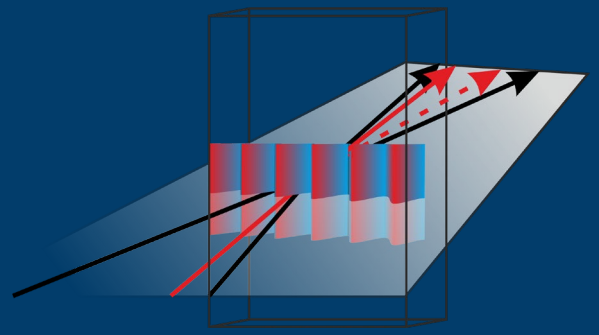
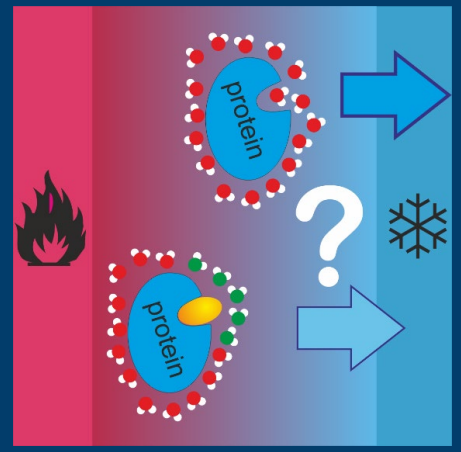
THERMOPHORESIS: THE CASE OF APOMYOGLOBIN

INSTITUTE OF BIOLOGICAL INFORMATION (IBI)
IBI-4:BIOMACROMOLECULAR SYSTEMS AND PROCESSES

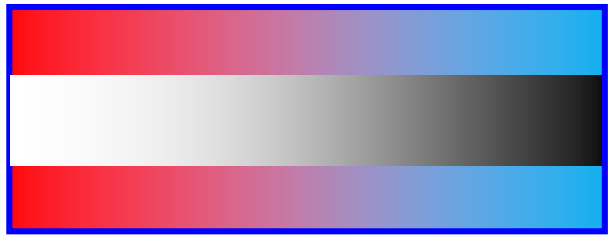




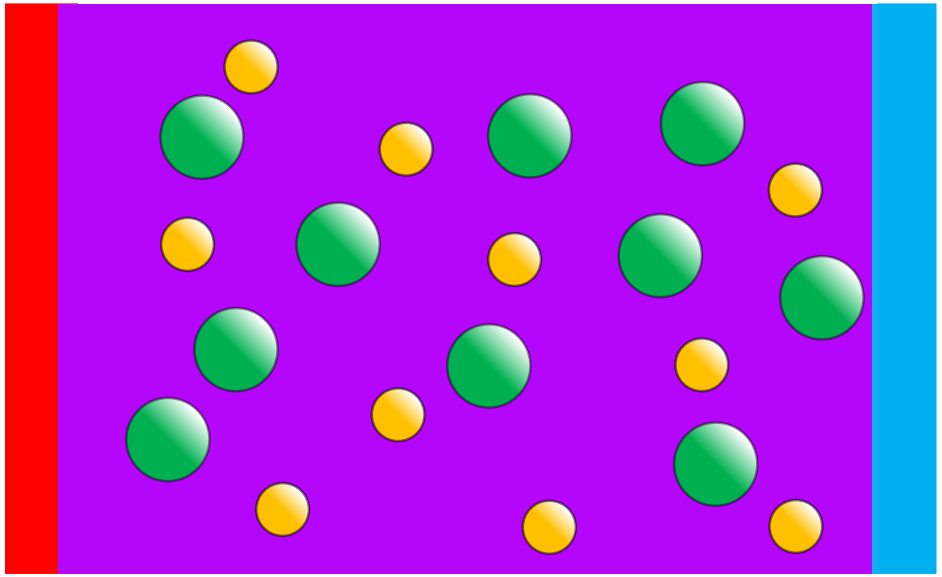
24.07.2024 I BINNY RUDANI

JÜLICH
Forschungszentrum

THERMODIFFUSION / THERMOPHORESIS



$$\vec{j} = \underbrace{-\rho D \nabla c}_{\text{mixing}} \underbrace{- c(1-c)\rho D_T \nabla T}_{\text{demixing}}$$



Steady state defines Soret coefficient S_T.

