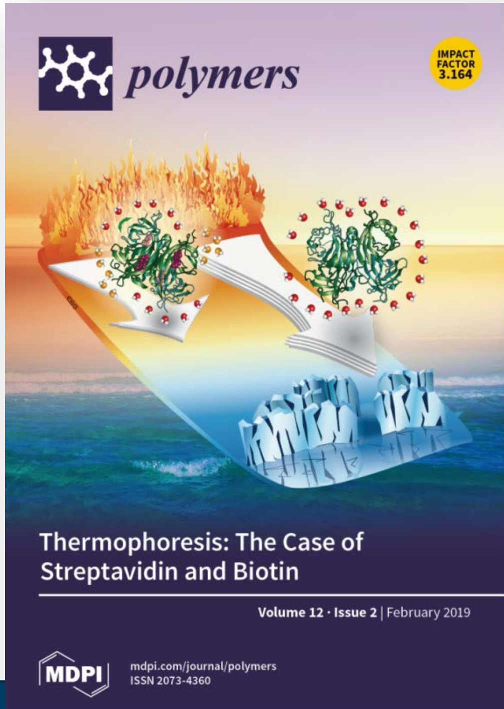

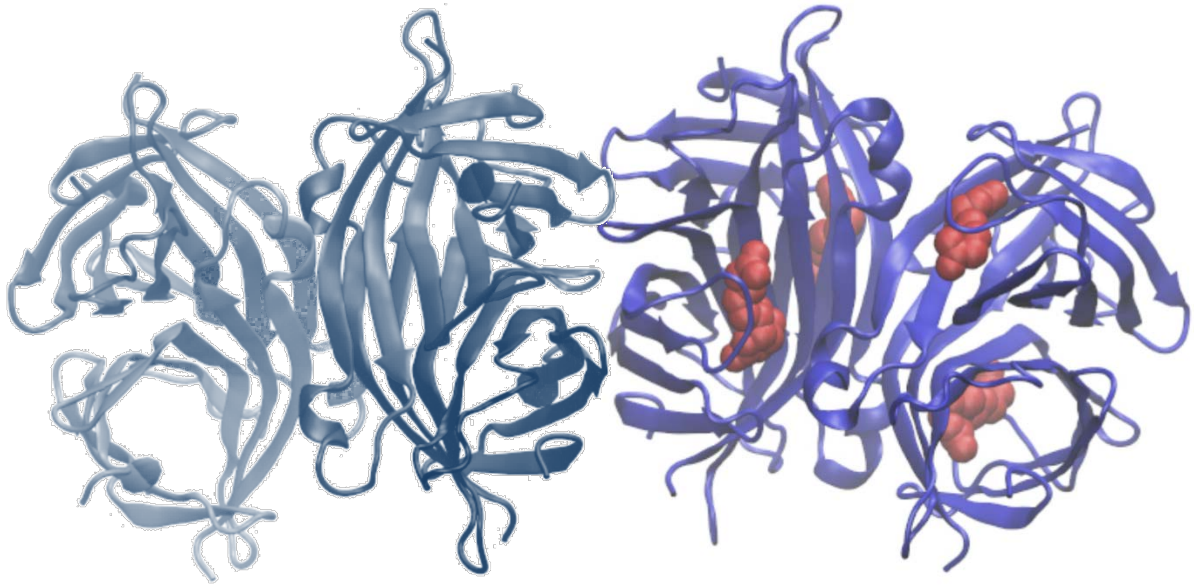

THERMOPHORESIS: THE CASE OF STREPTAVIDIN AND BIOTIN

19.11.2020 I **DOREEN NIETHER**, MONA SARTER, BERND W. KÖNIG, JÖRG FITTER, ANDREAS M. STADLER, SIMONE WIEGAND

2020 EUSMI User Meeting

JÜLICH
Forschungszentrum
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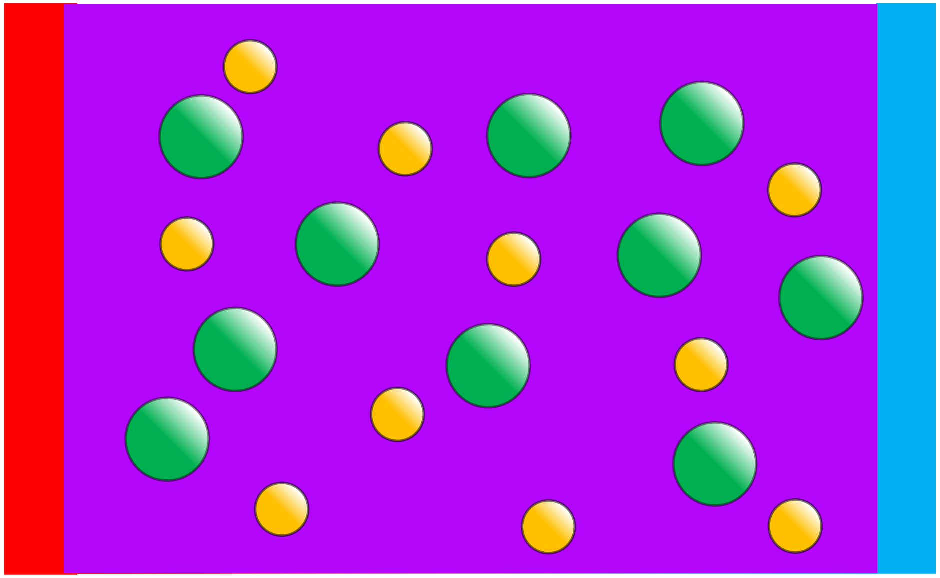
THERMOPHORESIS

Movement of particles driven by a temperature gradient

$$\vec{j} = -\rho D \vec{\nabla} w - w(1-w)\rho D_T \vec{\nabla} T$$

steady state $\vec{j} = 0$

$$\frac{D_T}{D} = -\frac{\vec{\nabla} w}{w(1-w)\vec{\nabla} T}$$



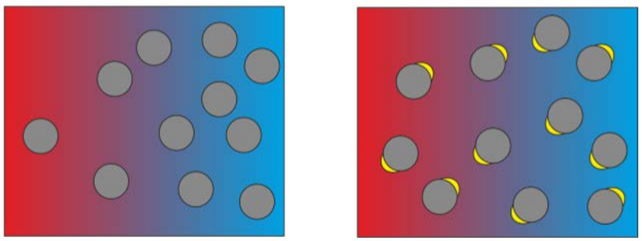
$$S_T = \frac{D_T}{D} \propto \frac{\Delta w}{\Delta T}$$

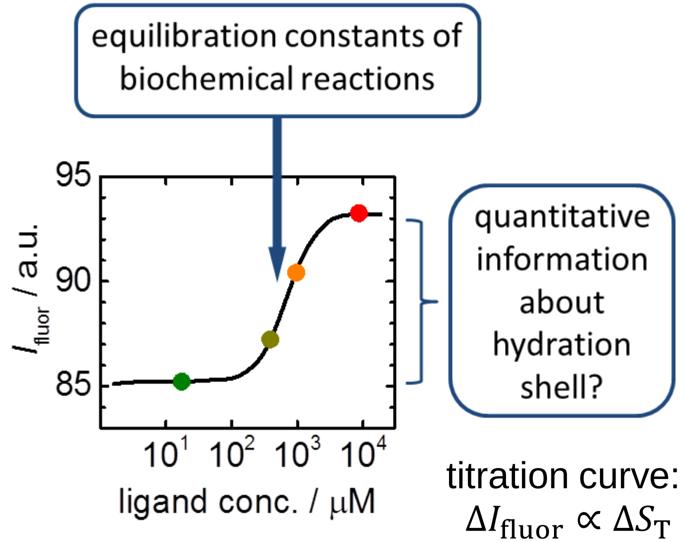
D	diffusion coefficient
w	weight fraction
ρ	mass density
D_T	thermodiffusion coefficient
$\vec{j}$	flux
T	temperature
S_T	Soret coefficient

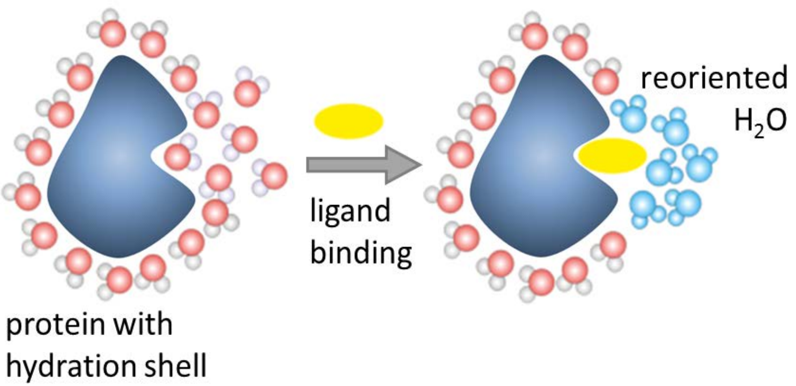
MOTIVATION

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

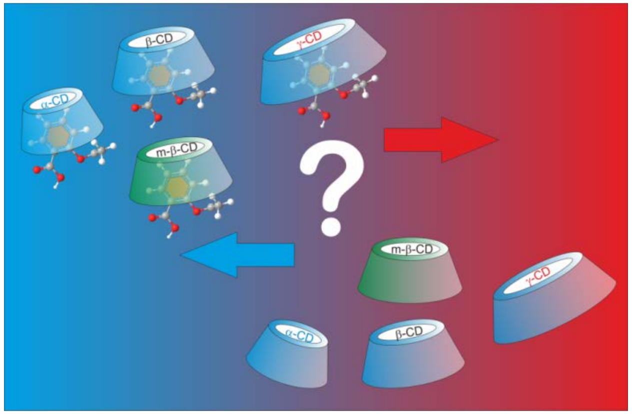






Hydration has strong influence on thermophoretic response.

