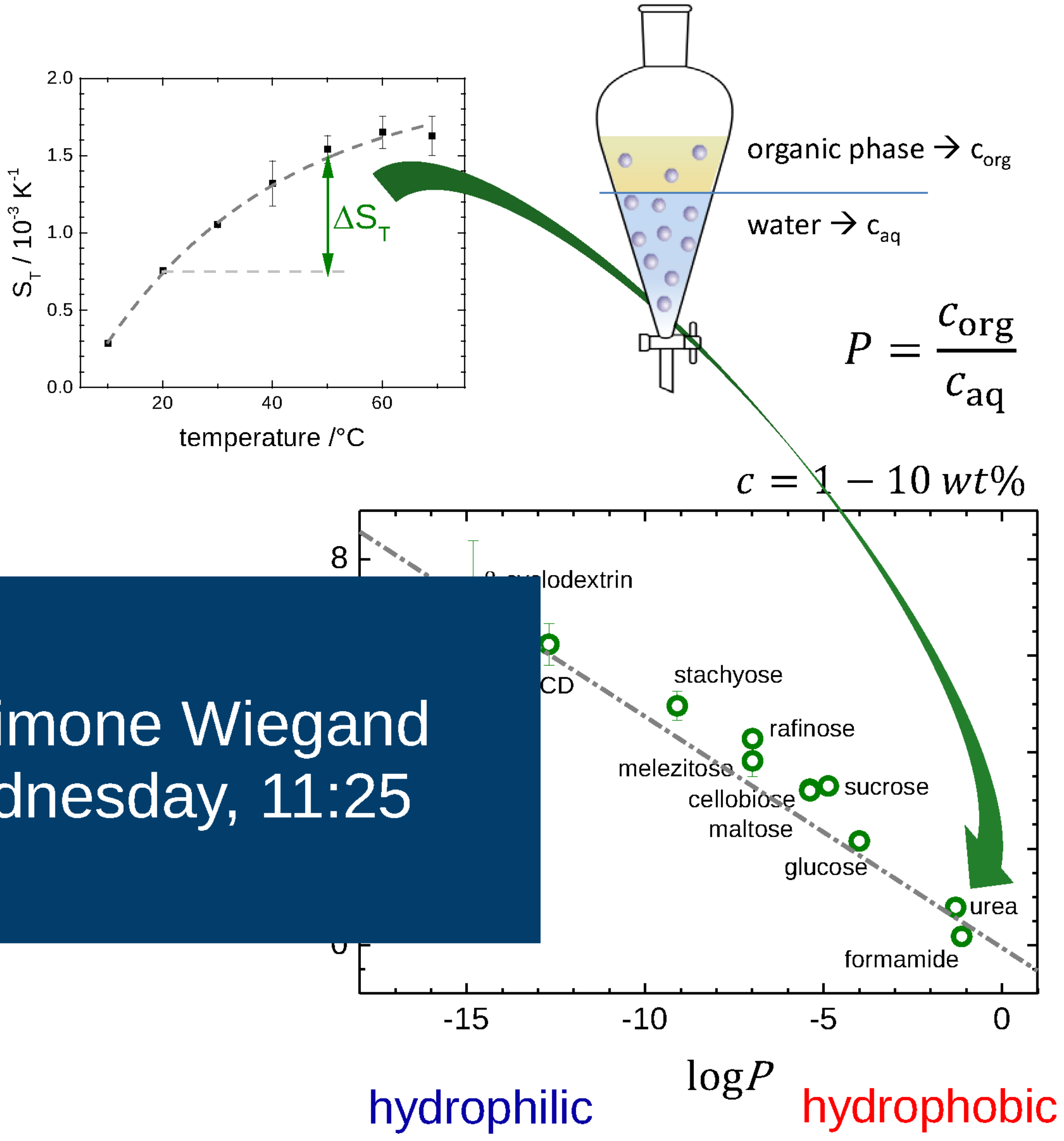


- aqueous systems
- S_{T} influenced by hydrogen bonds
  - HB network of water
  - HB between solute and water
- T-dependence of HB ⇔ thermodiffusion

RESULTS

Correlation with $\log P$

$$\Delta S_{\mathrm{T}} = S_{\mathrm{T}}(50°C) - S_{\mathrm{T}}(20°C)$$



$$P = \frac{c_{\mathrm{org}}}{c_{\mathrm{aq}}}$$

$$c = 1 - 10\ wt\%$$

- ΔS_{T} is measure for t
  dependence → prop
  chemical contributio
- ΔS_{T} correlates with h
  $(\log P)$
- connection between hydration and thermodiffusion

→ Simone Wiegand
Wednesday, 11:25

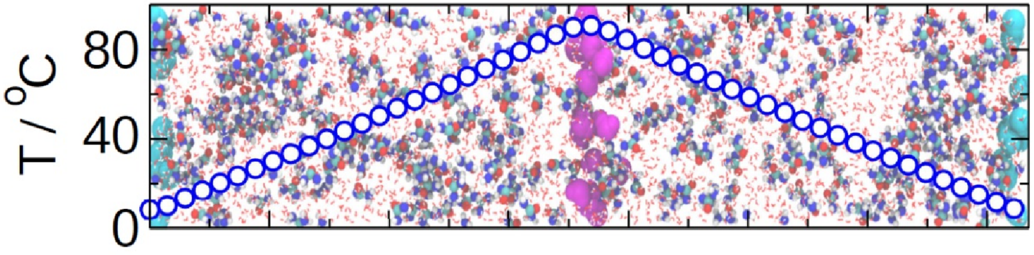
saccharides: P. Blanco et al., J. Phys. Chem. B 114, 2807 (2010)
urea: D. Niether et al., PCCP., 20, 1012 (2018).
formamide: D. Niether et al., PNAS, 113, 4272 (2016).

