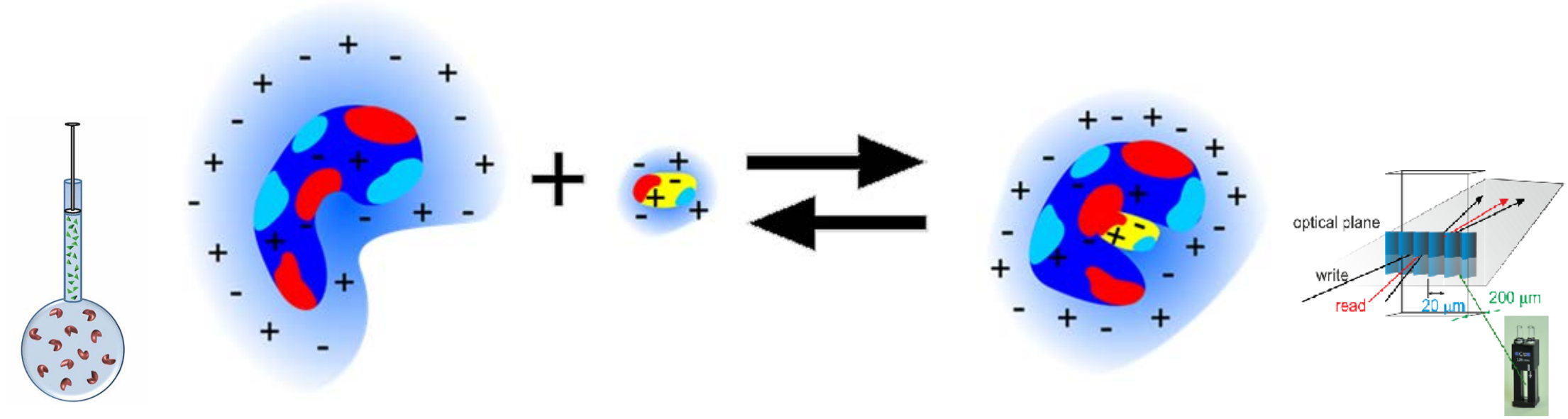


WHAT CAN WE LEARN ABOUT PROTEIN-LIGAND BINDING BY COMBINING ITC AND TDFRS?

29.06.2021 | SHILPA MOHANAKUMAR

JÜLICH
Forschungszentrum
Mitglied der Helmholtz-Gemeinschaft

MOTIVATION

Thermophoresis

- Motion of solute particles induced by thermal gradient.

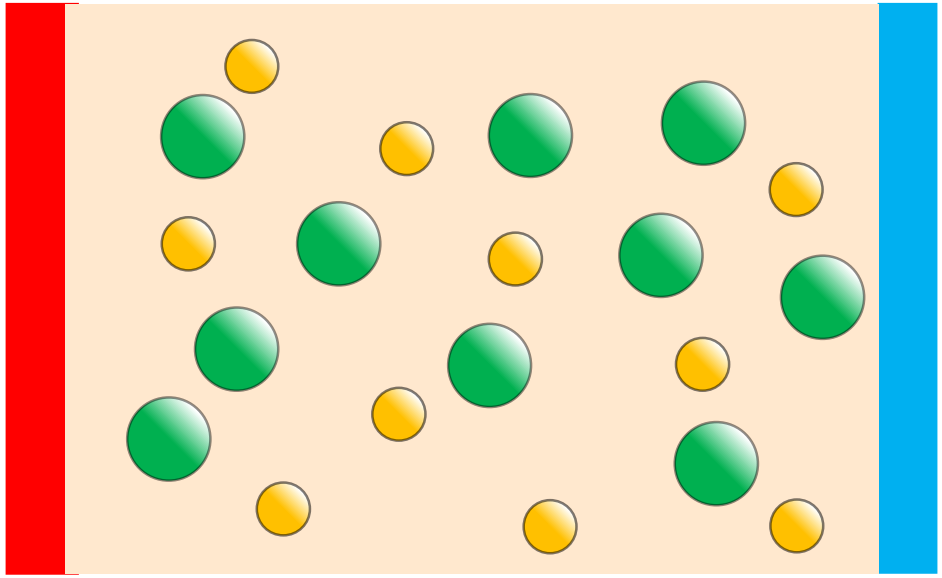
$$\vec{j} = -\rho D \vec{\nabla} w - w(1-w)\rho D_T \vec{\nabla} T$$

steady state $\vec{j} = 0$

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

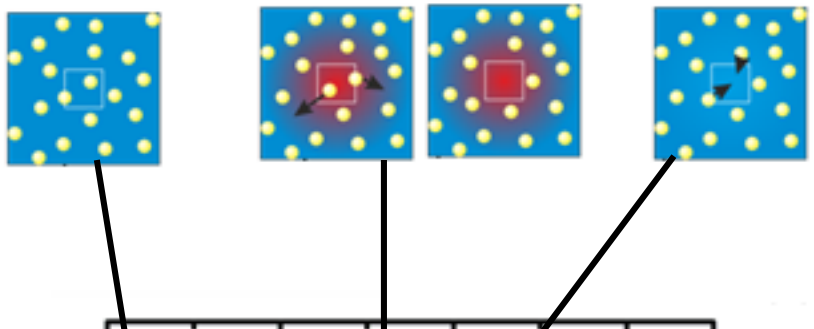
$$S_T \propto \frac{\Delta w}{\Delta T}$$

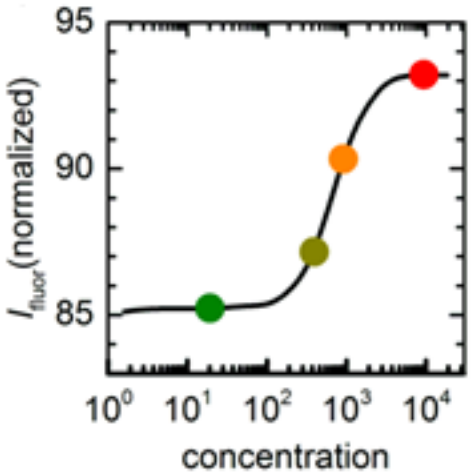
w	weight fraction
ρ	mass density
$\vec{j}$	flux
T	temperature

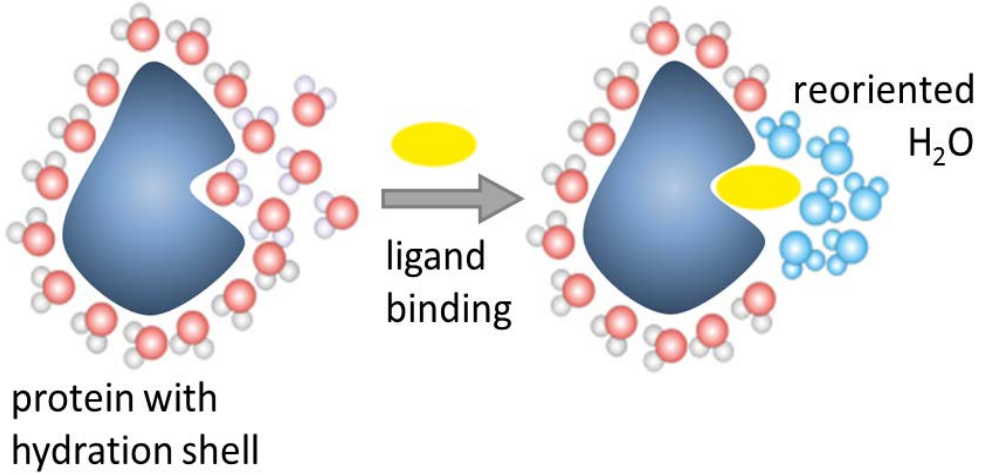


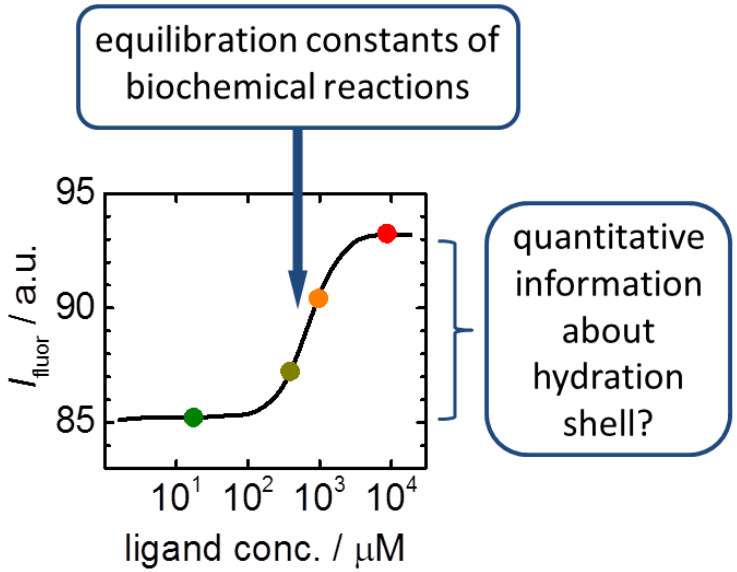
APPLICATIONS

Microscale Thermophoresis(MST)