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

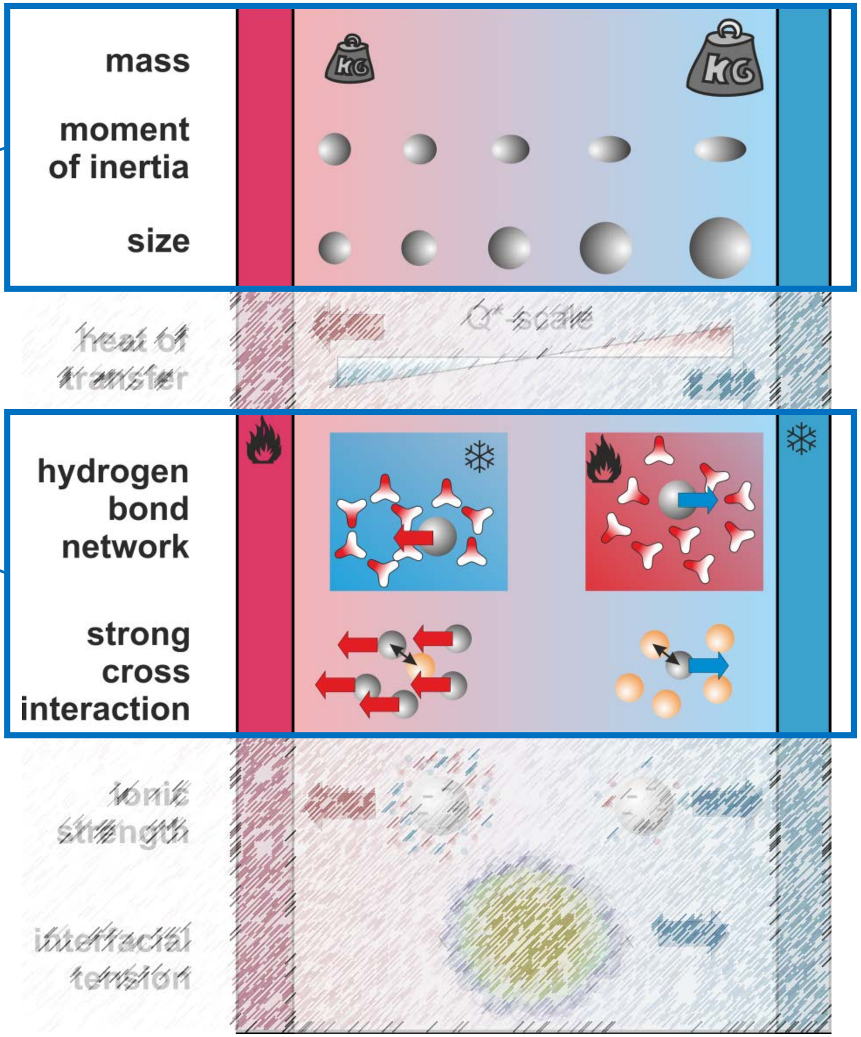


OPEN QUESTIONS

CONTRIBUTIONS

Aqueous systems

$$S_T \approx S_T^{\,i} + S_T^{\,chem}$$

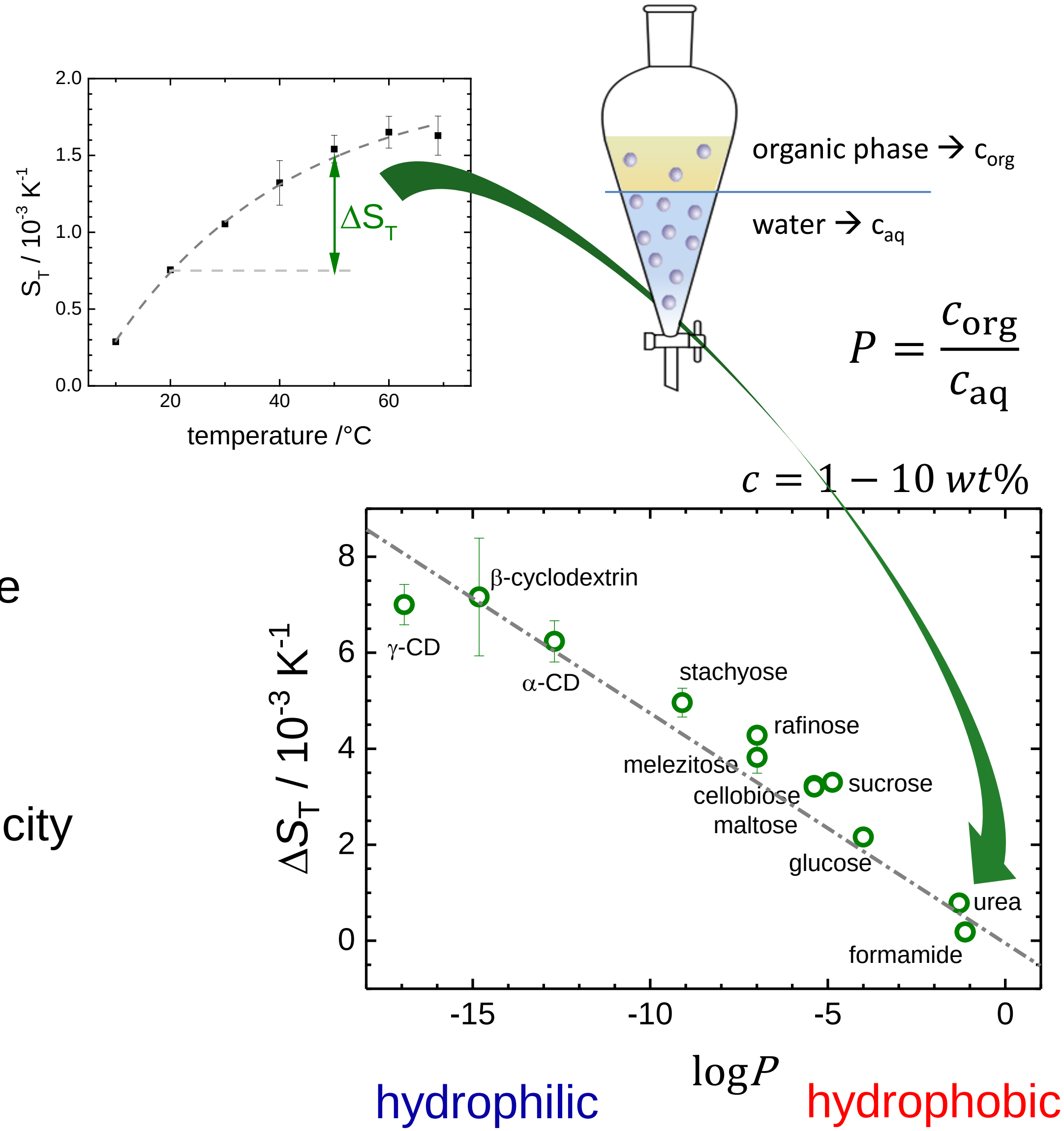


- aqueous systems
- S_T influenced by hydrogen bonds
  - HB network of water
  - HB between solute and water
- T-dependence of HB ⇔ thermodiffusion

RESULTS

Correlation with $\log P$

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



- ΔS_{T} is measure for temperature dependence → proportional to chemical contribution $S_{\mathrm{T}}^{\ chem}$
- ΔS_{T} correlates with hydrophobicity ($\log P$)
- connection between hydration and thermodiffusion

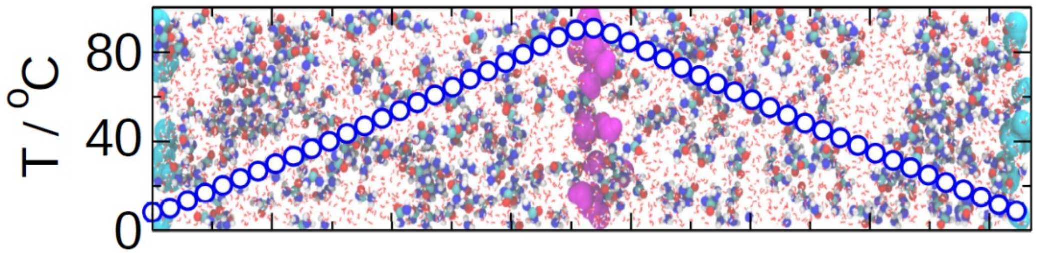
saccharides: P. Blanco et al., J. Phys. Chem. B (2010)
cyclodextrins: K. Eguchi et al., Eur. Phys. J. E (2016)
urea: D. Niether et al., PCCP. (2018)
formamide: D. Niether et al., PNAS (2016)