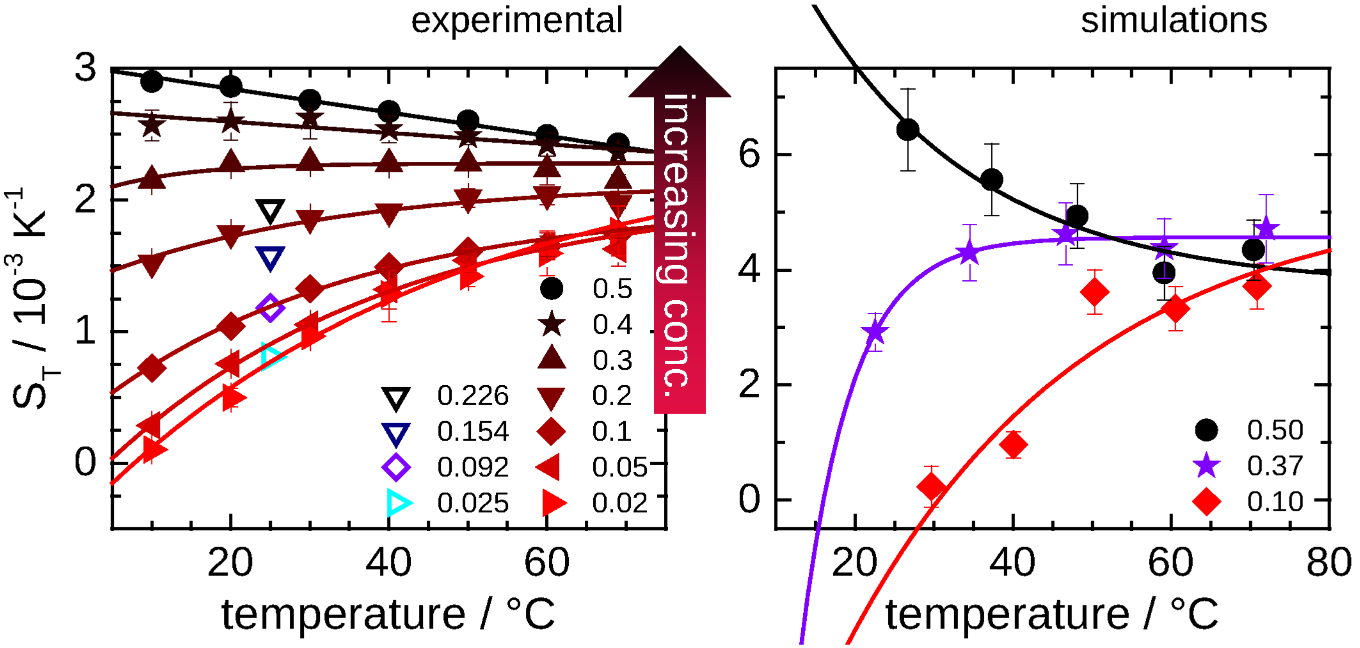
Collaboration with Fernando Bresme and
Silvia Di Lecce, Imperial College London

UREA + WATER

NEMD-simulations – microscopic understanding

- non-equilibrium molecular dynamics simulations
- T-dependence of S_T decreases with rising concentration




experimental

simulations

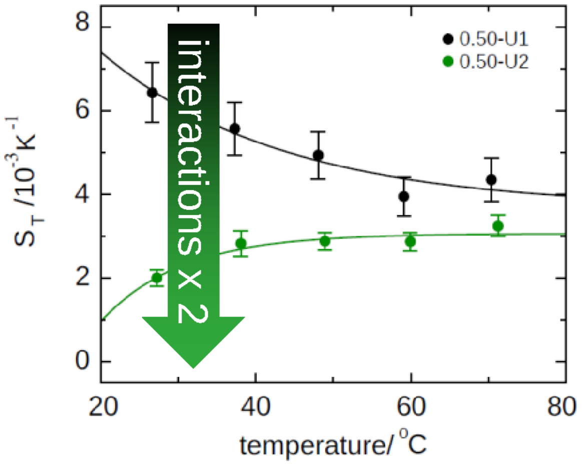
Collaboration with Fernando Bresme and
Silvia Di Lecce, Imperial College London

UREA + WATER

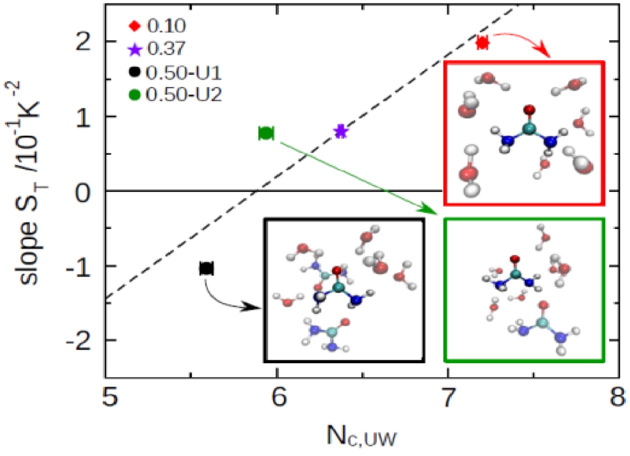
NEMD-simulations – microscopic understanding