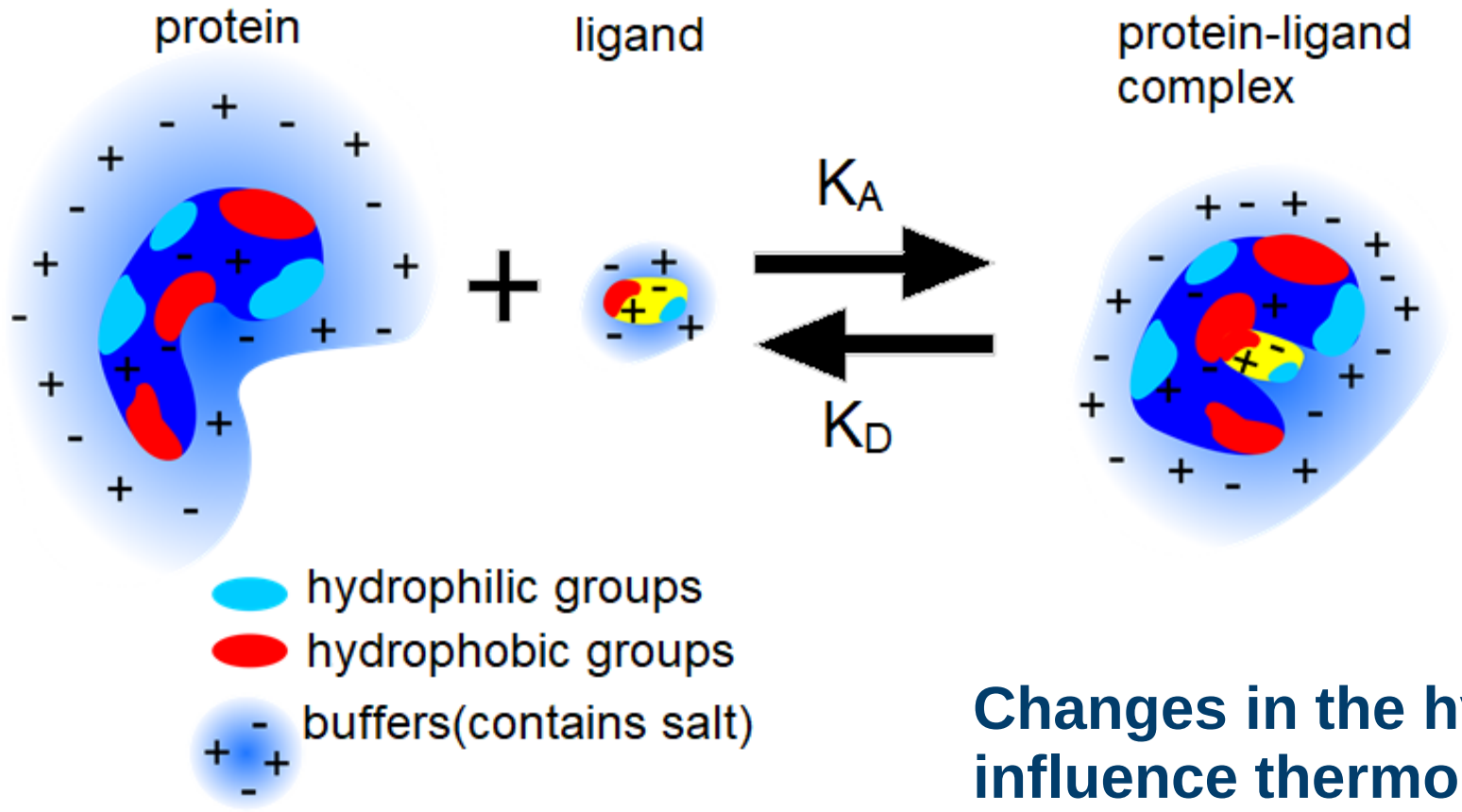






M. Jerabek-Willemsen et al, et al., *J. Mol. Struct.*, **1077**(2014), 101

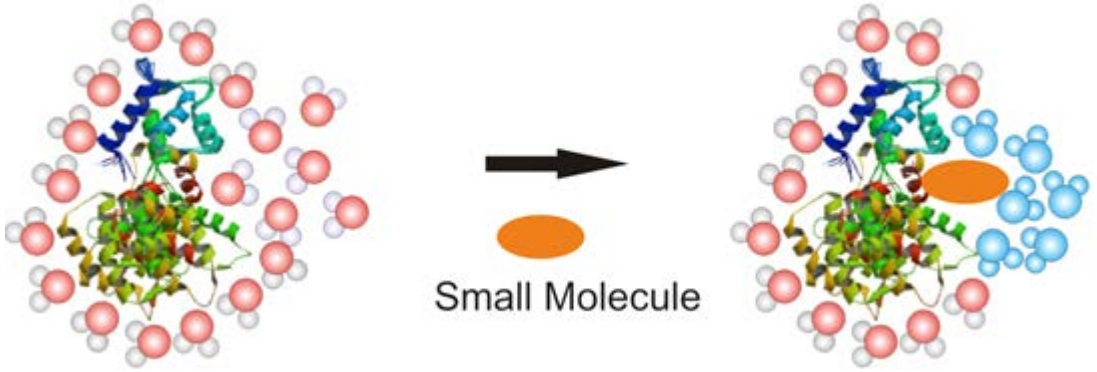
GOAL: PROTEIN-LIGAND SYSTEMS



Changes in the hydration layer influence thermophoresis!

Changes in the hydration layer can influence thermophoresis.

- Can this connection be quantified?
- Can it be used to gain information about change in hydration shell upon complex formation?
- Can free energy(ΔG), enthalpy(ΔH) and entropy(ΔS) be connected with the thermodiffusion properties?

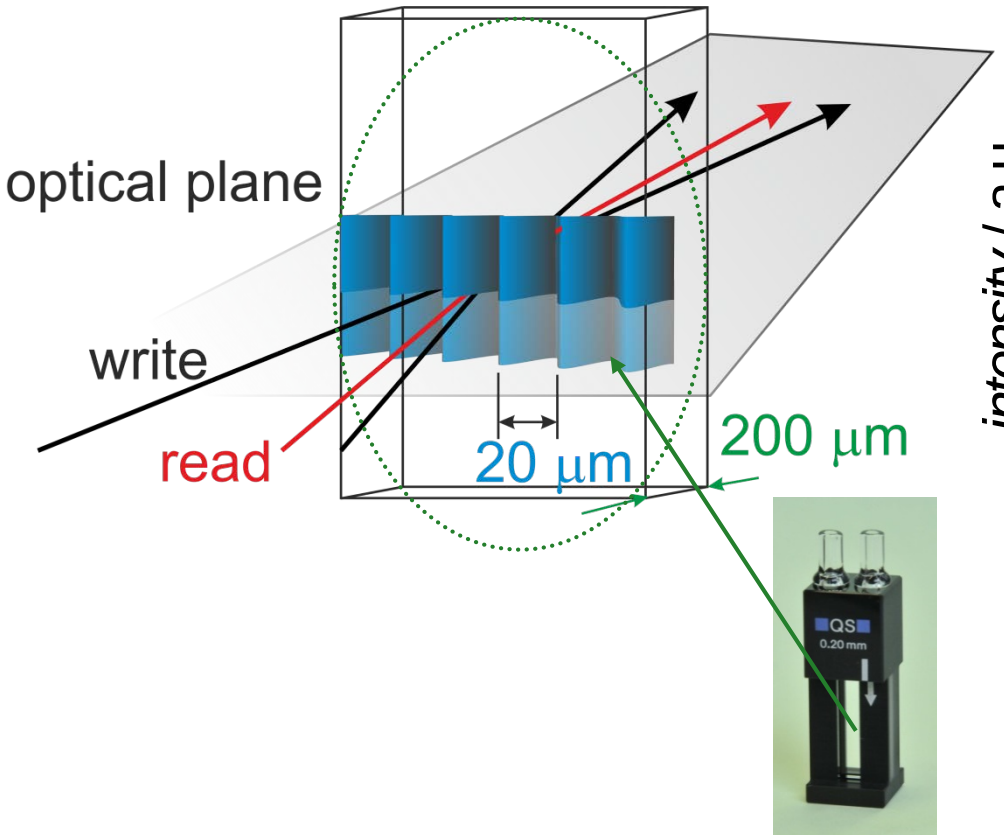


OPEN QUESTIONS

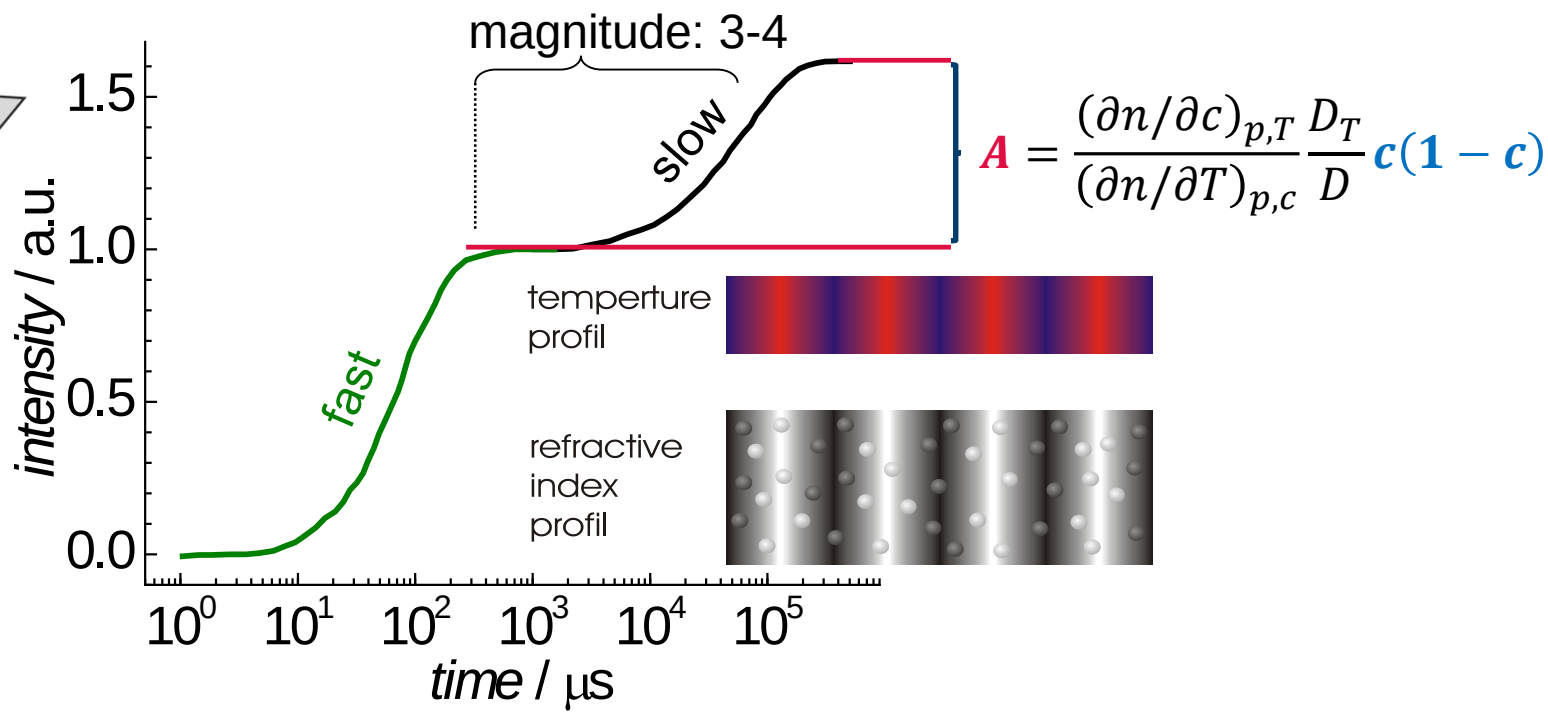
TECHNIQUES

TDFRS

Measured quantity:
Intensity of the diffracted beam



Molecules/colloids with higher refractive index moves to cold side



$$A = \frac{(\partial n/\partial c)_{p,T}}{(\partial n/\partial T)_{p,c}} \frac{D_T}{D} c(1-c)$$

$$\varsigma_{het} = \underbrace{\left(1 - e^{-t/\tau_{th}}\right)}_{\text{temperature}} - A\left(1 - e^{-t/\tau}\right)$$

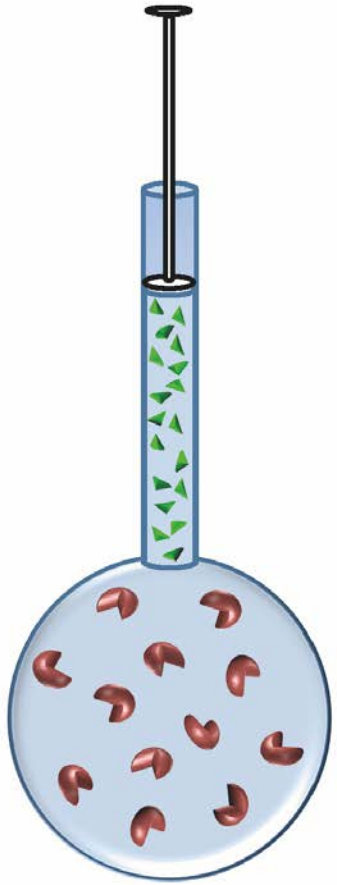
S. Wiegand et al., J Phys Chem B, **111** (2007), 14169