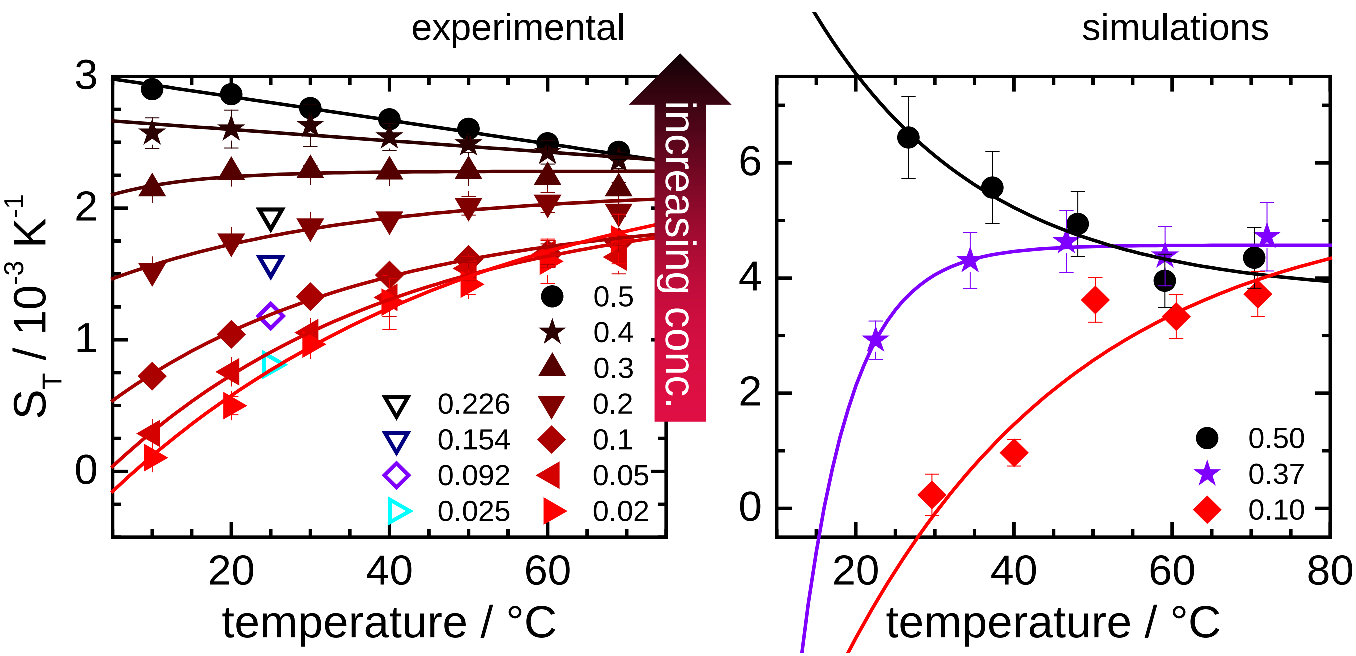
Collaboration with Fernando Bresme and
Silvia Di Lecce, Imperial College London

UREA + WATER

NEMD-simulations – microscopic understanding

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



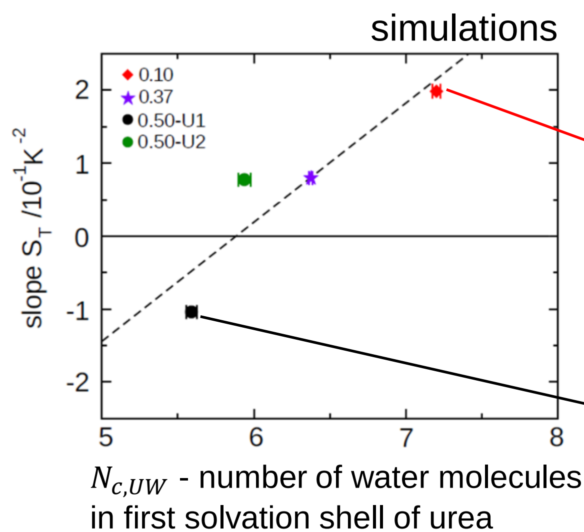


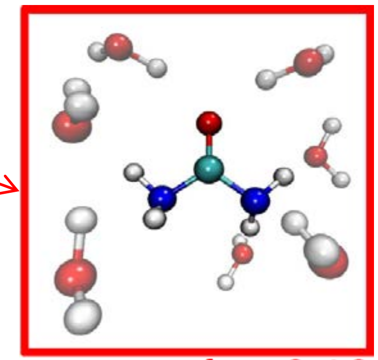
Collaboration with Fernando Bresme and
Silvia Di Lecce, Imperial College London

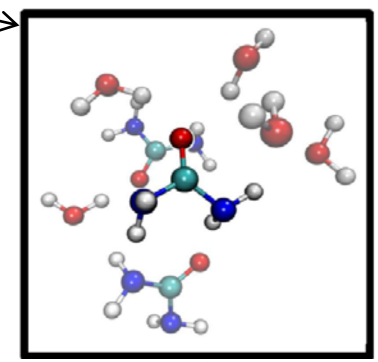
UREA + WATER

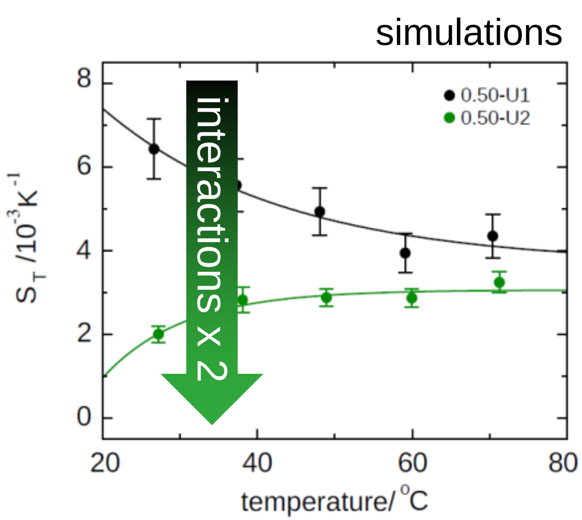
NEMD-simulations – microscopic understanding

NEMD simulations show that interactions between **urea and water** decrease with rising urea conc.









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

D. Niether *et al.*, *PCCP* (2018)

HYDROPHILICITY AND S_T

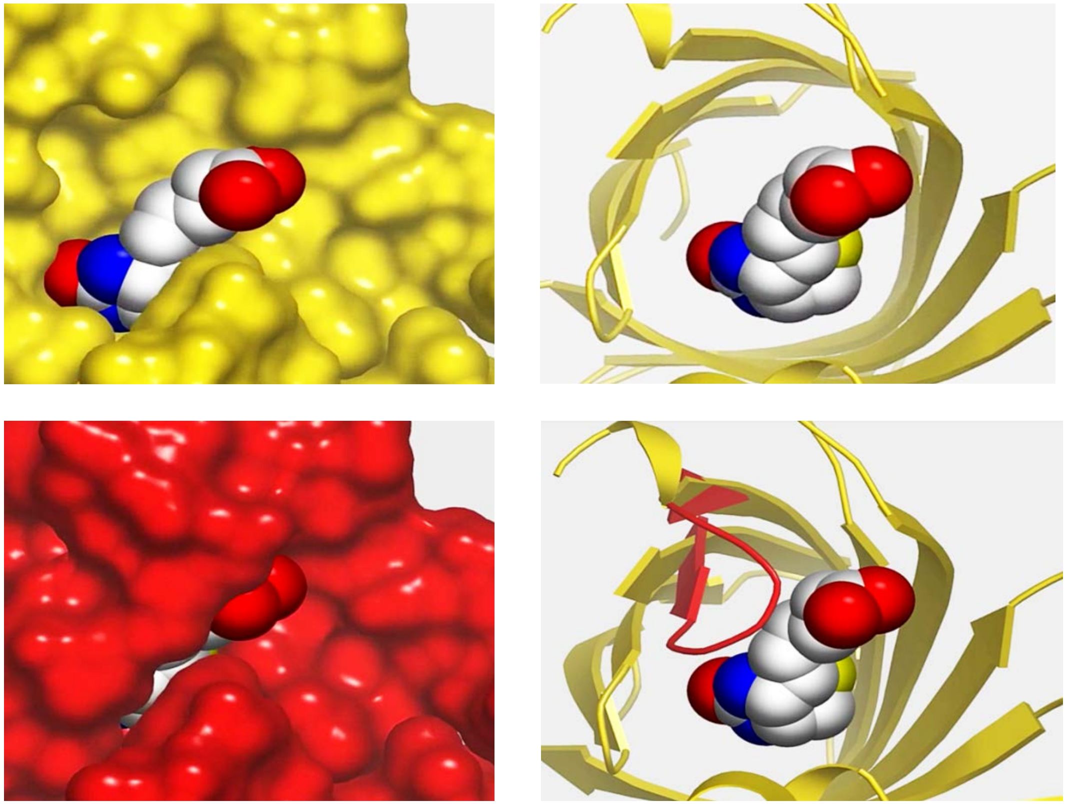
$$S_T \approx S_T^{\,i} + S_T^{\,chem}$$

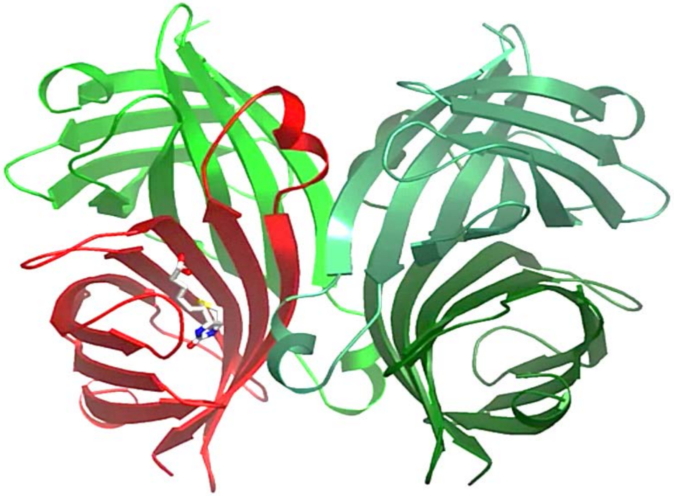
- dominated by hydrogen bonds in aqueous systems
→ proportional to ΔS_T (T-dependence of S_T)

- Correlation with logP
  Hydrophilic substances → high ΔS_T
  Hydrophobic substances → low/negative ΔS_T