- NEMD simulations show that interactions between **urea and water** decrease

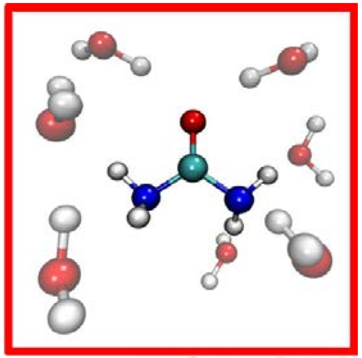
simulations



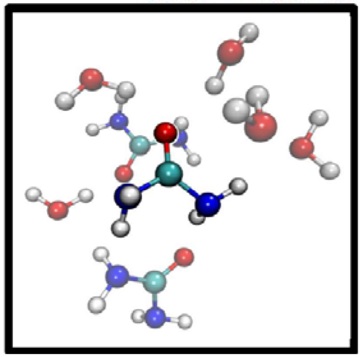
simulations



- increasing U-W interactions: slope goes from negative to positive



w.f. = 0.10



w.f. = 0.50

D. Niether *et al.*, *PCCP* **20**, 1012 – 1020 (2018)

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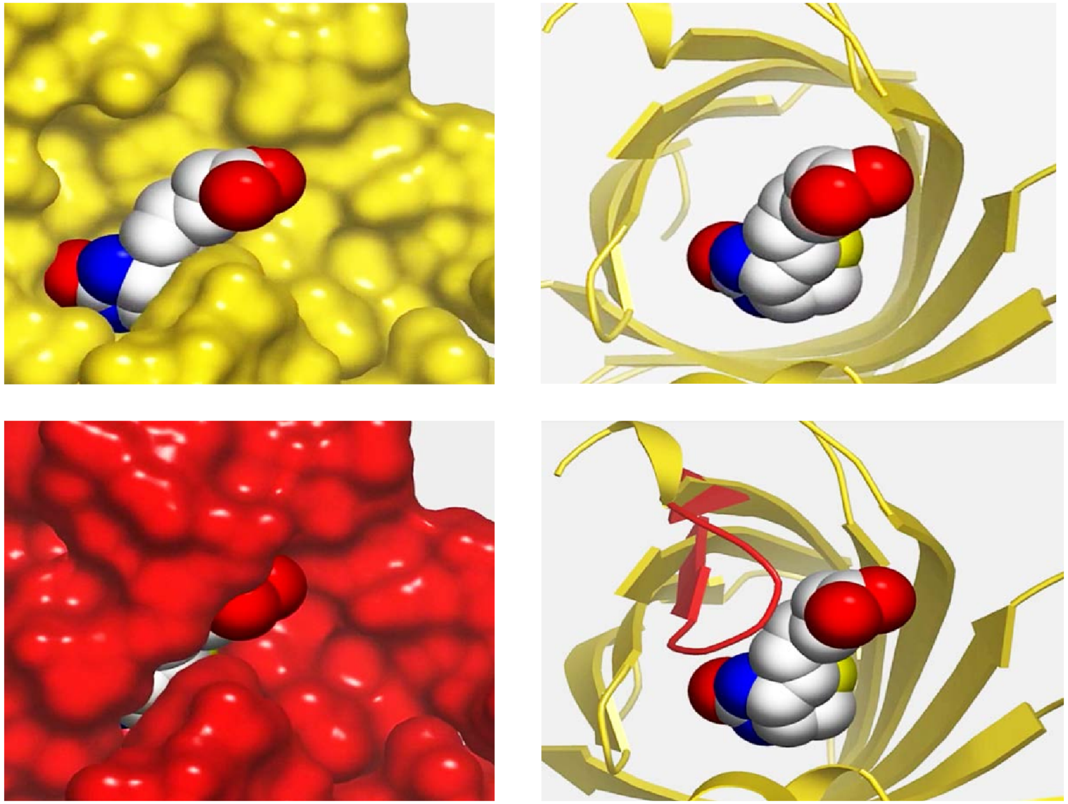
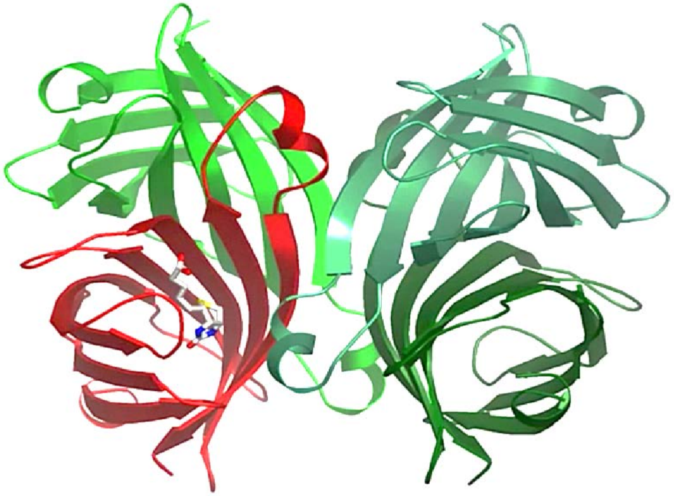
STREPTAVIDIN + BIOTIN