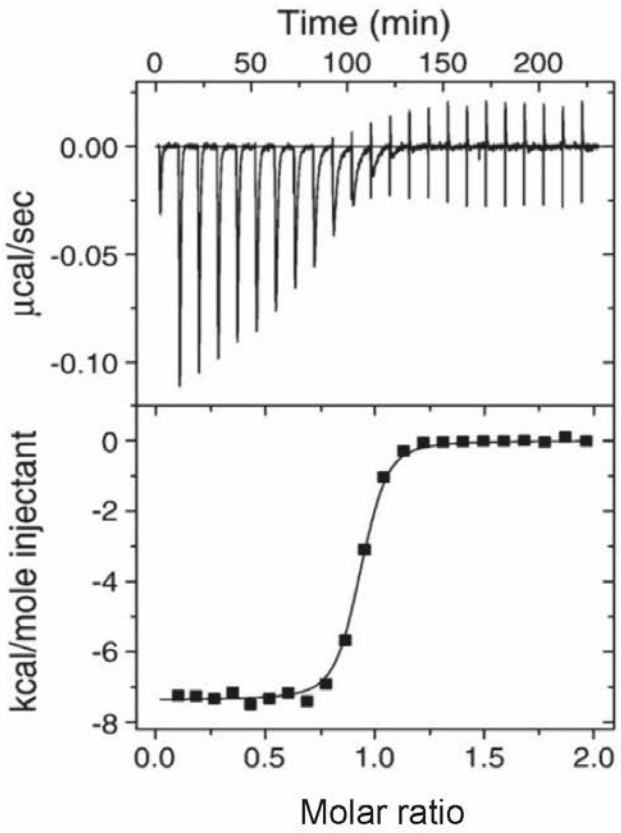
TECHNIQUES

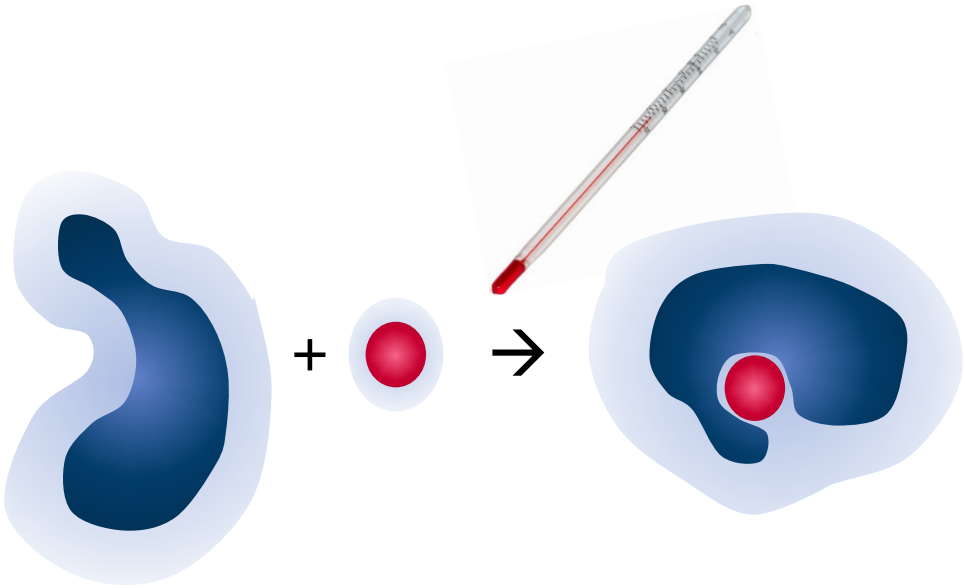
ITC

Syringe:
ligand

Cell:
protein



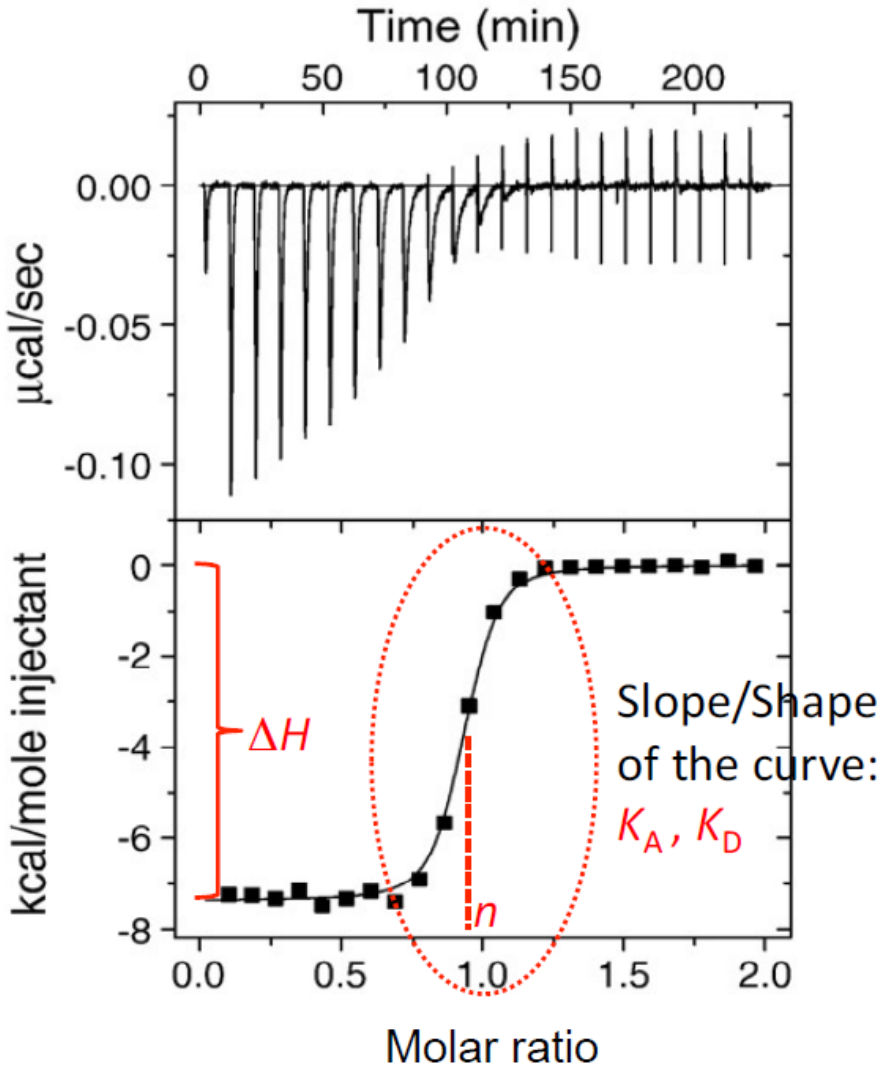




Directly measures heat associated with binding!

TECHNIQUES

ITC: What do we gain?



- Binding affinity(K_a, K_d)
- Stochiometry(n)
- Binding enthalpy(ΔH)
- Free energy of binding(ΔG)
- Binding entropy(ΔS)

$$\Delta G = -RT \ln K$$

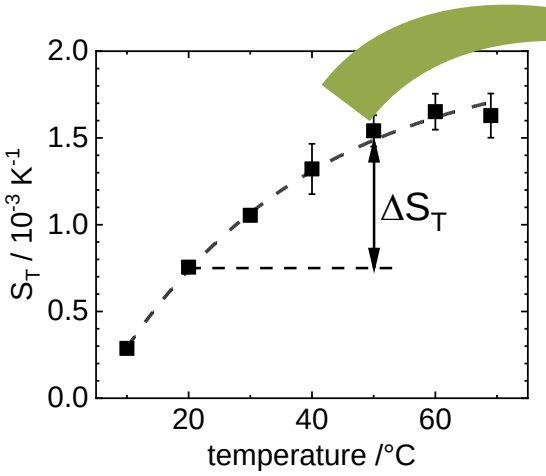
$$\Delta G = \Delta H - T\Delta S$$

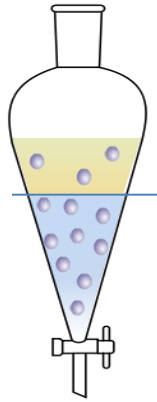
AQEOUS SOLUTIONS OF NON-IONIC SOLUTES

Temperature dependence of S_T

ΔS_T is measure for temperature dependence

$$\Delta S_T = S_T(50°C) - S_T(20°C)$$



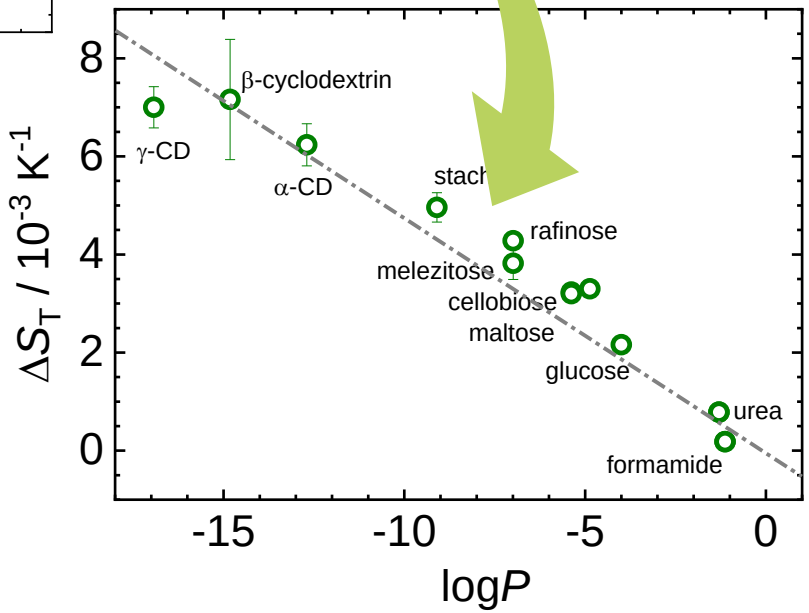


organic phase → c_{org}

water → c_{aq}

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

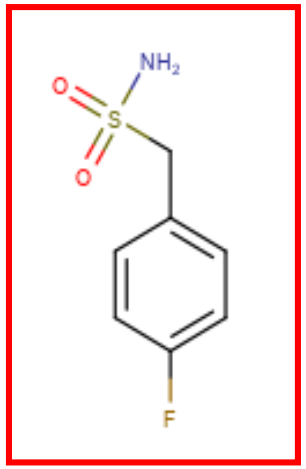
ΔS_T correlates with hydrophobicity(log *P*)



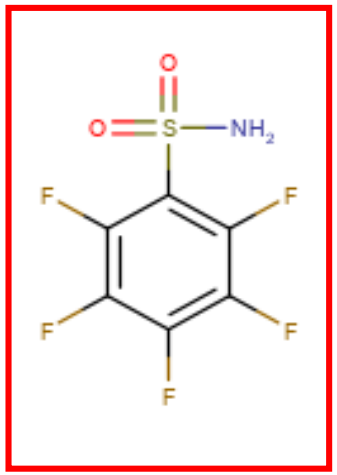
saccharides: P. Blanco et al., J. Phys. Chem. B (2010)
cyclodextrins: K. Eguchi et al., Eur. Phys. J. E (2016)
urea: D. Niether et al., PCCP. (2018)
formamide: D. Niether et al., PNAS (2016)

PROTEIN-LIGAND SYSTEMS

Bovine Carbonic Anhydarse II + Ligand



4-fluorobenzene Sulfonamide(4-FBS)



Pentafluorobenzene Sulfonamide(PFBS)

Buffer used: sodium phosphate(20 mM, pH=7.4)

Salt components: $Na_2HPO_4.7H_2O$

$NaH_2PO_4.H_2O$

Why fluorinated ligands?