STREPTAVIDIN + BIOTIN

Protein-ligand system

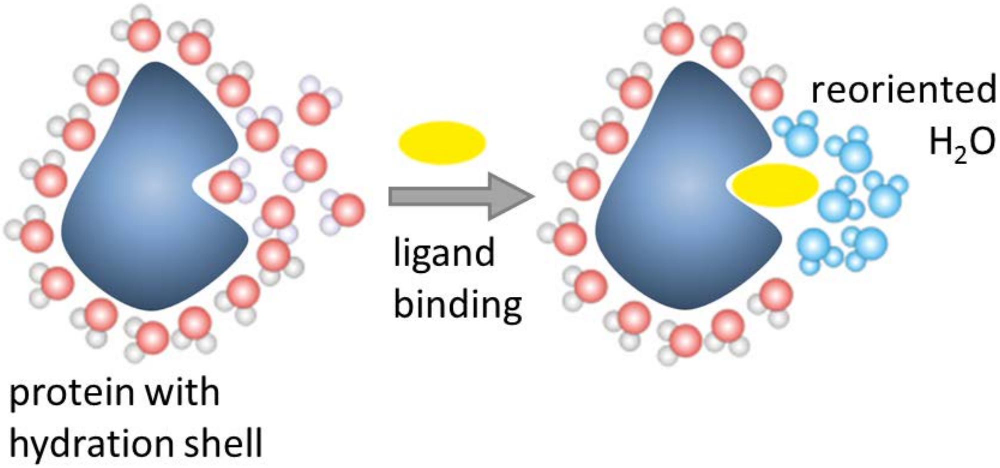




streptavidin tetramer

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

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

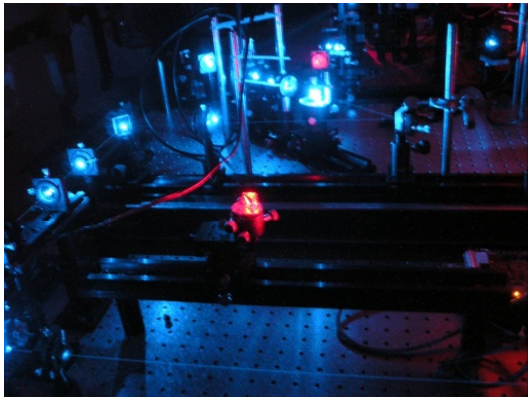
COMBINING THREE DIFFERENT METHODS TO UNDERSTAND THE BINDING

ITC

measures the entropy and free enthalpy changes due to the binding

QENS at SPHERES [Garching]

neutron back scattering probes dynamic processes



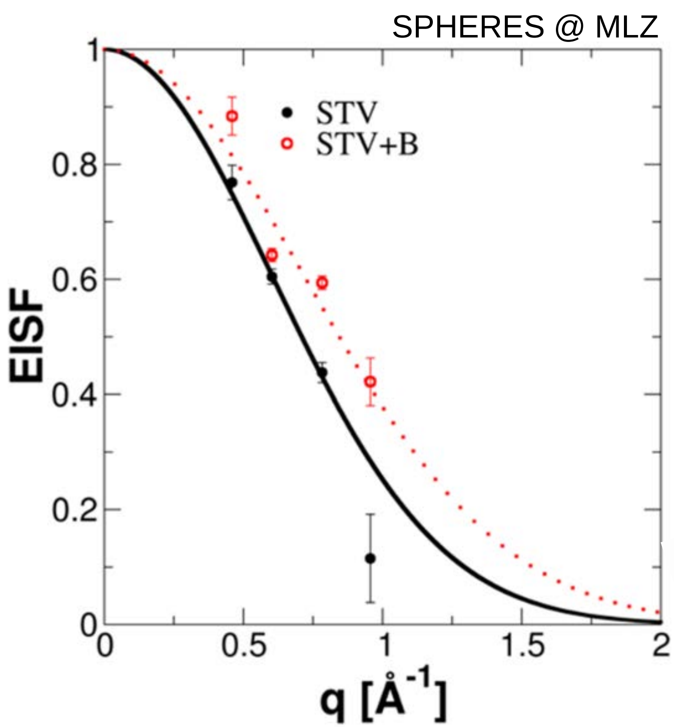
TDFRS

measures the diffusion of solute molecules in a temperature gradient

QUASI-ELASTIC NEUTRON SCATTERING

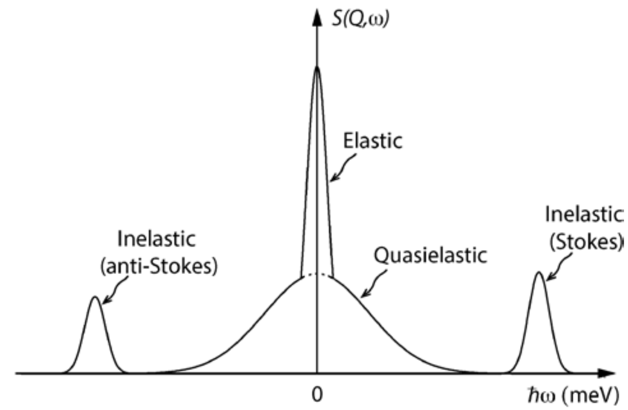
Determination of protein dynamics in solution

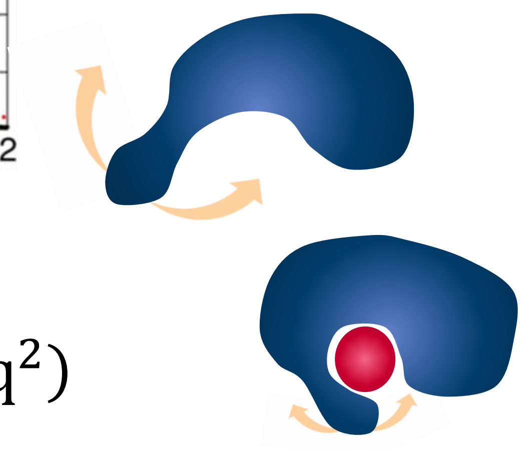
Andreas Stadler and Mona Sarter,
ICS-1, JCNS and RWTH Aachen



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, ...)

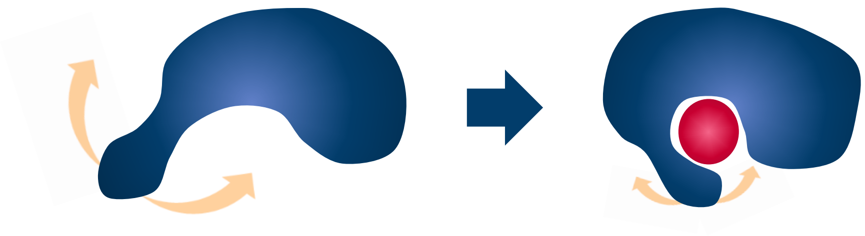
$$A_0(q) = \exp(-\langle x^2\rangle * q^2)$$

Sarter *et al.*, J.Phys.Chem.B (2020)

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	ΔSconf $[\frac{\text{kJ}}{\text{mol K}}]$
ns	-2.2 ± 0.3
ps	-1.3 ± 0.2

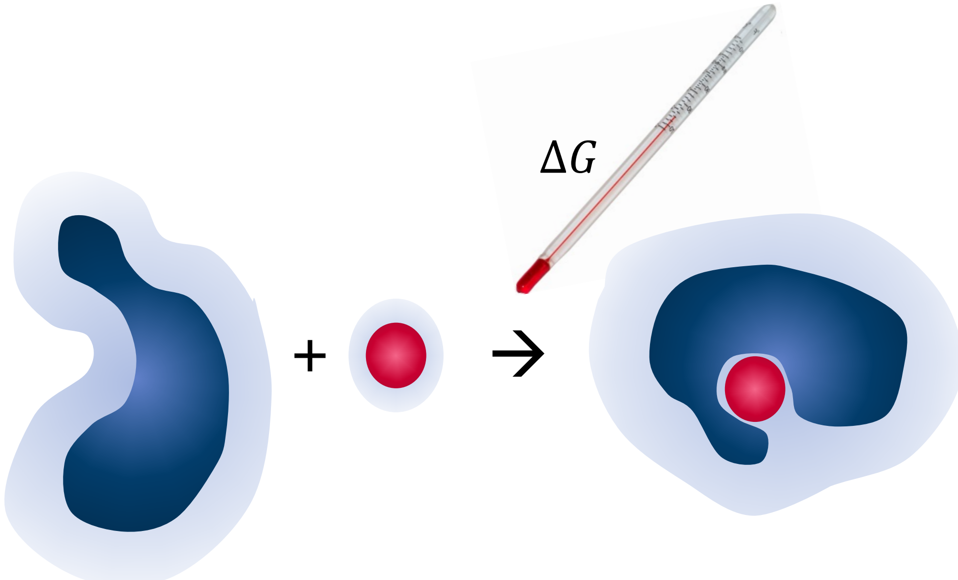
- streptavidin is more mobile than streptavidin+biotin complex
- fast motions attributed to side-chains
- conformational entropy negative on ps to ns time-scale
- entropy change free protein → complex:

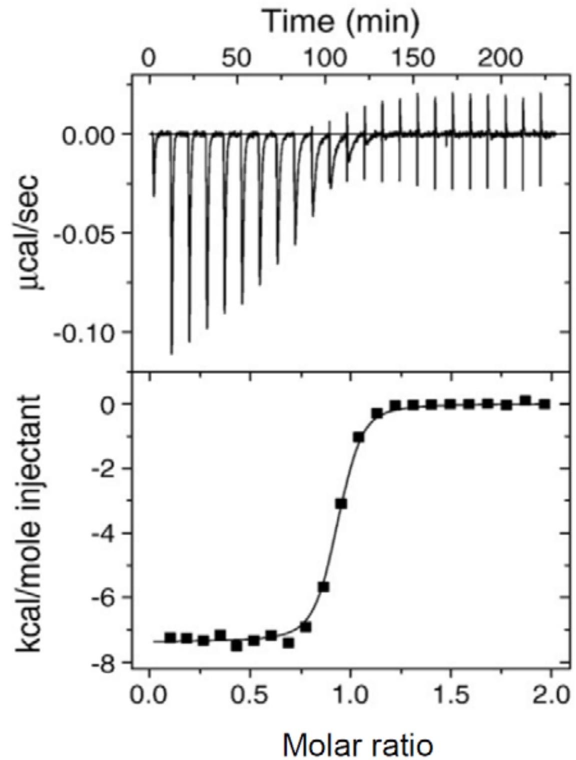
$$\Delta S^{QENS} = -2.2 \pm 0.3 \text{ kJ mol}^{-1} \text{ K}^{-1}$$