Protein-ligand system

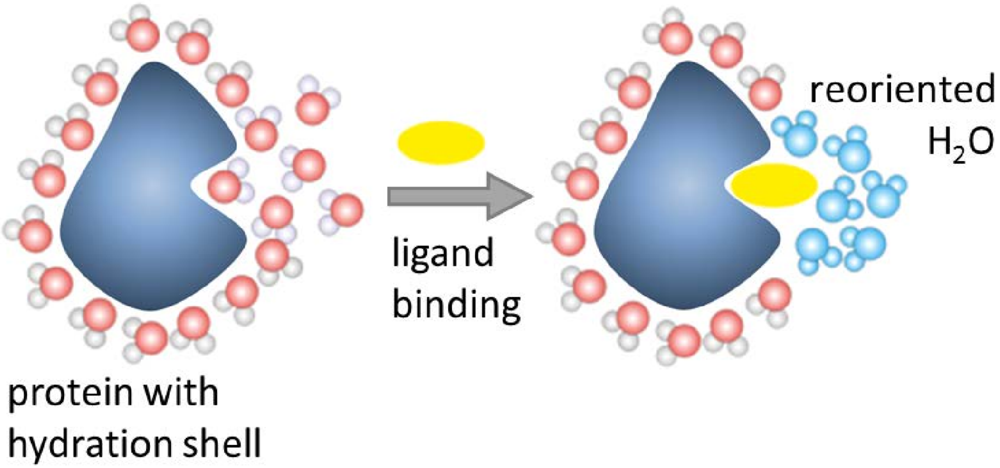




streptavidin tetramer

PROTEIN LIGAND INTERACTIONS

Influence of hydration layer



ΔH enthalpy: forces in the protein

VdW interactions
H-bonding
screened charges

ΔS entropy: number of accessible states

$$\Delta S = \Delta S_{protein} + \Delta S_{hydration}$$

< 0 ?

ligand binding determined by change of free energy

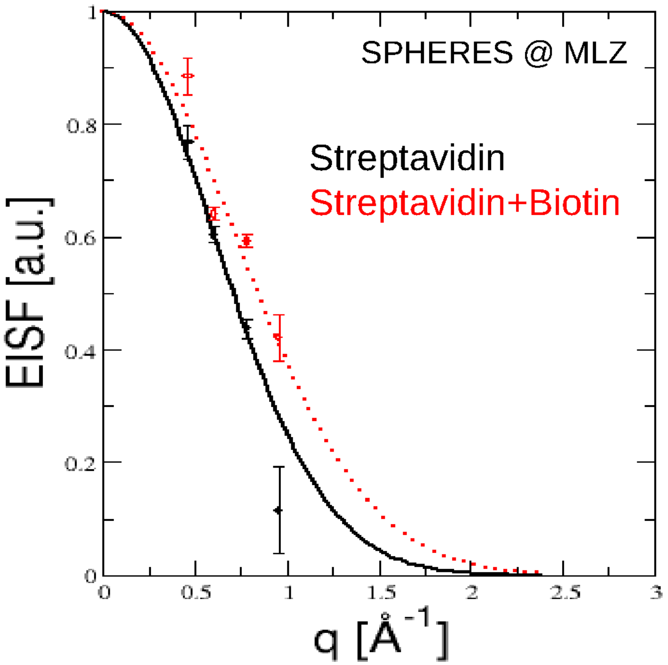
$$\Delta G = G^{bound} - G^{free} = \Delta H - T\Delta S$$

QUASI-ELASTIC NEUTRON SCATTERING

Determination of protein dynamics in solution

Andreas Stadler and Mona Sarter,
ICS-1, JCNS and RWTH Aachen

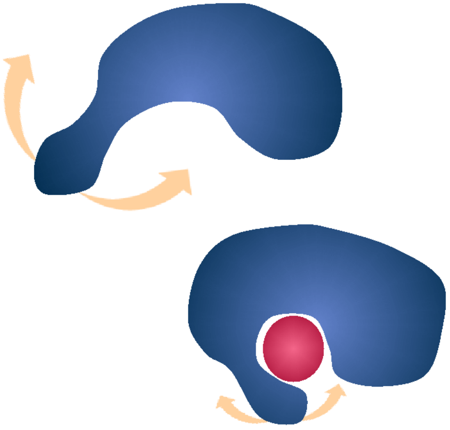
EISF SPHERES

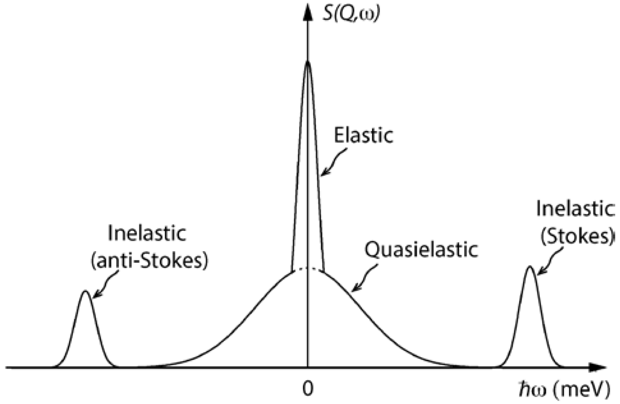


$$A_0(q) = \exp(-\langle x^2 \rangle * q^2)$$

measured in D_2O: only dynamic of **protein**

EISF –
elastic
incoherent
structure
factor →
amplitudes
of motion





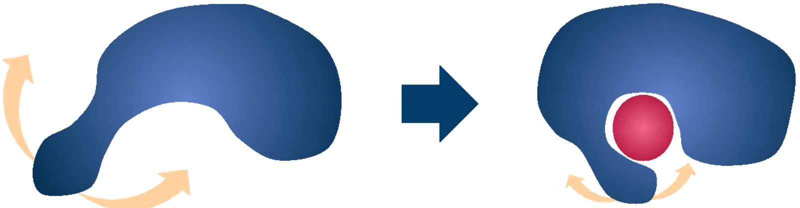
Quasi-elastic scattering:

- small energy exchange between neutron and particle
- processes with distribution of energies (translations, rotations, ...)