- Larger size compared to hydrogen
- High electronegativity
- Low polarizability

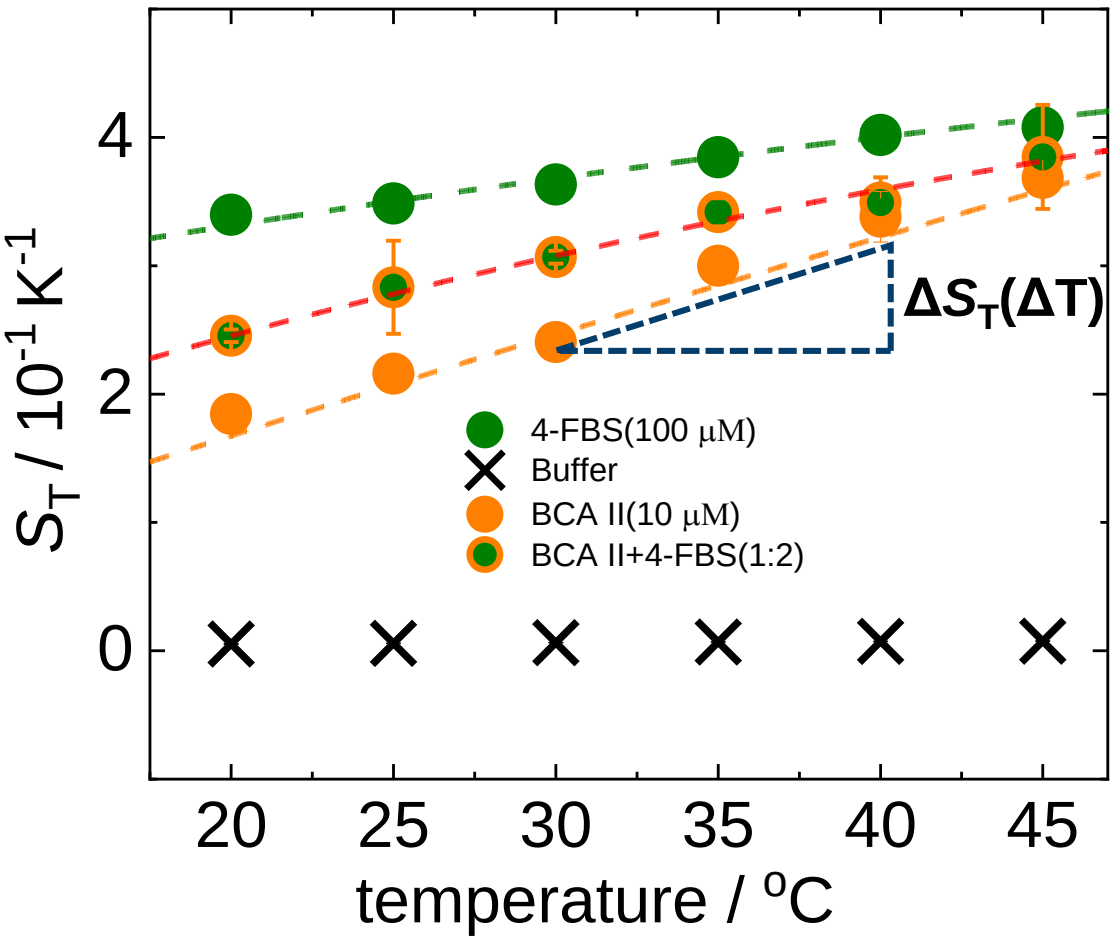
K. Uneyama, Organofluorine Chemistry, Blackwell Publishing, Oxford, 2006.

J. Gao, S. Qiao, G. M. Whitesides, J. Med. Chem., 1995, 38, 2292 –2301.

A. Ikai et al., Acta Cryst., 2004, D60, 792-795

TDFRS MEASUREMENT

Bovine Carbonic Anhydarse II + 4-FBS



- reduced $\Delta S_T(\Delta T)$ for BCA II + 4-FBS

  → surface of complex is less hydrophilic
- indicates breaking of HB
- Larger $\Delta S_T(\Delta T)$ for BCA II

  → surface of protein is more hydrophilic
- Difference between S_T of free protein and complex decreases with increasing temperature

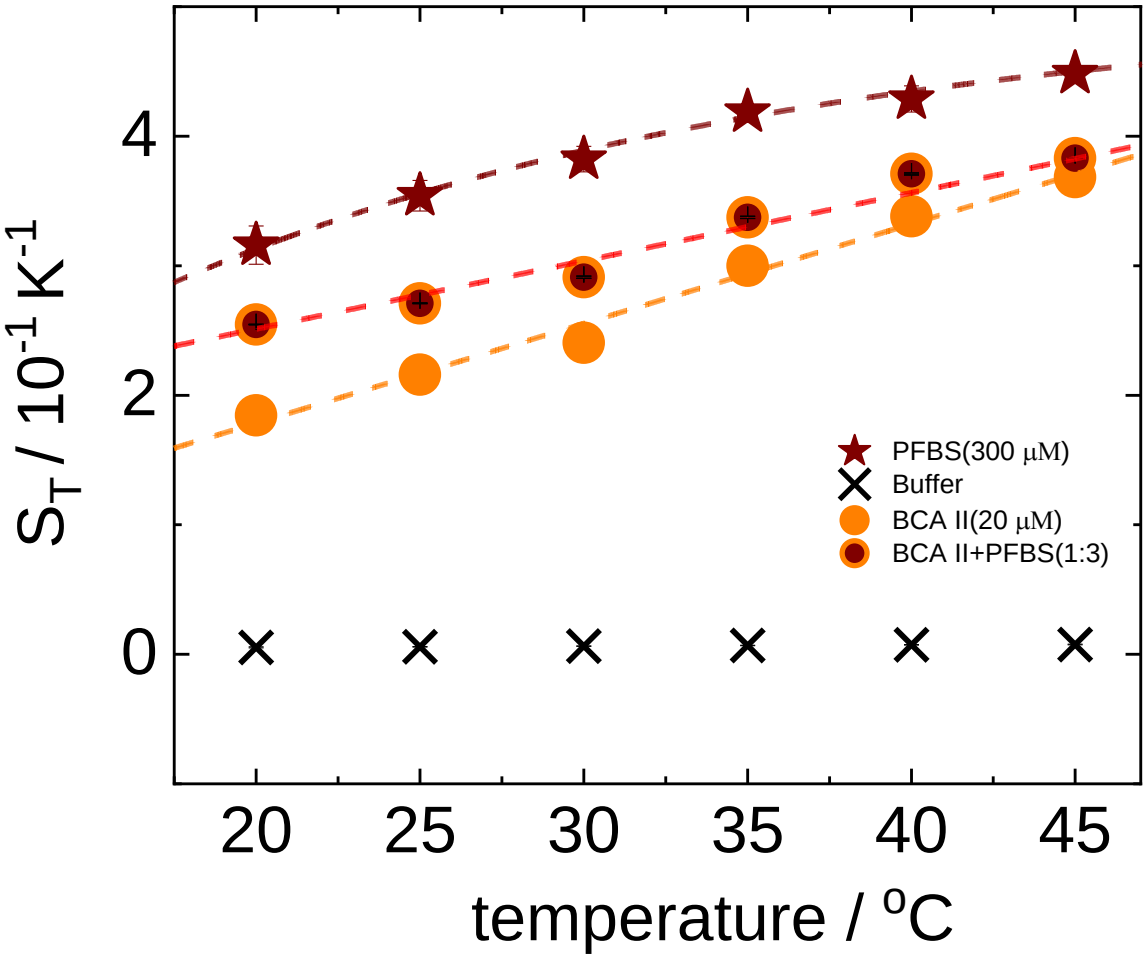
Solvent Accessible Surface Area (PYMOL):