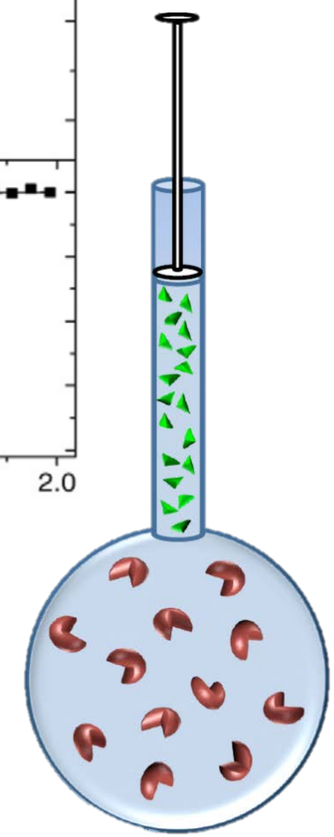
Sarter *et al*., J.Phys.Chem.B (2020)

ITC

Isothermal titration calorimetry







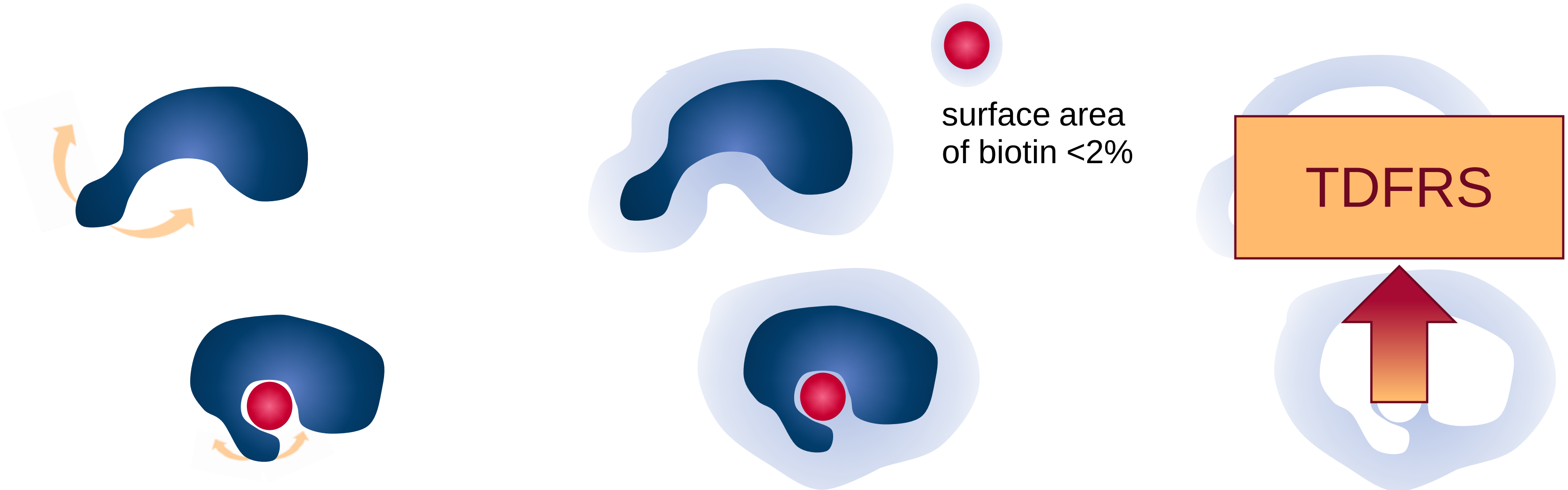
measures free energy change upon complex formation (**protein + hydration shell**)

$$\Delta S^{ITC} = -0.35 \pm 0.13 \text{ kJ mol}^{-1} \text{ K}^{-1}$$

Sarter *et al.*, J.Phys.Chem.B (2020)

RESULTS

Streptavidin + biotin

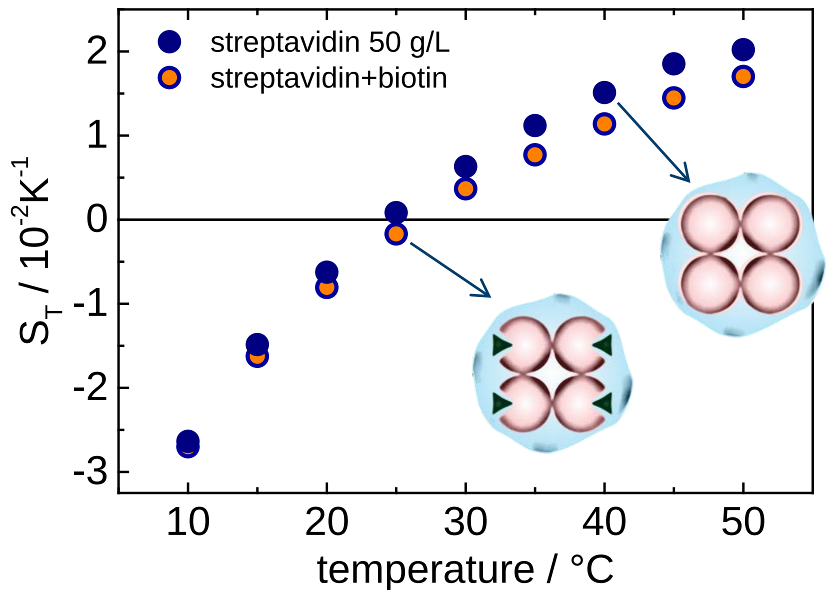


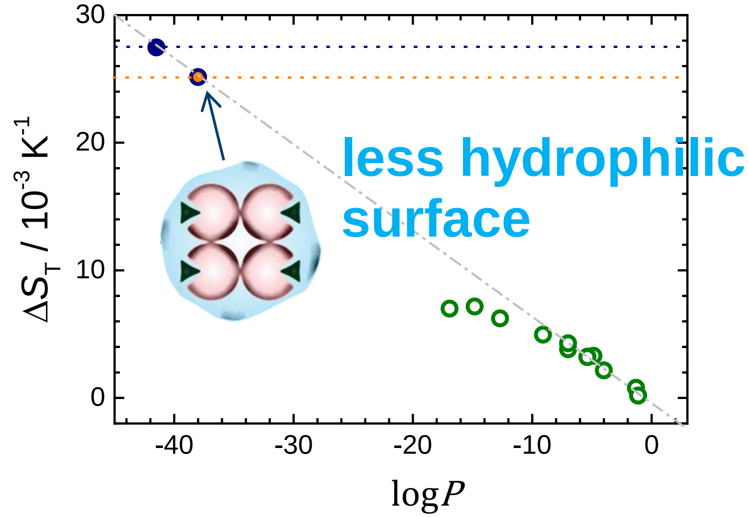
QENS	ITC	Difference
$\Delta S^{QENS} =$ -2.2 ± 0.3 kJ mol^{-1} K^{-1}	$\Delta S^{ITC} =$ -0.4 ± 0.1 kJ mol^{-1} K^{-1}	$\Delta S^{hs} = \Delta S^{ITC} - \Delta S^{QENS} =$ 1.8 ± 0.4 kJ mol^{-1} K^{-1}
entropy change of **protein**	entropy change of **protein + hydration shell**	→ entropy of **hydration shell** increases when biotin binds

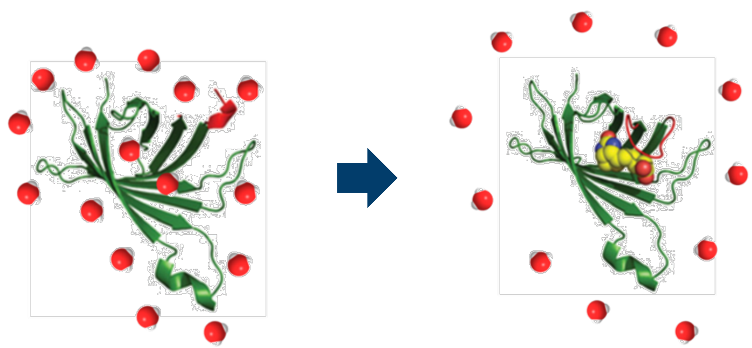
RESULTS

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



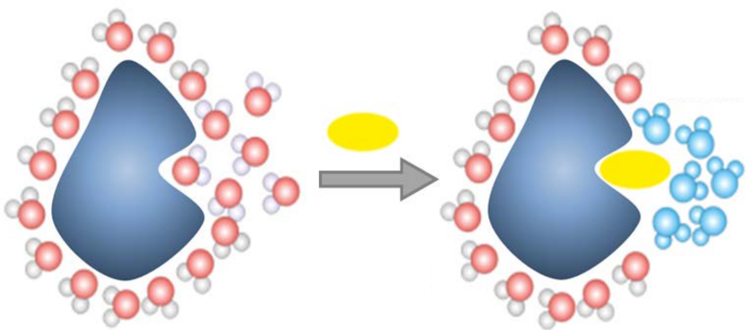




D. Niether *et al.*, Polymers (2020)

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



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ACKNOWLEDGEMENT

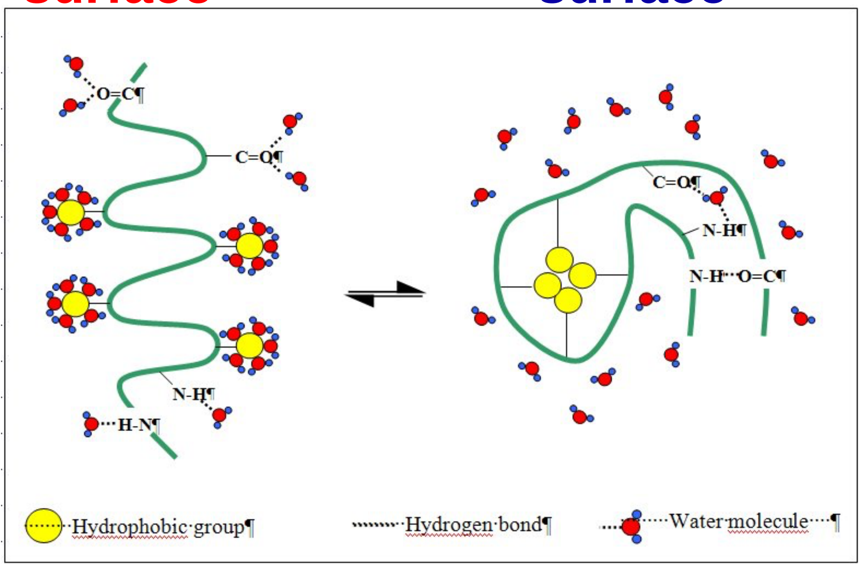
Simone Wiegand, Jan K.G. Dhont, and the IBI-4

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!