CONFORMATIONAL ENTROPY

Reduction of protein conformational entropy due to biotin binding

$$\Delta S_{conf} = 3R \cdot ln\left[\sqrt{\left(\frac{\langle x^2\rangle_{bound}}{\langle x^2\rangle_{free}}\right)}\right]$$



Time-scale measured	$\Delta \mathrm{Sconf}\ [\frac{\mathrm{kJ}}{\mathrm{mol\ K}}]$
ps	-1.8 ± 0.1
100 ps	-1.4 ± 0.5
ns	-2.2 ± 0.3

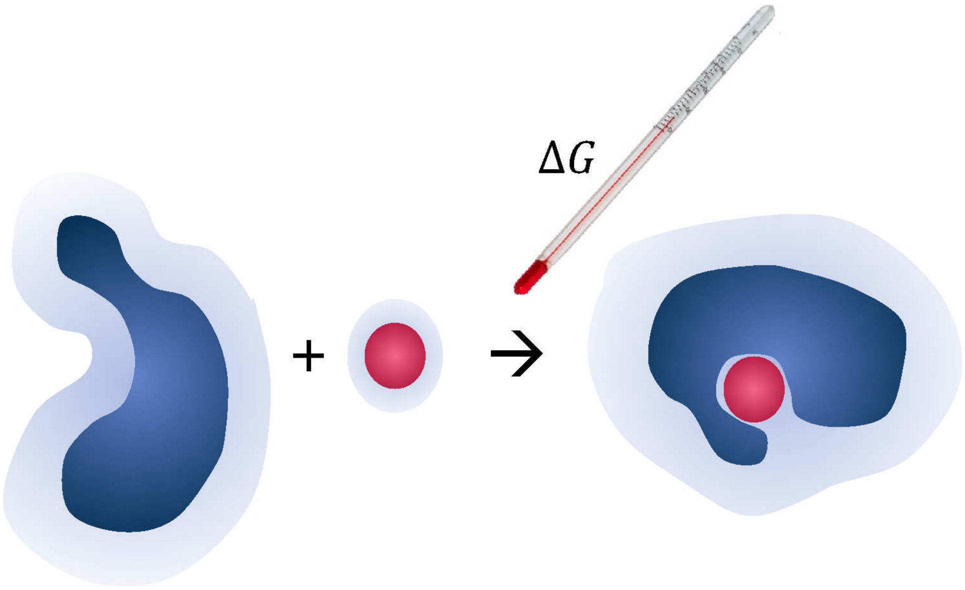
- streptavidin is more mobile than streptavidin+biotin complex
- fast motions attributed to side-chains
- conformational entropy negative on ps to ns time-scale
- entropy change free protein → complex:

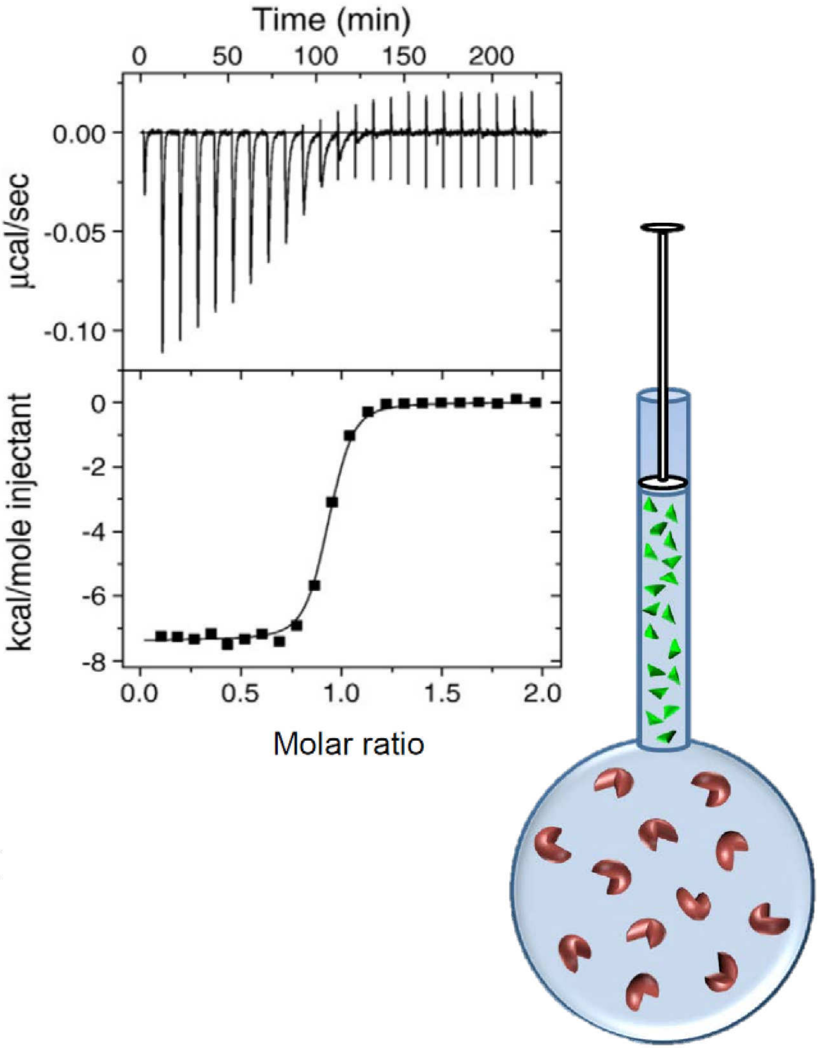
$$\Delta S^{QENS} = -2.0\ \pm 0.2\ \mathrm{kJ\ mol^{-1}\ K^{-1}}$$