	Hydrophilic(Å²)	Hydrophobic(Å²)
HCA II	8500	2020
HCA II + 4-FBS	8250	2280

Calculations done using PyMOL

TDFRS MEASUREMENT

Bovine Carbonic Anhydarse II + PFBS



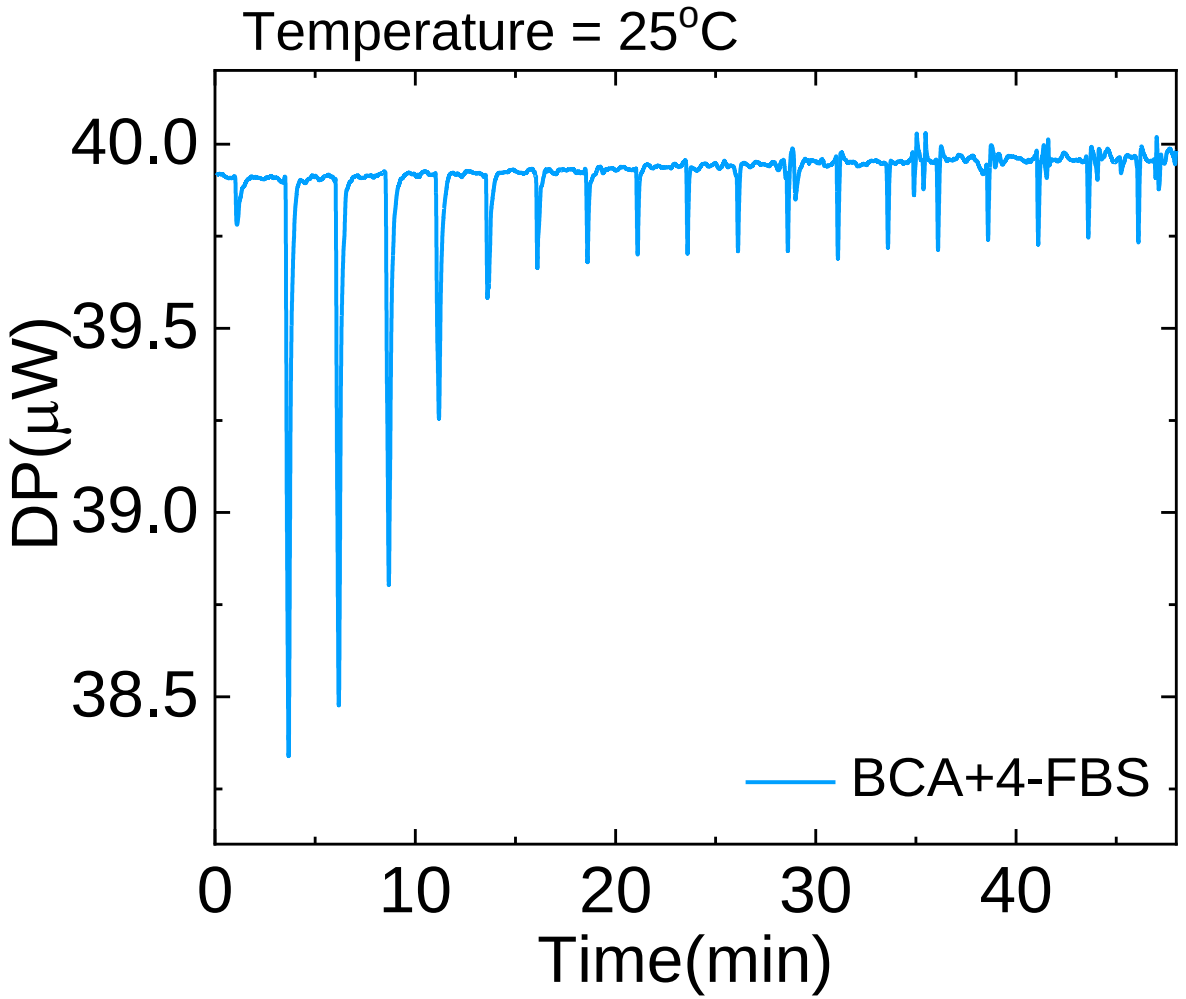
- reduced ΔS_T for BCA II + PFBS

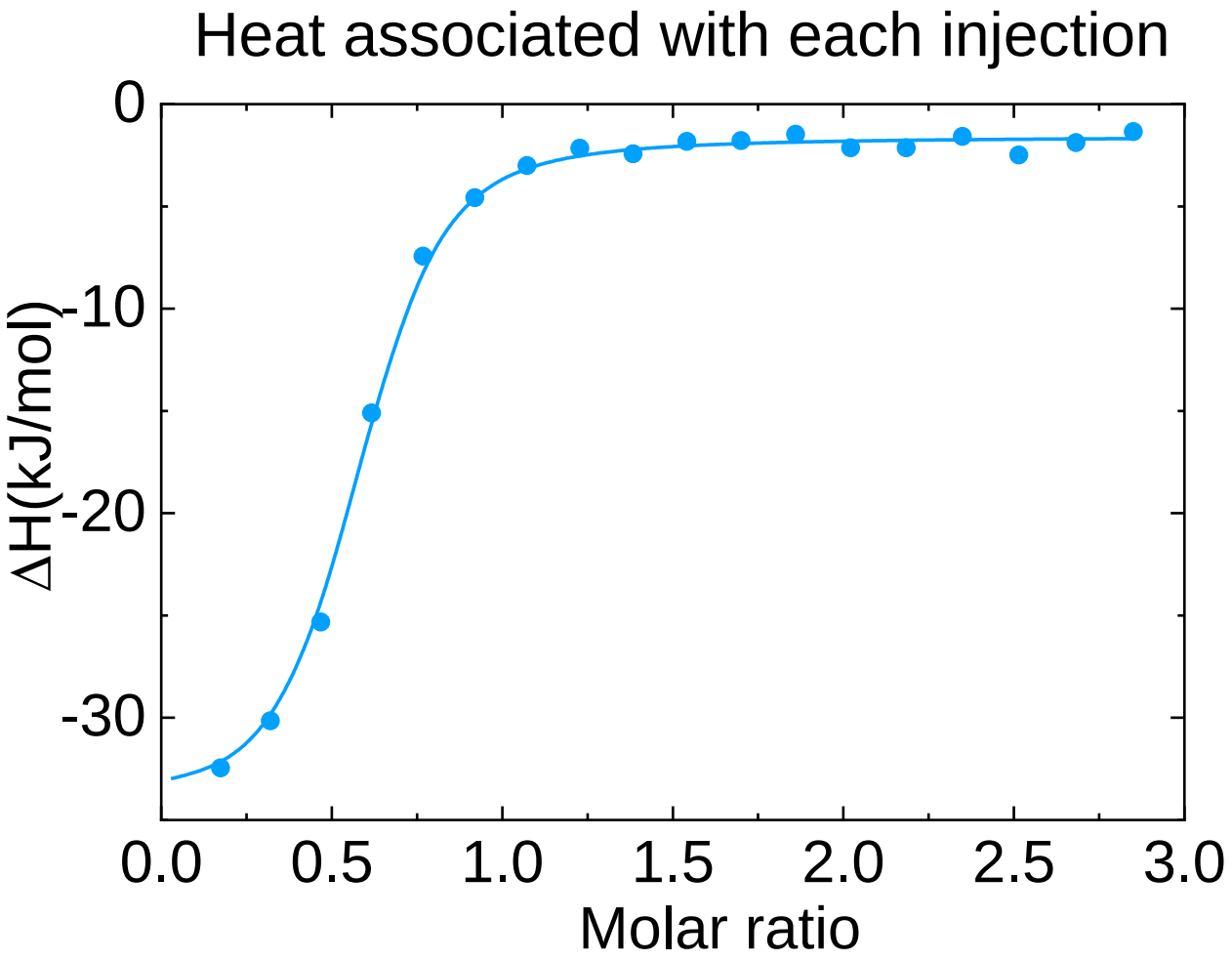
  → surface of complex is less hydrophilic
- indicates breaking of HB
- More ΔS_T for BCA II

  → surface of protein is more hydrophilic
- Difference between S_T of free protein and complex decreases with increasing temperature

ITC MEASUREMENT

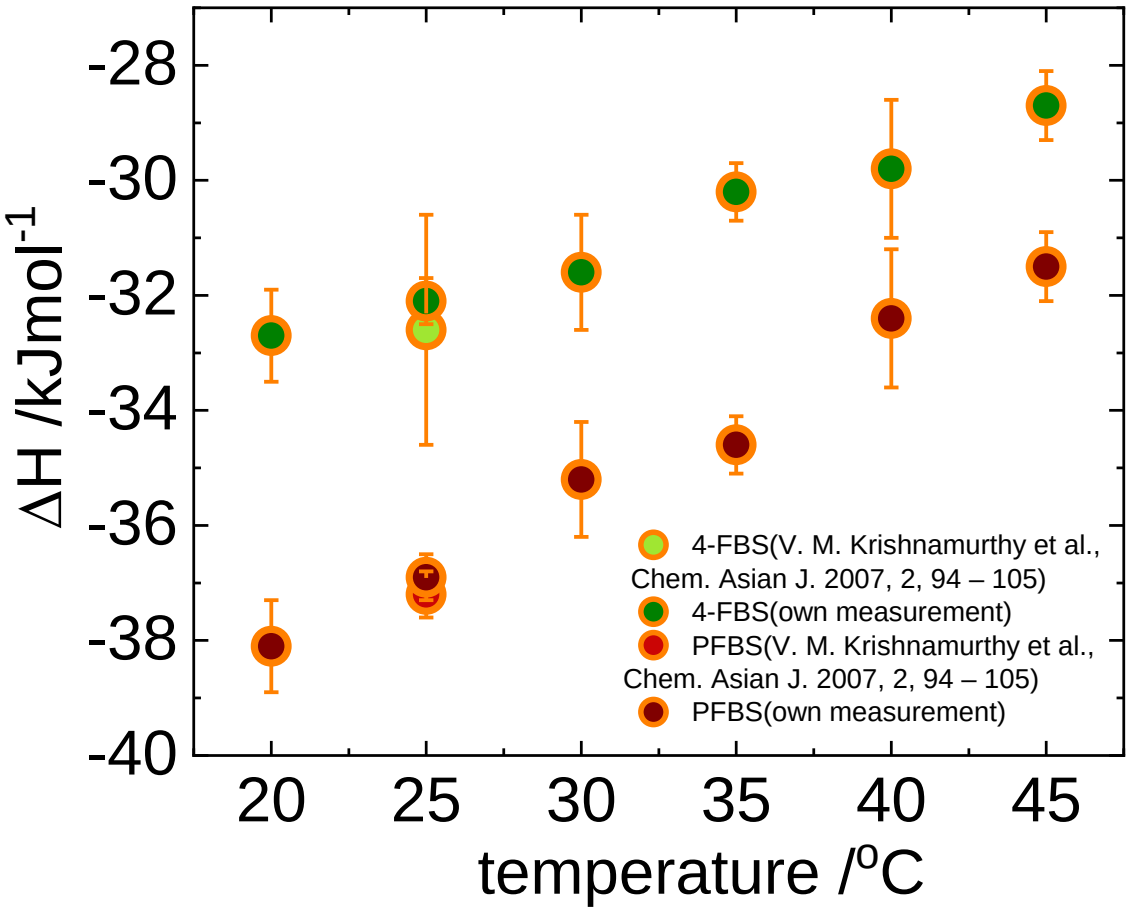
Bovine Carbonic Anhydarse II + 4-FBS

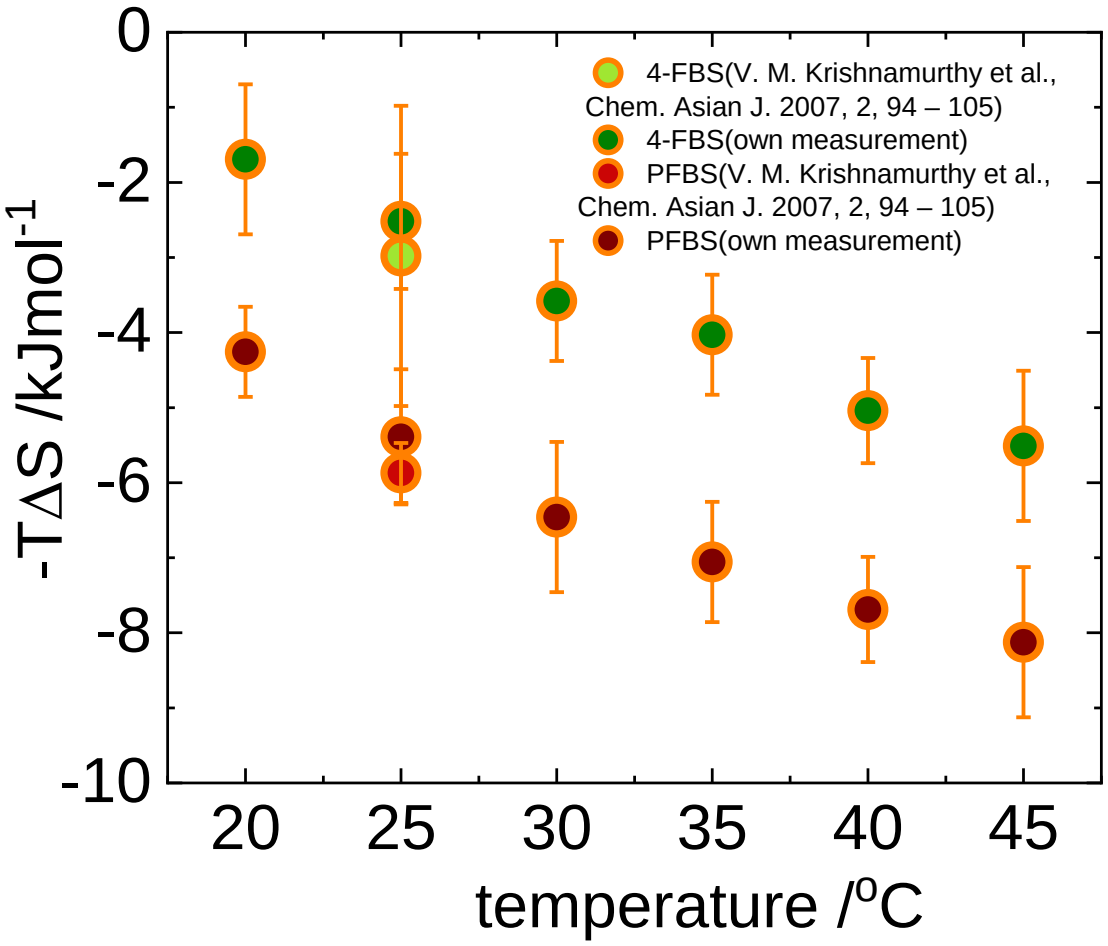




ITC MEASUREMENT

Bovine Carbonic Anhydarse II + Ligand

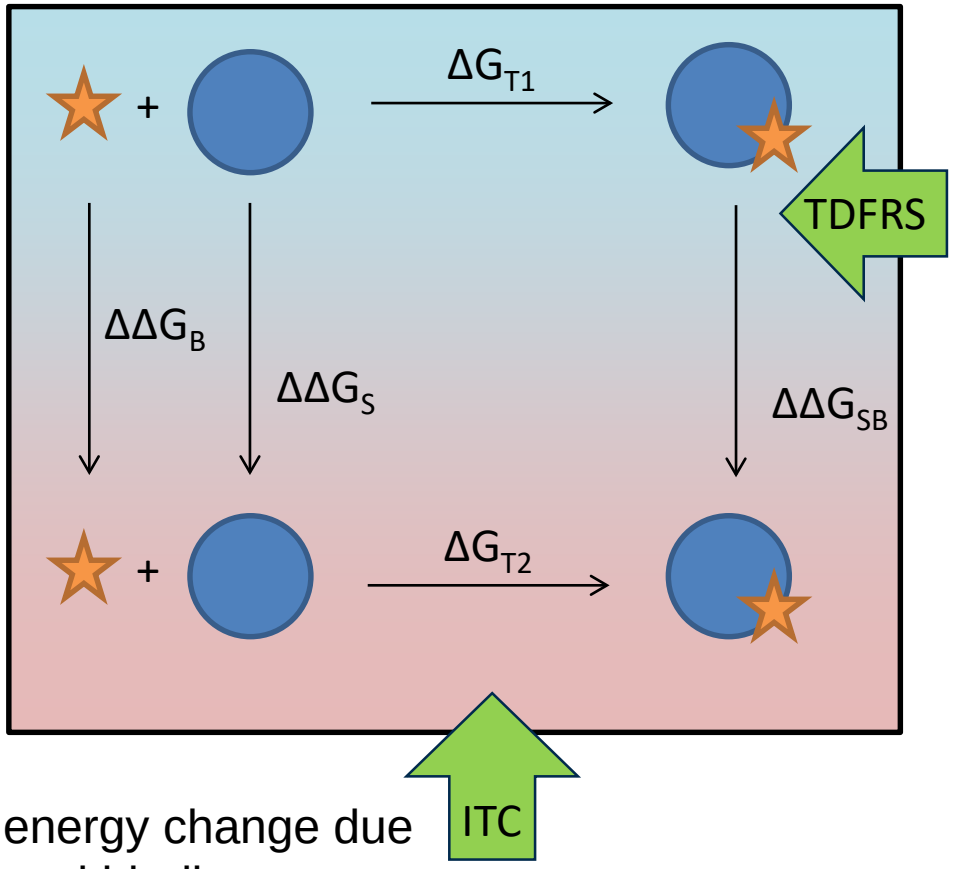




V. M. Krishnamurthy et al., Chem. Asian J. 2007, 2, 94 – 105

ANALYSIS OF ITC AND TDFRS MEASUREMENTS

Bovine Carbonic Anhydarse II + Ligand



free energy change due to ligand binding

change of solvation free energy between T1 and T2

$$\Delta G_{T2} = \Delta G_{T1} + \Delta\Delta G_{SB} - \Delta\Delta G_B - \Delta\Delta G_S$$