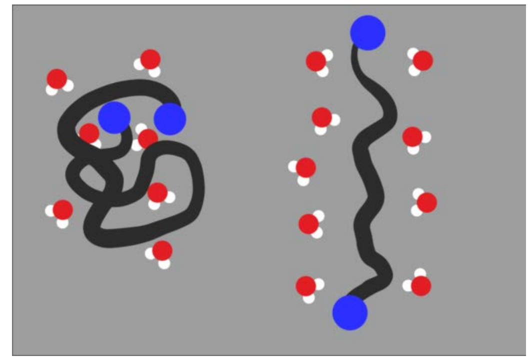
MICROSCOPIC PICTURE

hydrophobic surface | **hydrophilic surface**



- protein flexible
- hydration water less mobile

- protein less flexible
- hydration water mobile



flexible coil

lower entropic contribution from the hydration shell

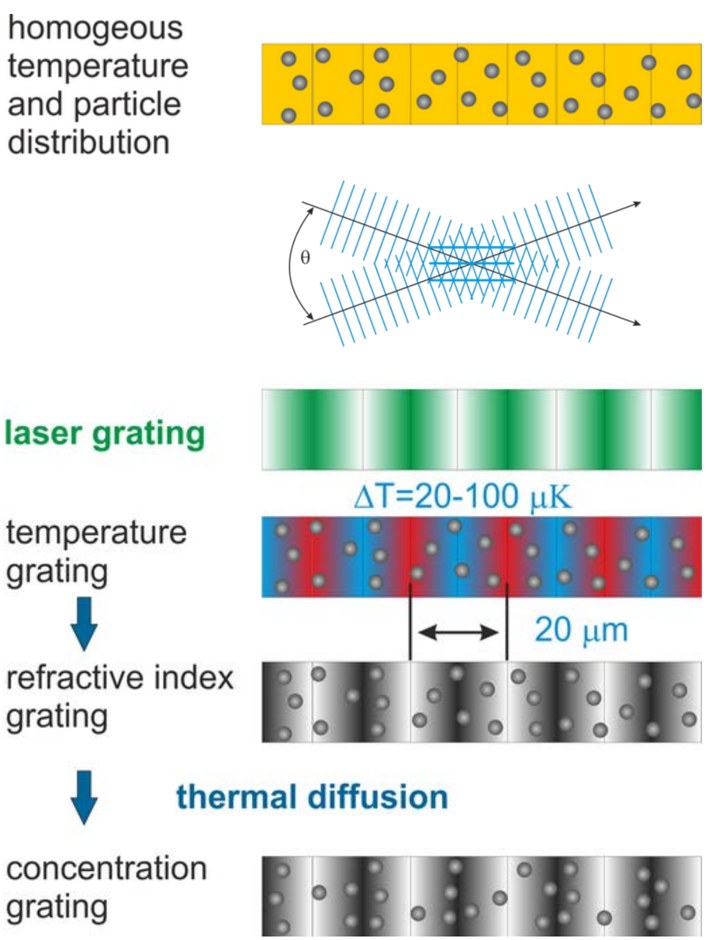
stretched chain

larger entropic contribution from the hydration shell

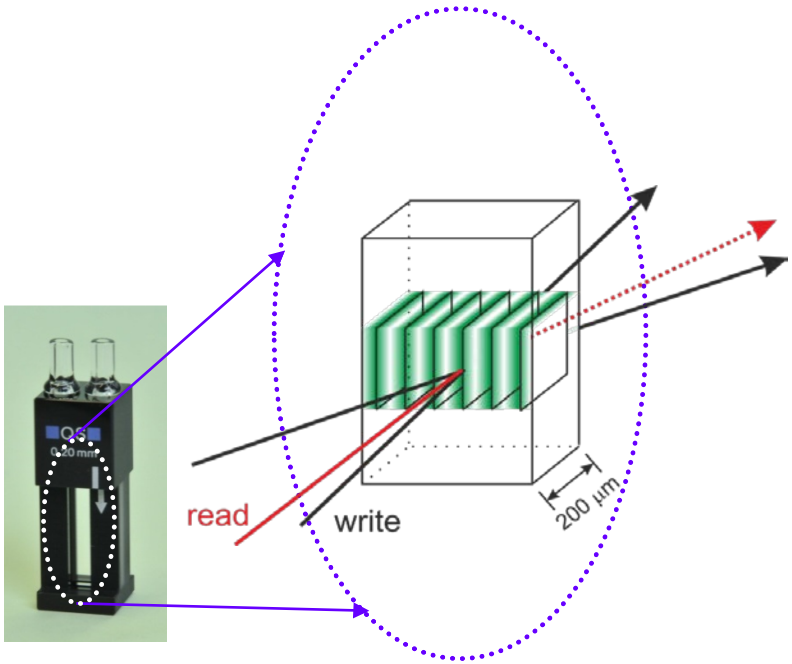
Gallat et al. Biophys J. 2012
Liese, S. *et al.*, ACS Nano, **11** (2017) 702-712

SETUP

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



Measured quantity:
Intensity of the diffracted beam



SETUP

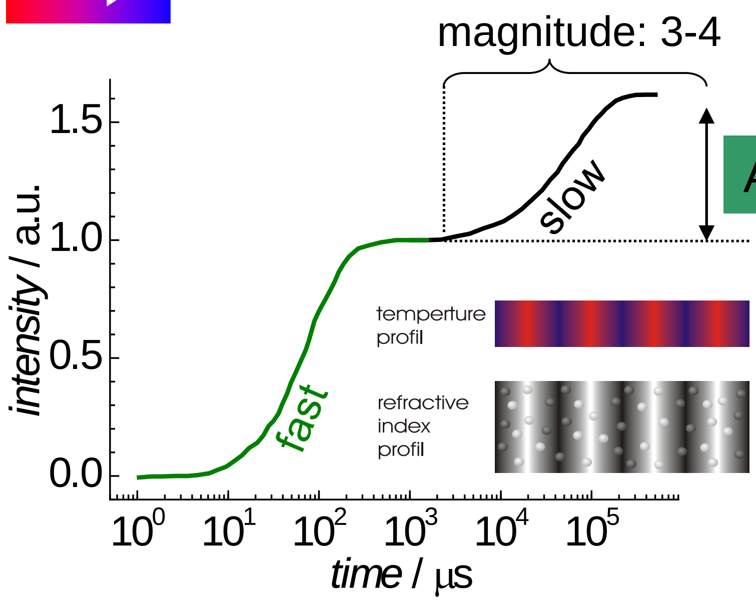
Signal

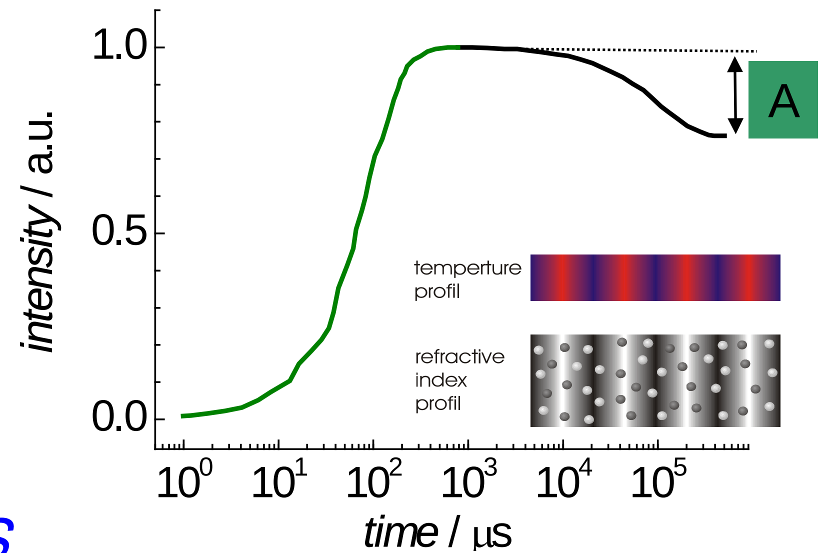
Measured quantity:
Intensity of the diffracted beam