Receveur et al. JMB 1997
Fitter Biophys J 2003

ITC

Isothermal titration calorimetry





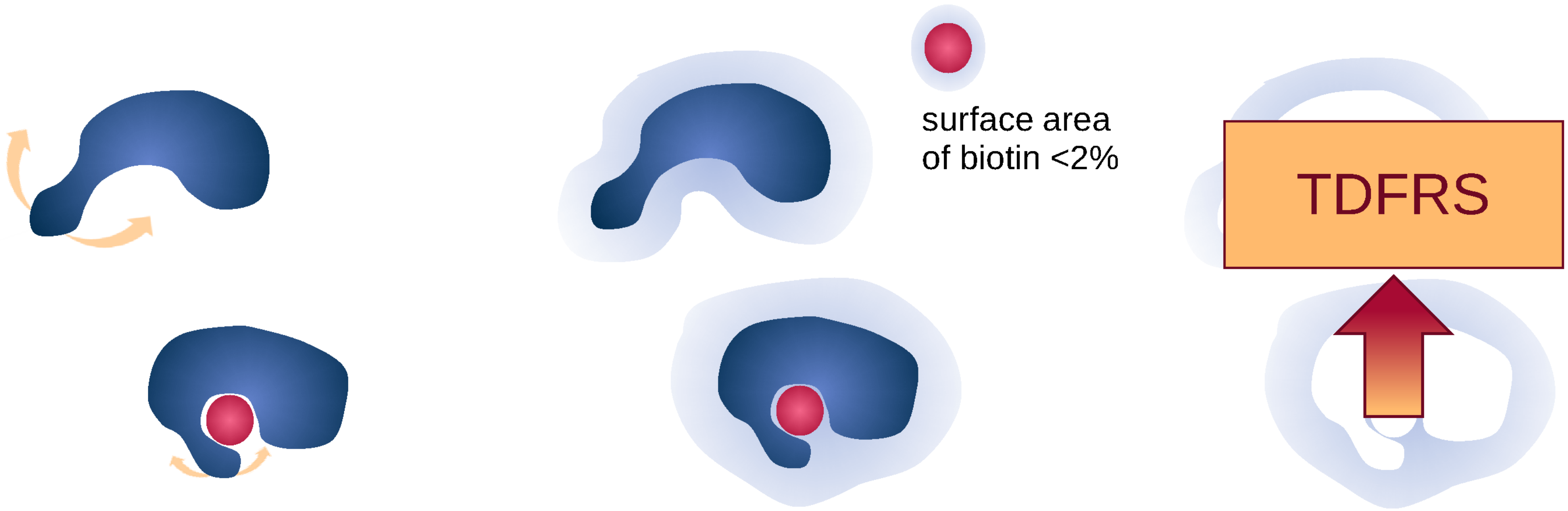
measures free energy change upon complex formation (**protein + hydration shell**)

$$\Delta S^{ITC} = -0.1 \pm 0.1 \text{ kJ mol}^{-1} \text{ K}^{-1}$$

Kuo, T.C. *et al.*, J. Mol. Recognit., **28** (2015) 125-128
Williams et al JMB 2003

RESULTS

Streptavidin + biotin



surface area
of biotin <2%

TDFRS

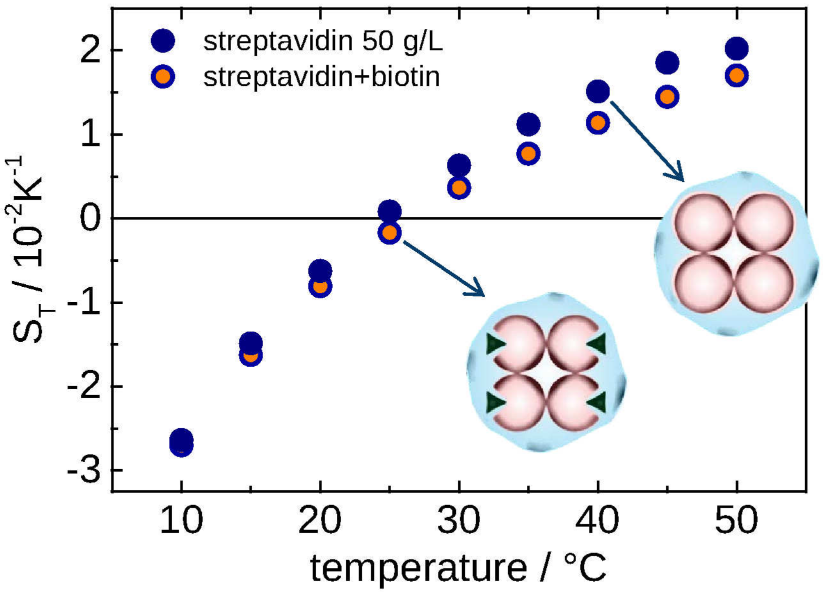
QENS	ITC	Difference
$\Delta S^{QENS} =$ $-1.8 \ \pm 0.2 \text{ kJ mol}^{-1}\text{ K}^{-1}$	$\Delta S^{ITC} =$ $-0.1 \ \pm 0.1 \text{ kJ mol}^{-1}\text{ K}^{-1}$	$\Delta S^{hs} = \Delta S^{ITC} - \Delta S^{QENS} =$ $1.9 \pm 0.3 \text{ kJ mol}^{-1}\text{ K}^{-1}$
entropy change of **protein**	entropy change of **protein + hydration shell**	→ entropy of **hydration shell** increases when biotin binds