When concentration and temperature differences are small;

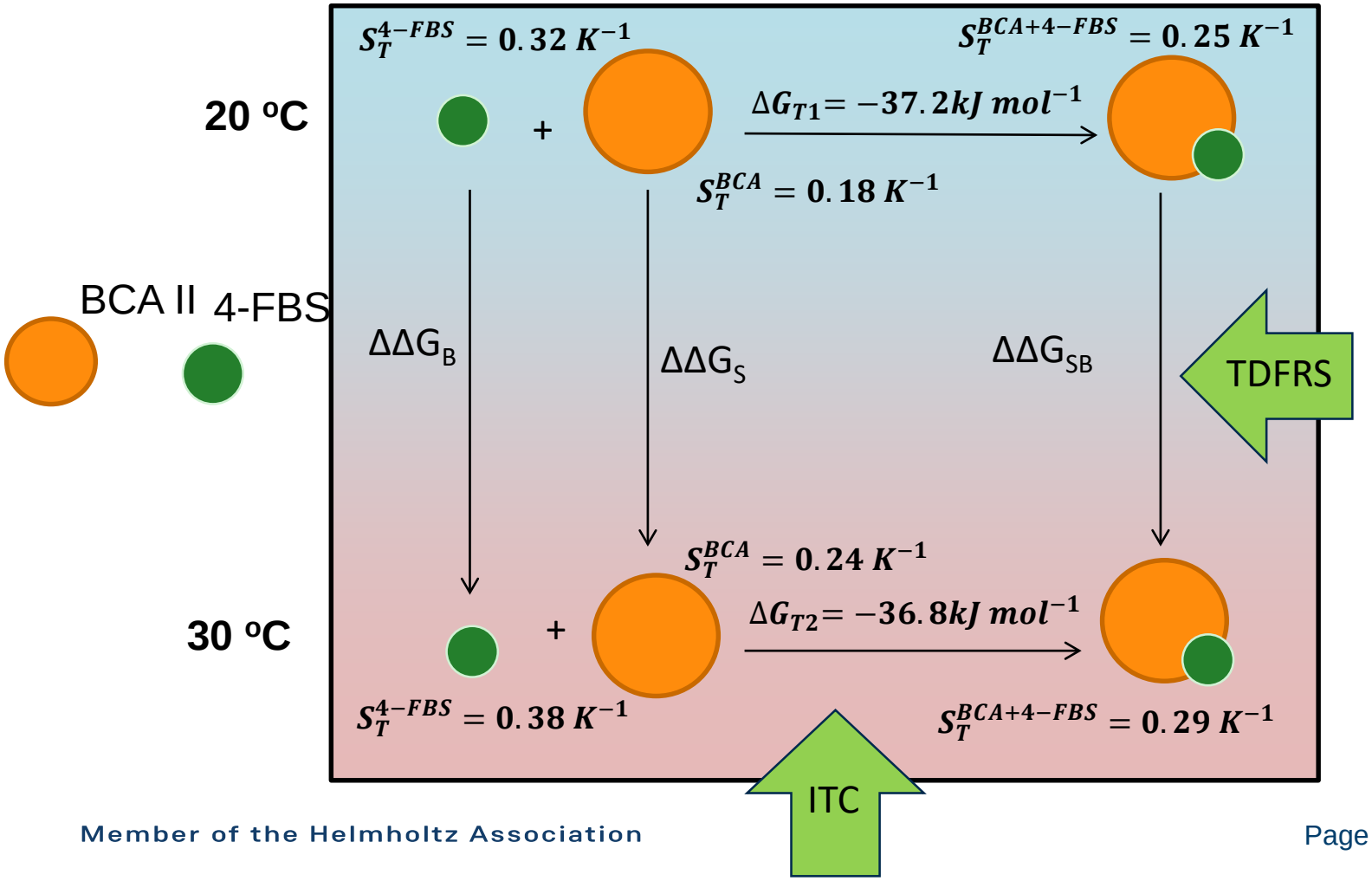
$$\frac{dc}{c} = \frac{dG}{kT} = S_T dT$$

$$\Delta\Delta G = \frac{k}{2}(S_{T2}T_2^2 - S_{T1}T_1^2)$$

A. Würger, Cr Mecanique **341**, 438 (2013).
D. Niether, 2018, Thermophoresis of biological and biocompatible systems

ANALYSIS OF ITC AND TDFRS MEASUREMENTS

Bovine Carbonic Anhydarse II + Ligand



From calculations:

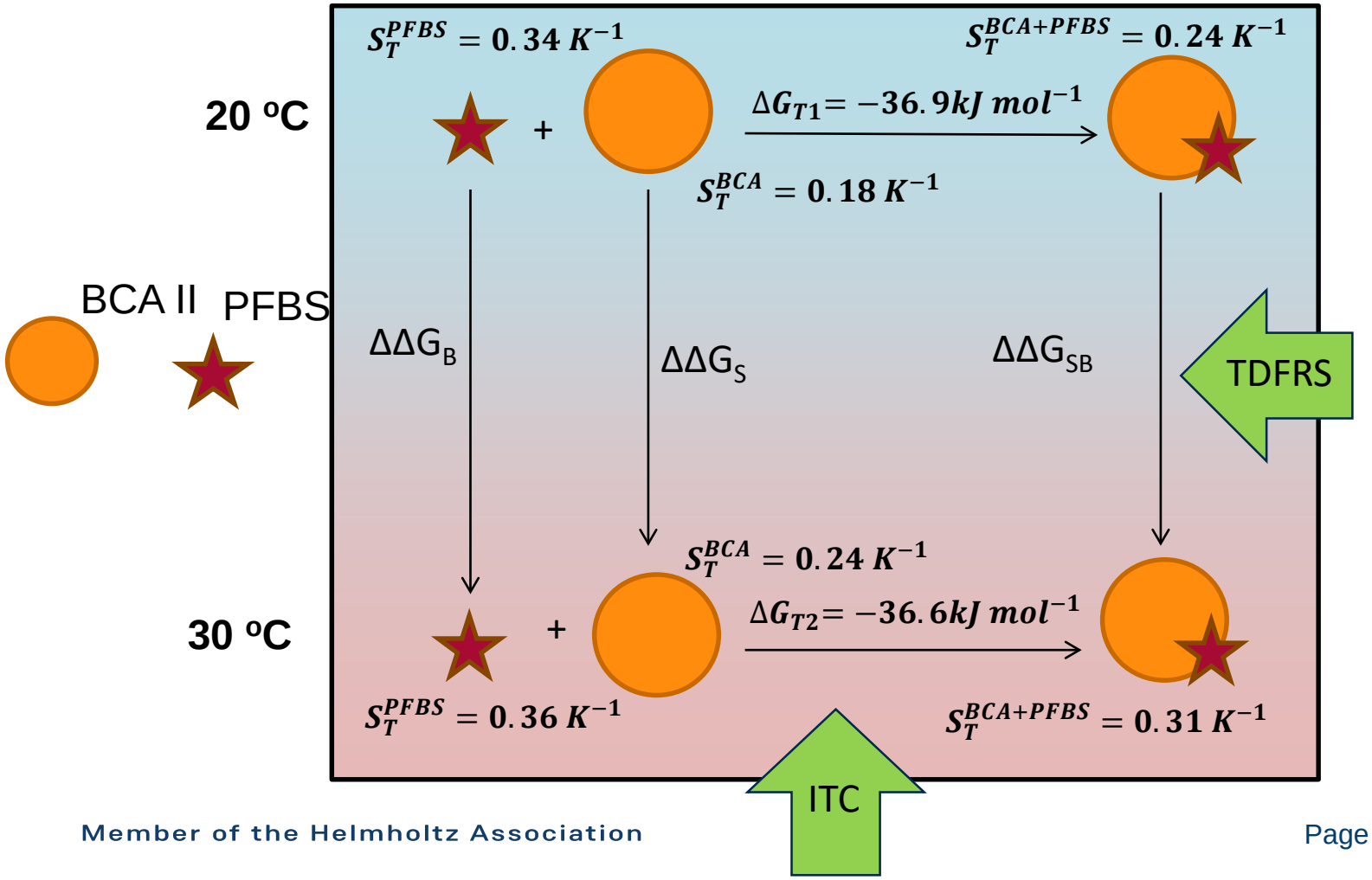
$$\Delta G_{T2} = -37.2 \pm 2.8\ kJ\ mol^{-1}$$

From ITC:

$$\Delta G_{T2} = -36.8 \pm 0.2\ kJ\ mol^{-1}$$

ANALYSIS OF ITC AND TDFRS MEASUREMENTS

Bovine Carbonic Anhydarse II + Ligand



From calculations:

$$\Delta G_{T2} = -36.9 \pm 3.1\ kJ\ mol^{-1}$$

From ITC:

$$\Delta G_{T2} = -36.6 \pm 0.2\ kJ\ mol^{-1}$$

Still needs to be validated for other systems!

CONCLUSION

- Preliminary study on the complex formation of BCA II with fluorinated ligands
- Temperature dependence of S_T shows that complex is less hydrophilic compared to free protein
- Free energy of binding(ΔG) determined using ITC and calculated from TDFRS measurements agree within the error limits.

ACKNOWLEDGEMENT

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

Doreen Niether, IEK-8

Thank you and Stay Healthy

BioSoft
Biophysics and Soft Matter

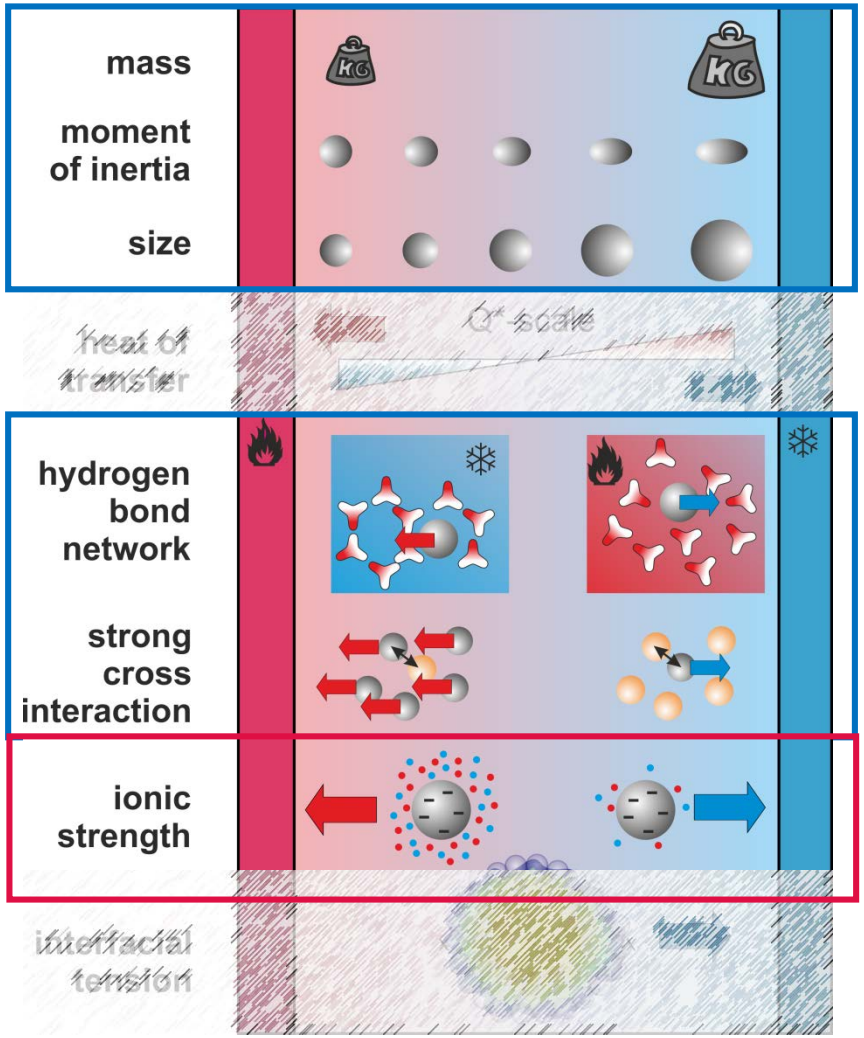
Universität zu Köln

JÜLICH
Forschungszentrum

CONTRIBUTIONS

$$S_T \approx S_T^i + \underbrace{S_T(\text{H–bond}) + S_T(\text{ionic})}_{S_T^{chem}}$$