Molecules/colloides with higher refractive index moves to…

…cold side

…warm side



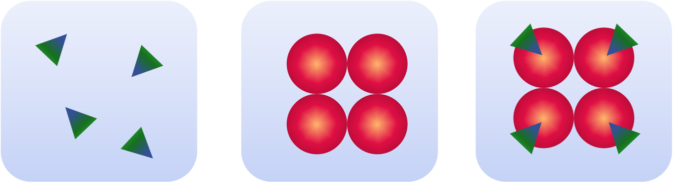


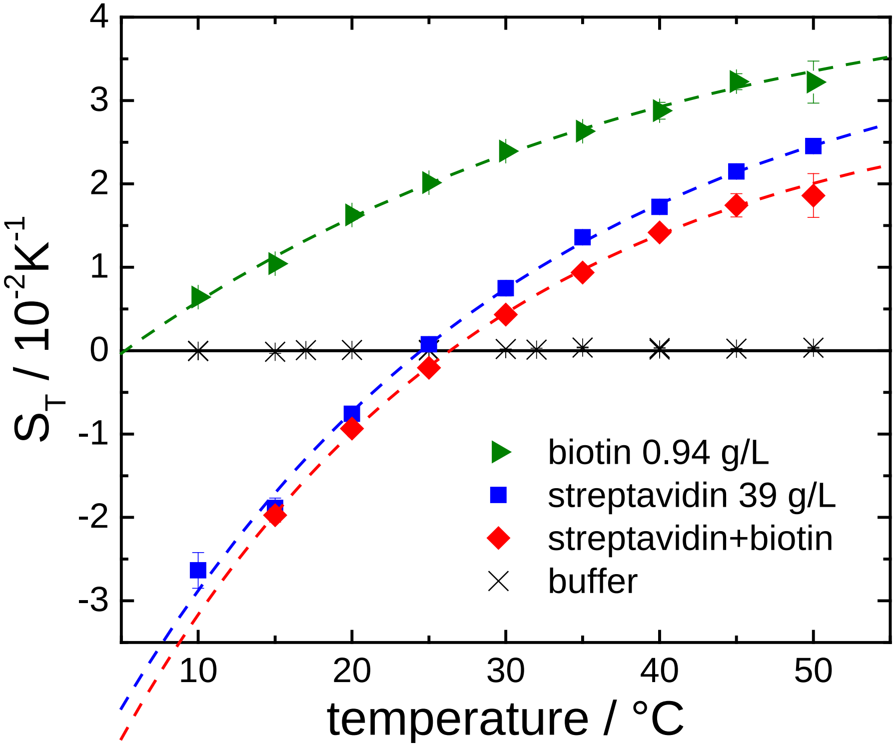
S_T

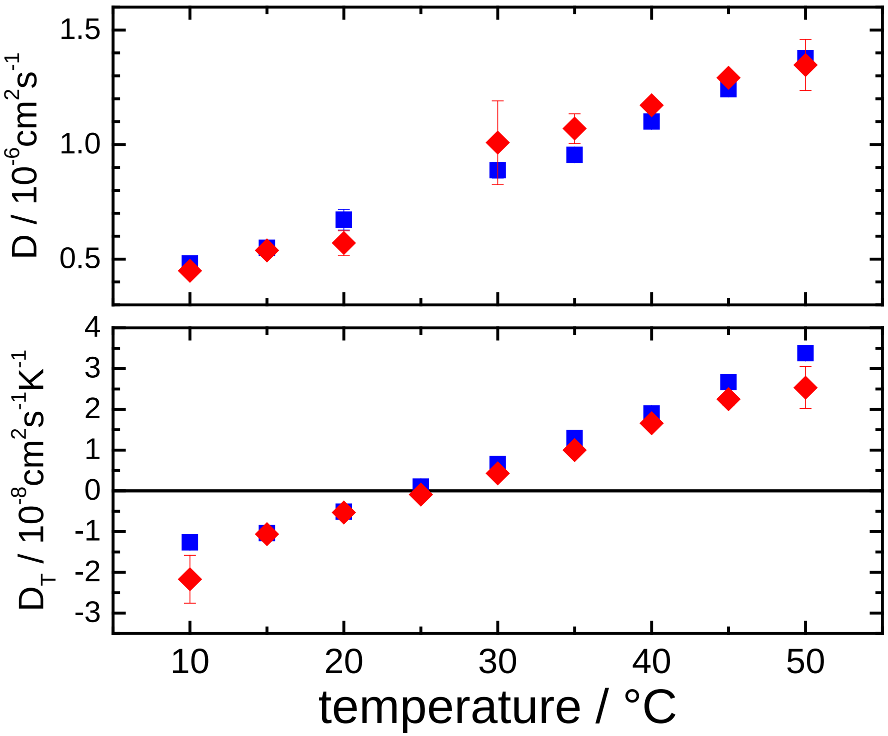
$$\varsigma_{het} = \underbrace{\left(1-e^{-t/\tau_{th}}\right)}_{\text{temperature}} - \underbrace{\frac{(\partial n/\partial c)_{p,T}}{(\partial n/\partial T)_{p,c}} \frac{D_T}{D} c(1-c)\frac{1}{\tau-\tau_{th}}}_{A} \left[\tau\left(1-e^{-t/\tau}\right) - \tau_{th}\left(1-e^{-t/\tau_{th}}\right)\right]$$

RESULTS

Streptavidin + biotin in H_2O-buffer







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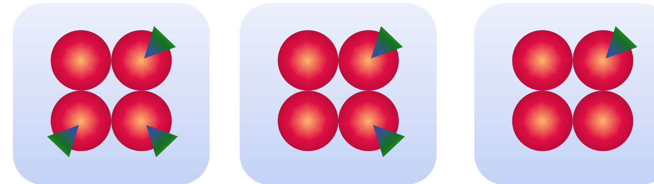
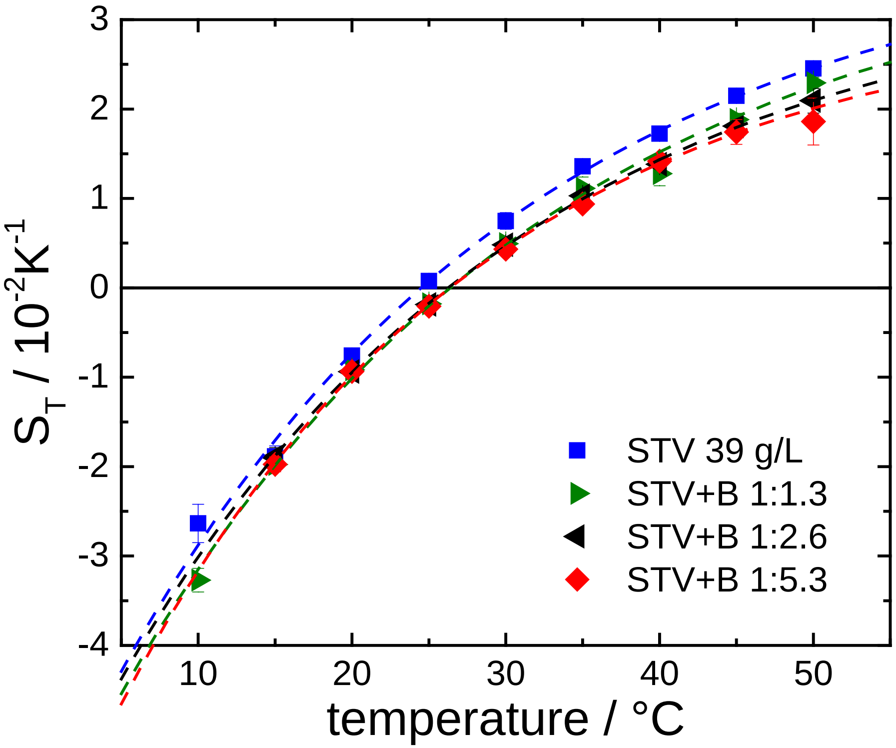
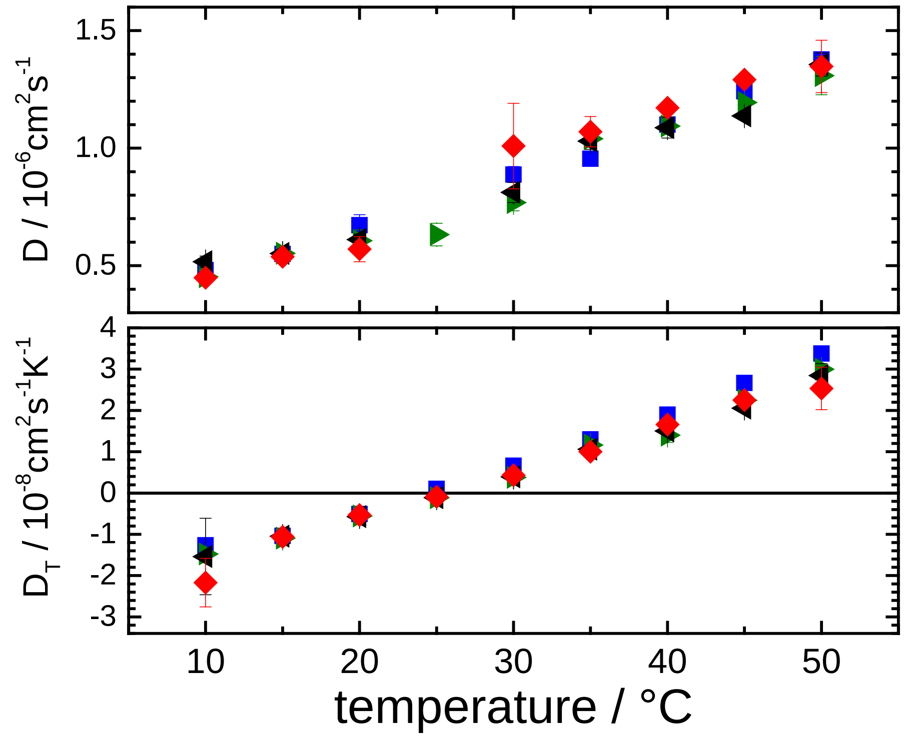
RESULTS