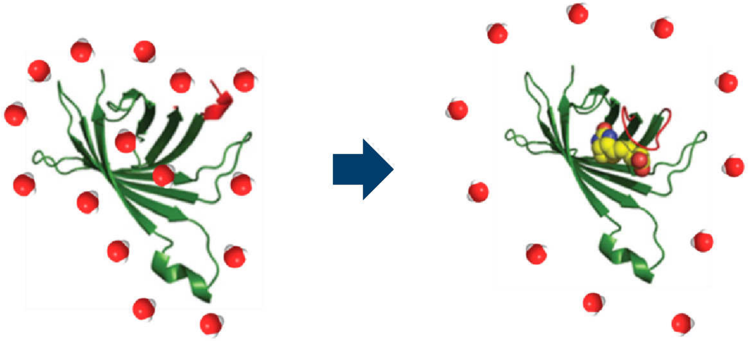
RESULTS

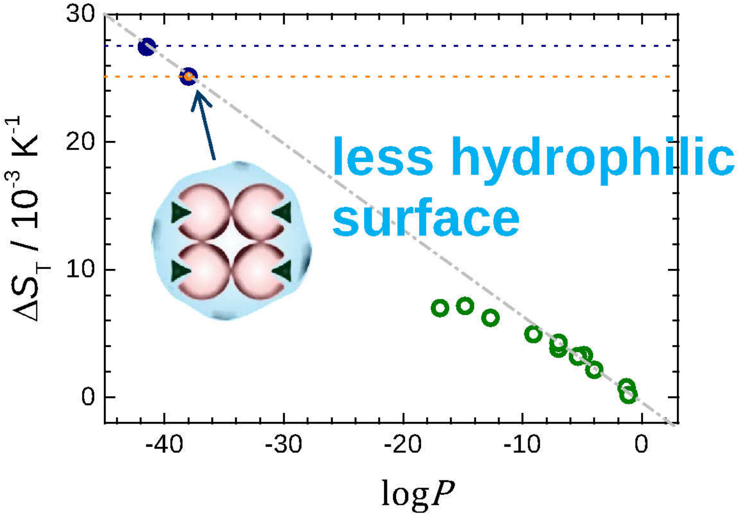
Streptavidin + biotin

- reduced ΔS_T for strep + biotin

  → surface of complex less hydrophilic
- indicates breaking of HB
- results from neutron scattering data:
  - protein-complex more rigid
  - entropy increase in hydration shell





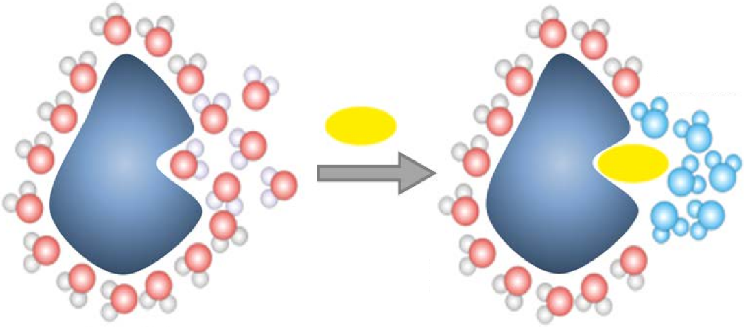


less hydrophilic surface

D. Niether *et al.*, AIP Conference Proceedings **1929**, 020001 (2018)

SUMMARY

- Influence of hydration on S_T was investigated
- Conclusion on complex formation of streptavidin: disordered hydration layer compensates less flexible protein complex
- Clear change in ΔS_T when biotin binds on streptavidin, connection to entropy change in hydration shell



ACKNOWLEDGEMENT

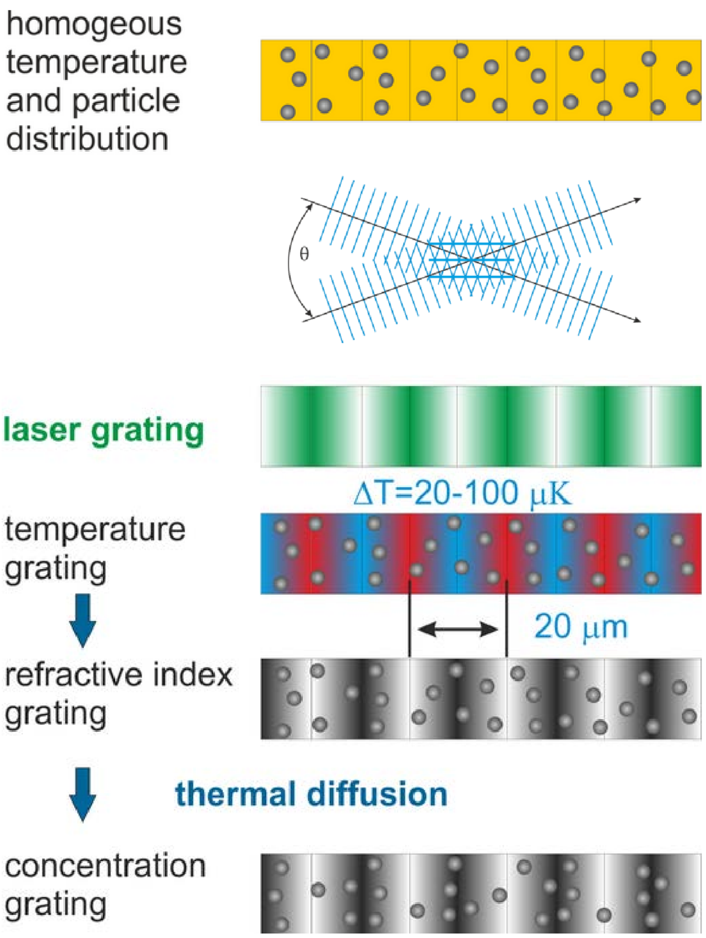
Simone Wiegand, Jan K.G. Dhont, and the ICS-3

collaborators	Silvia Di Lecce and Fernando Bresme, Imperial College London, UK – NEMD simulations
	Mona Sarter, Andreas Stadler, Bernd König, and Jörg Fitter, FZJ and RWTH Aachen – streptavidin QENS

Thank you for your attention!