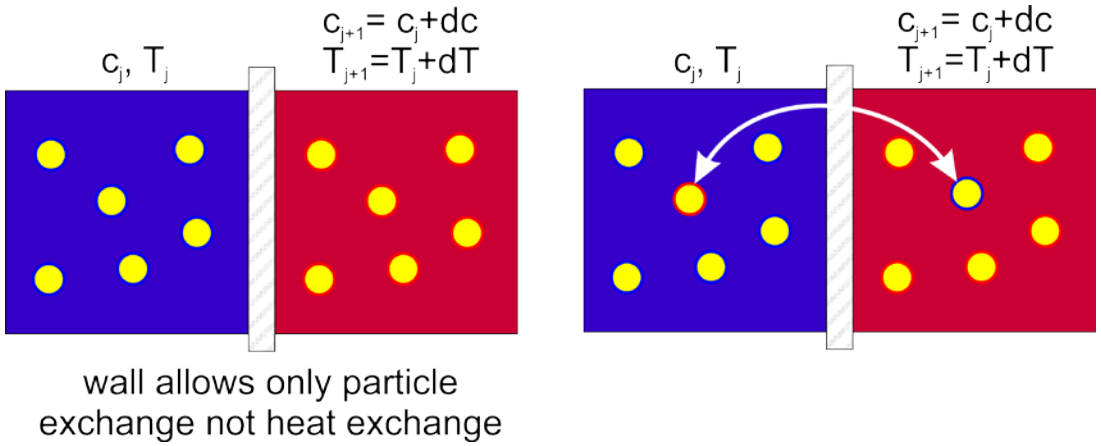
- S_T influenced by hydrogen bonds
  - HB network of water
  - HB between solute and water
- T-dependence of HB ⇔ thermodiffusion



S_T(ionic solutes)

EASTMAN

Thermodynamics of non-isothermal systems

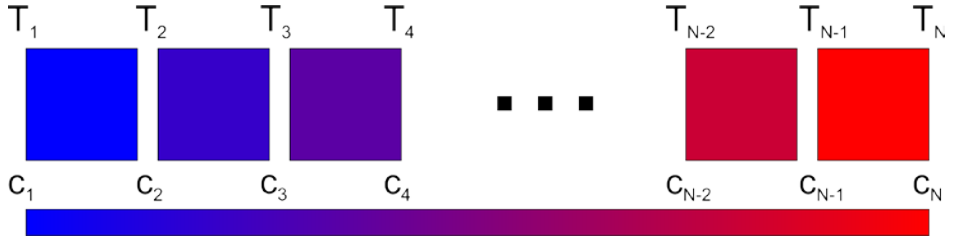


Eastman …

- calculates the entropy of transfer S^* (unknown)
- replaces S^* by the Soret coefficient S_T (unknown)
- Identifies it with the single-particle entropy S

$$S_T \mathrm{d}T = \frac{\mathrm{d}G}{k_B T}$$

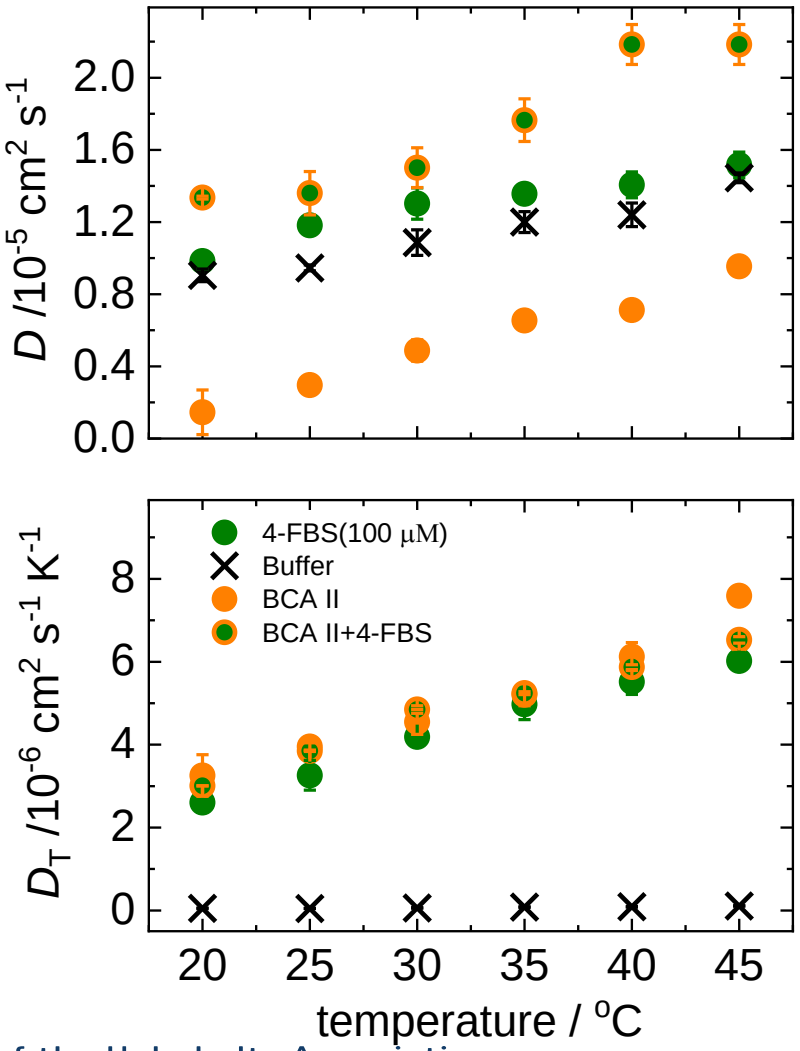
Series of heat reservoirs differing infinitesimally in temperature



E. D. Eastman, J. Am. Chem. Soc. **48**, 1482 (1926).
E. D. Eastman, J Am Chem Soc **50**, 292 (1928).
E. D. Eastman, J Am Chem Soc **50**, 283 (1928).
A. Würger, Cr Mecanique **341**, 438 (2013).

PROTEIN-LIGAND SYSTEMS

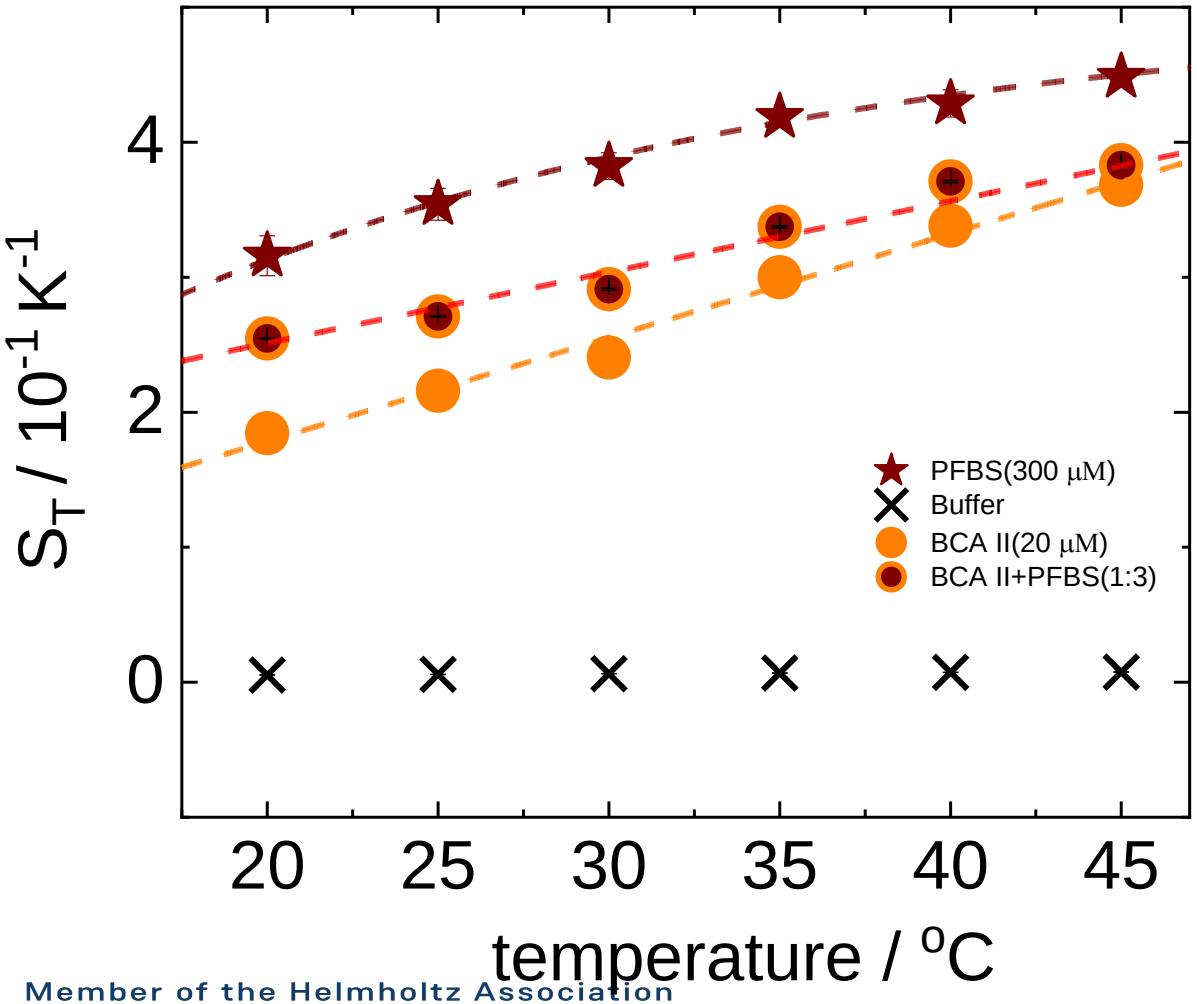
Bovine Carbonic Anhydarse II + 4-FBS

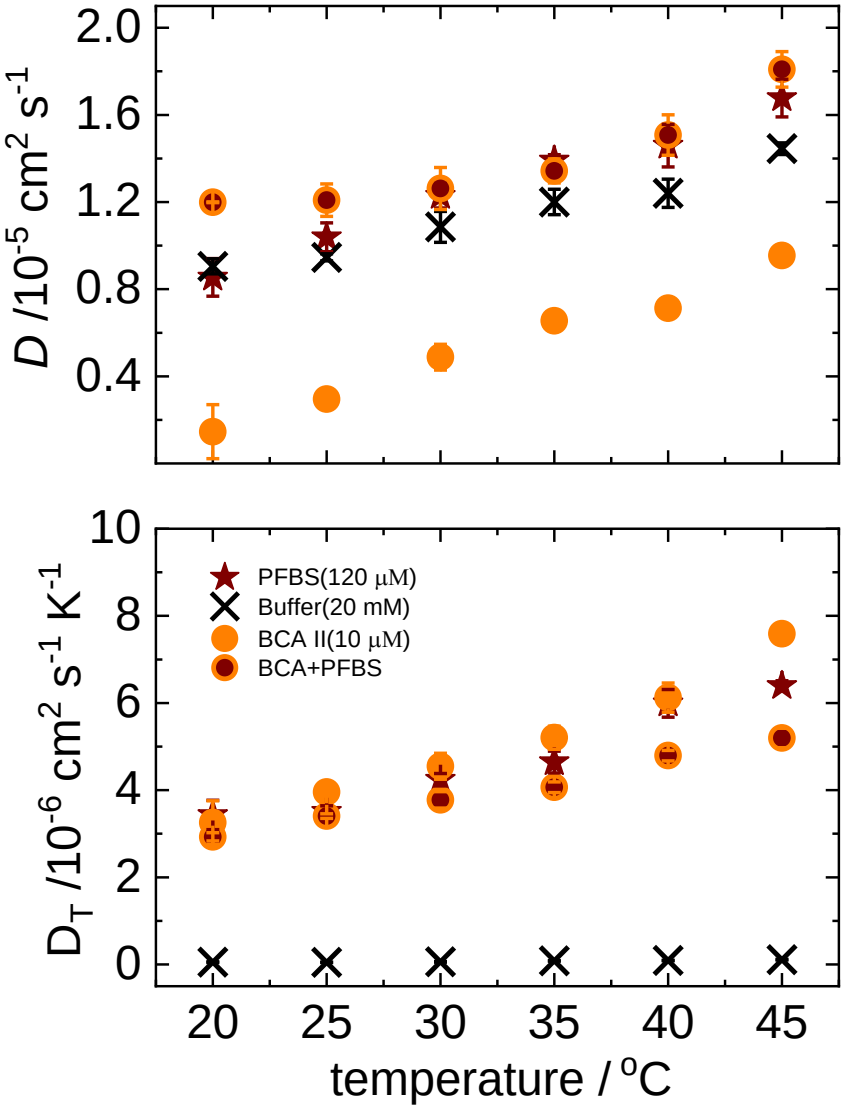


- Diffusion(*D*) of BCA II increases with ligand binding
- Thermal diffusion coefficient(D_T) of complex is reduced compared to the protein
- Difference between D_T of free protein and complex increases with increasing temperature