Streptavidin + biotin - stoichiometry



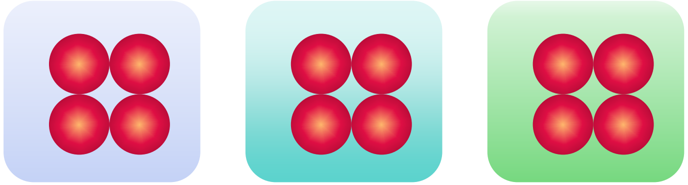


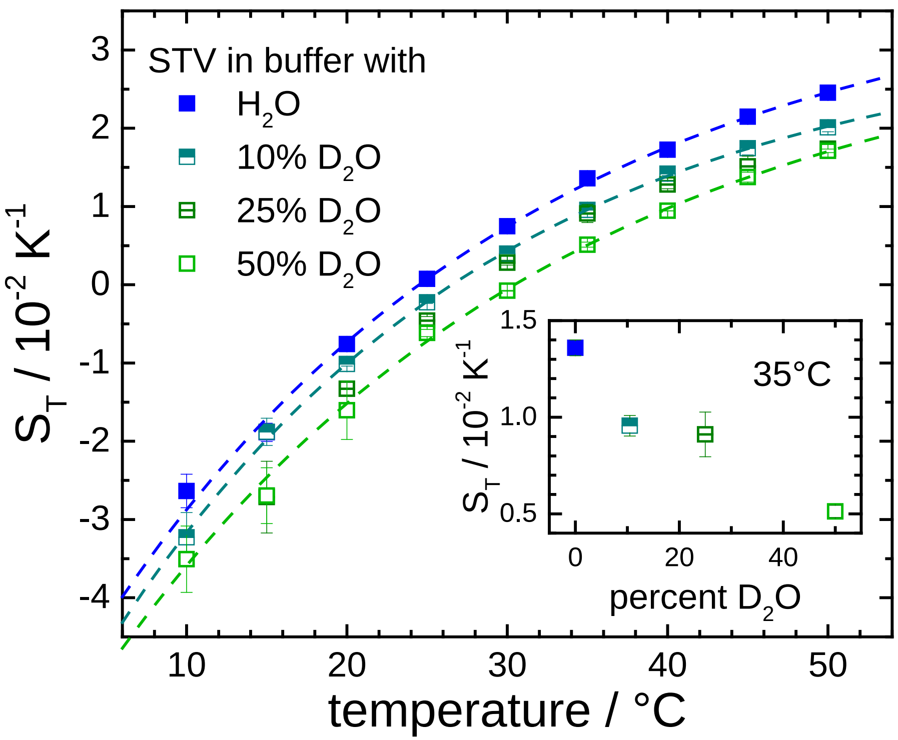


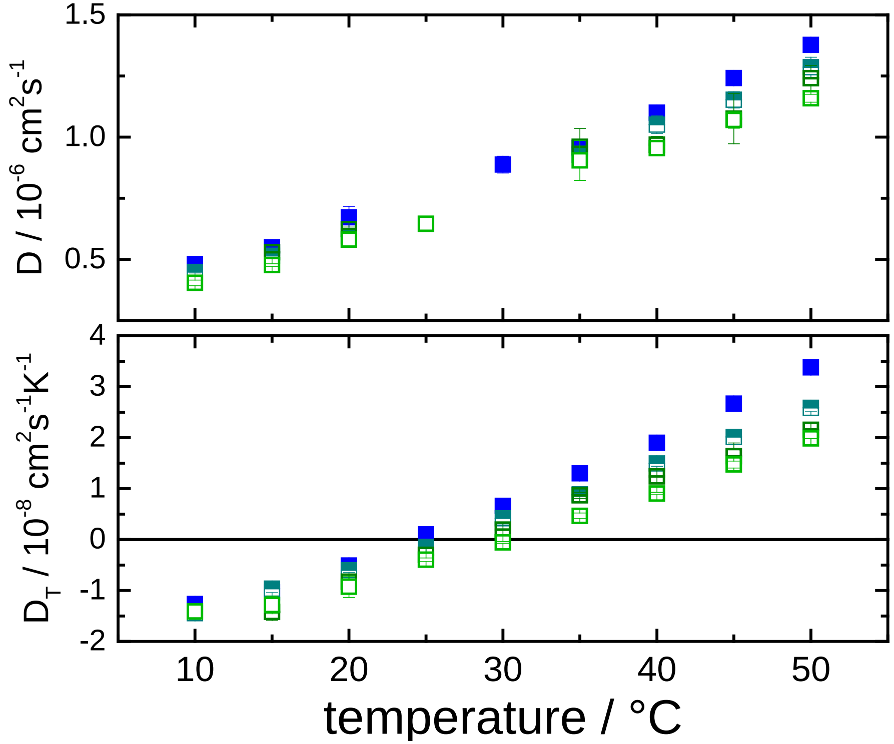
non-linear dependence on biotin concentration might point to cooperative binding

RESULTS

Streptavidin in buffer with D_2O



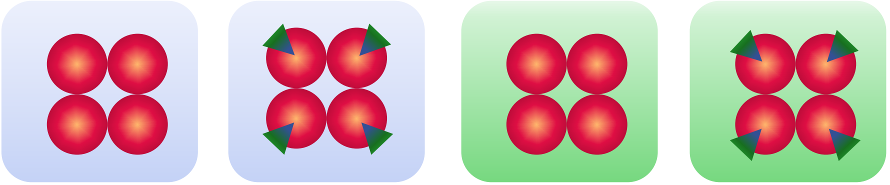


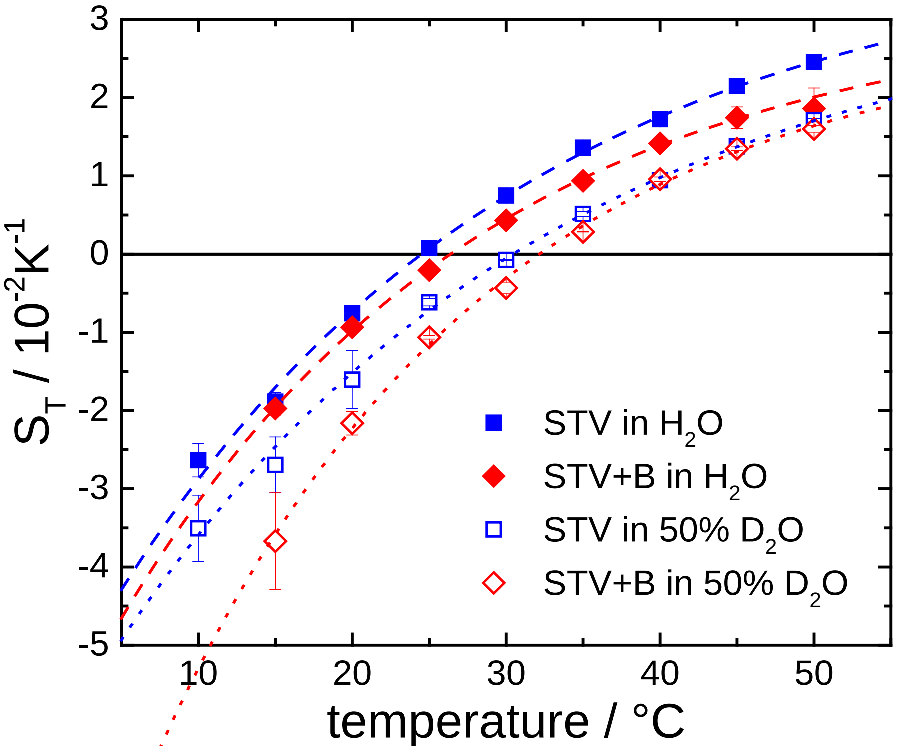


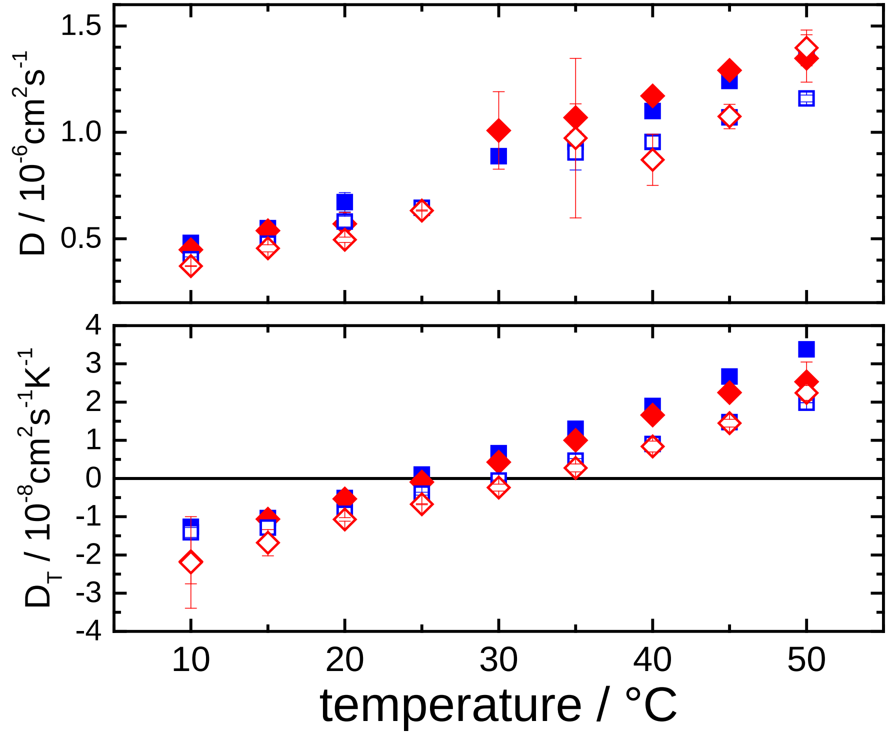
Measurement above ~50% D_2O not possible due to reduced IR-absorption of solvent

RESULTS

Streptavidin + biotin – H_2O and D_2O







It‘s likely that the behaviour of hydration shell differs between H_2O and D_2O