SETUP

Infra-red Thermal Diffusion Forced Rayleigh Scattering (IR-TDFRS)



homogeous temperature and particle distribution

laser grating

ΔT=20-100 μK

temperature grating

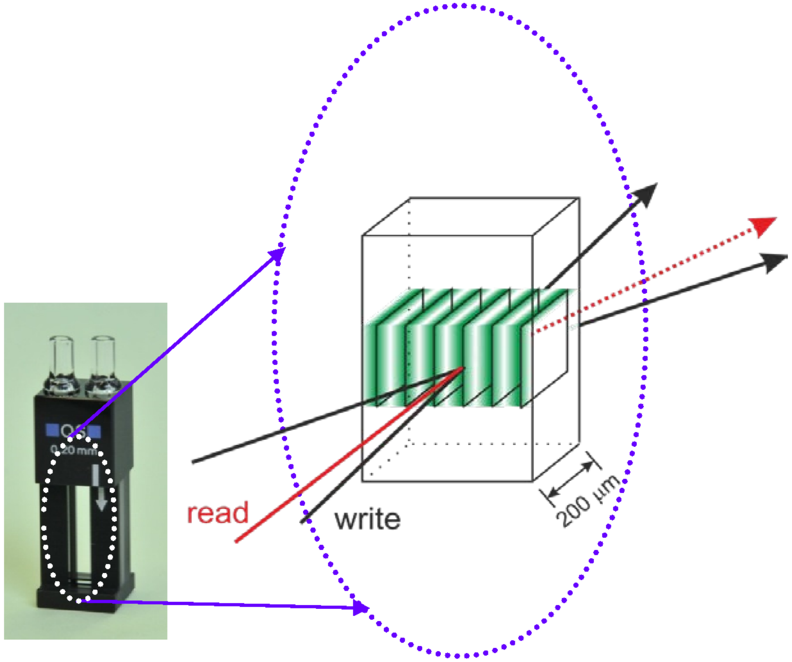
20 μm

refractive index grating

thermal diffusion

concentration grating

Measured quantity:
Intensity of the diffracted beam



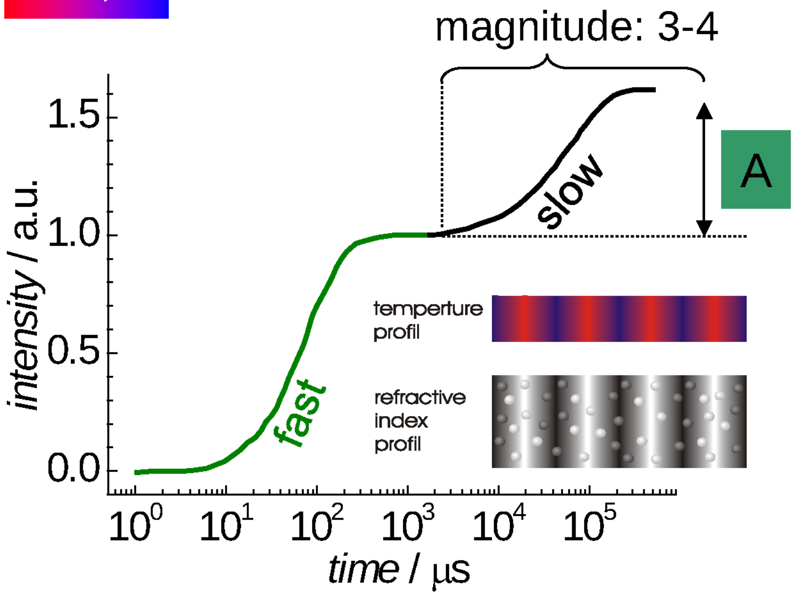
SETUP

Signal

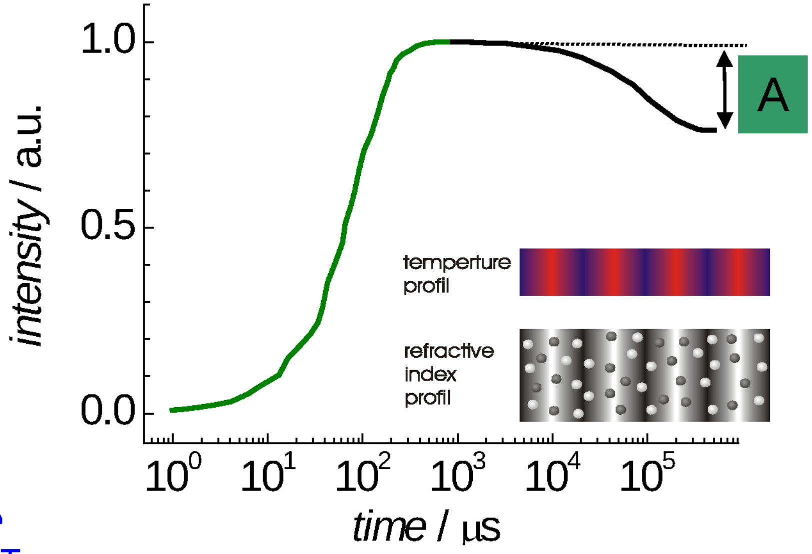
Measured quantity:
Intensity of the diffracted beam

Molecules/colloides with higher refractive index moves to…

…cold side



…warm side



S_T

$$\varsigma_{het} = \underbrace{\left(1-e^{-t/\tau_{th}}\right)}_{\text{temperature}} - \underbrace{\frac{(\partial n/\partial c)_{p,T}}{(\partial n/\partial T)_{p,c}} \boxed{\frac{D_T}{D}}\, c\,(1-c)\,\frac{1}{\tau-\tau_{th}}}_{A} \left[\tau\left(1-e^{-t/\tau}\right)-\tau_{th}\left(1-e^{-t/\tau_{th}}\right)\right]$$

A