PROTEIN-LIGAND SYSTEMS

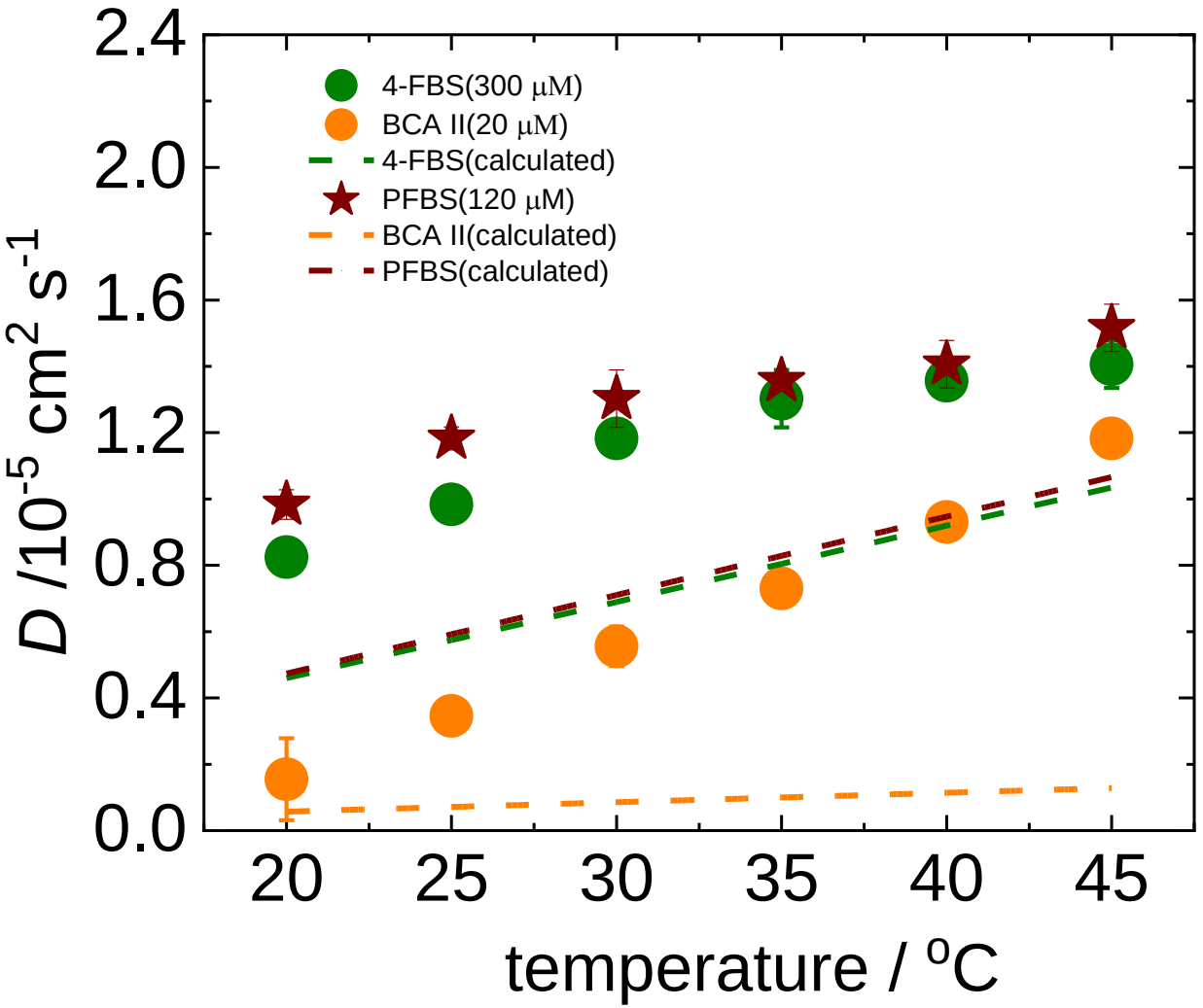
Bovine Carbonic Anhydarse II + PFBS





PROTEIN-LIGAND SYSTEMS

Diffusion coefficient



- Estimated the diffusion coefficient using Stokes-Einstein relation

$$D = \frac{k_B T}{6\pi\eta R}$$

- Calculated diffusion coefficients are below experimentally obatined values.

D. Niether et al., Polymers. 2020, 12, 376

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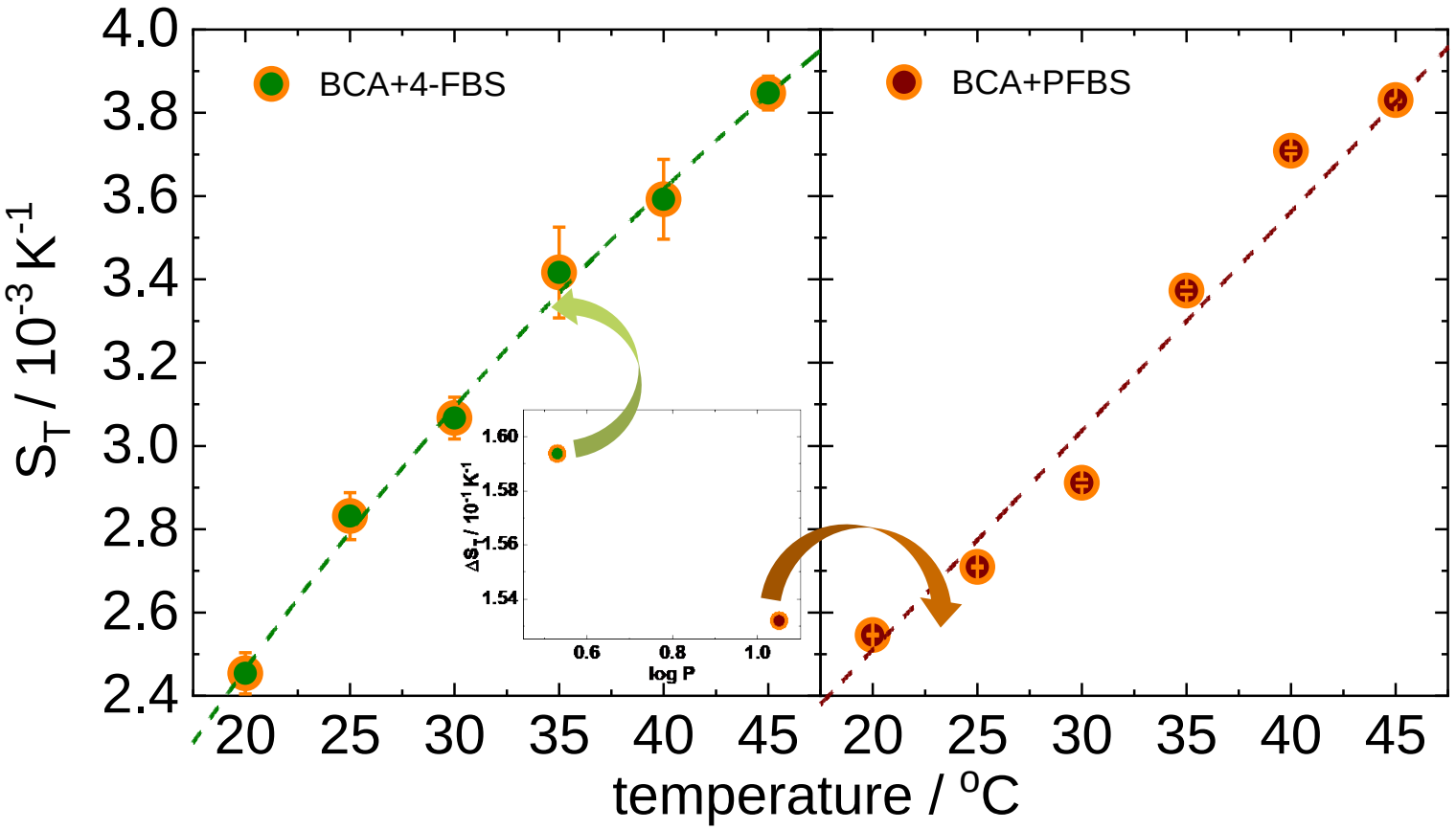
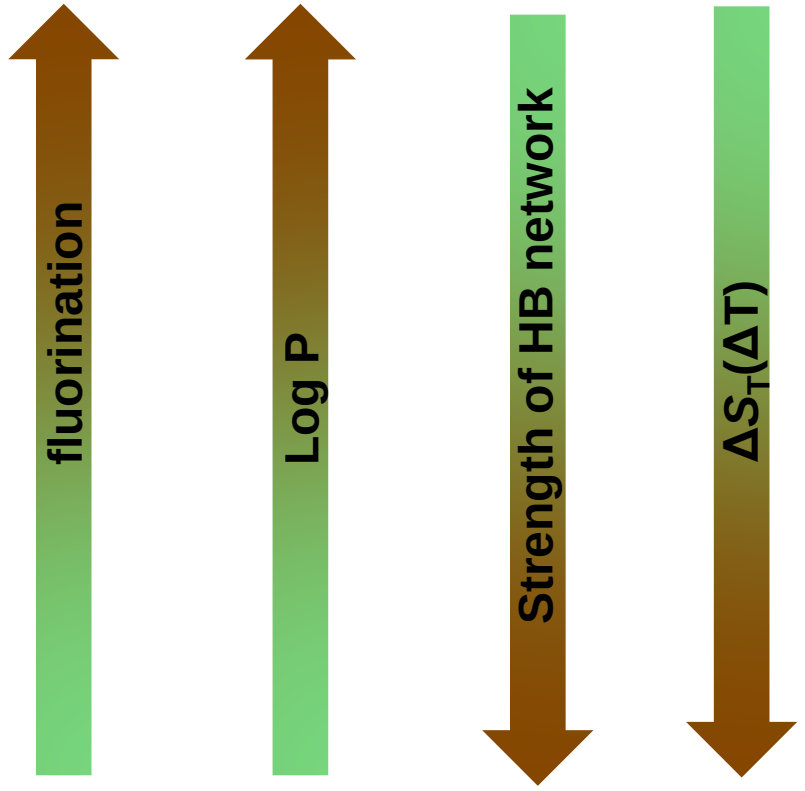
BCA II + SULFONAMIDES

Effect of fluorination





Effect of fluorination was validated from TDFRS measurements!

V. M. Krishnamurthy et al., Chem. Asian J. 2007, 2, 94 – 105