$$S_T \equiv \frac{D_T}{D}$$

D diffusion coefficient

D_T thermal diffusion coefficient

$S_T = 10^{-3} K^{-1}$ – $1 K^{-1}$

molecules colloids

FACTORS INFLUENCING S_T

mass

moment of inertia

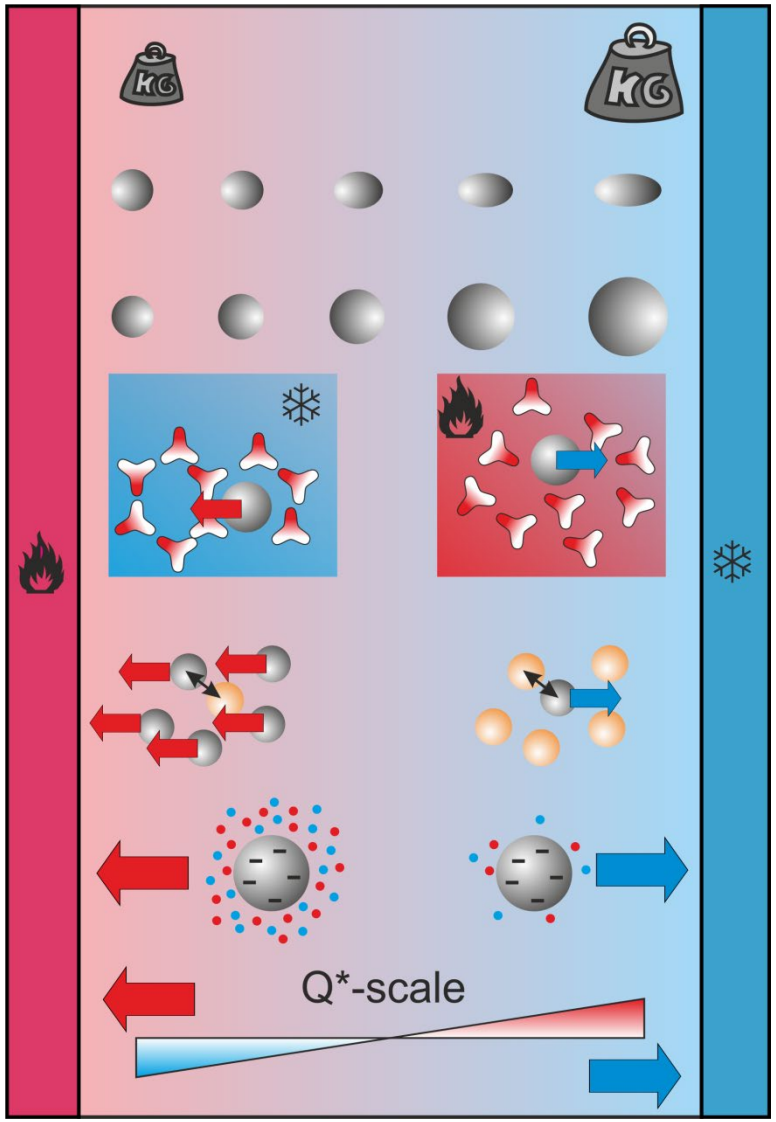
size

hydrogen bond network

strong cross interaction

ionic strength

heat of transfer



Ethanol/water

S_T>0

S<0

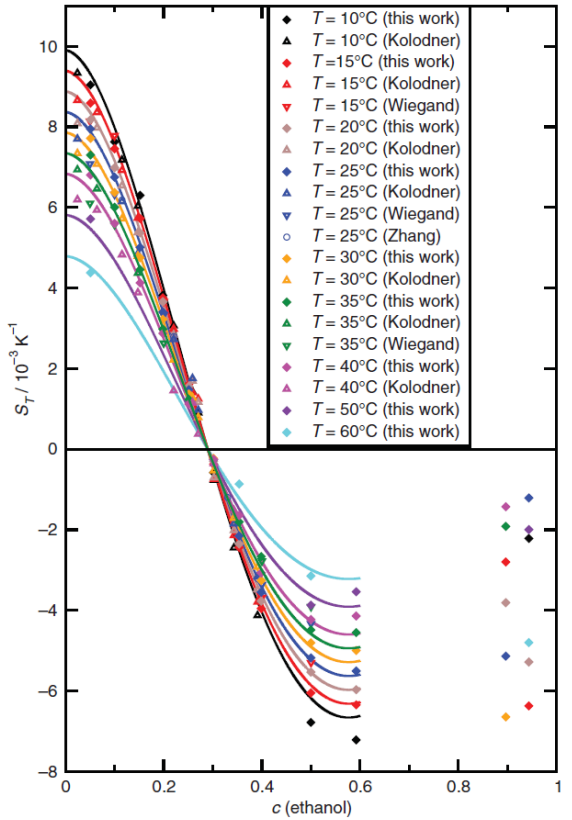


Figure 5. (Colour online). Soret coefficient S_T of ethanol–water as a function of ethanol mass fraction at various temperatures. See text for references.

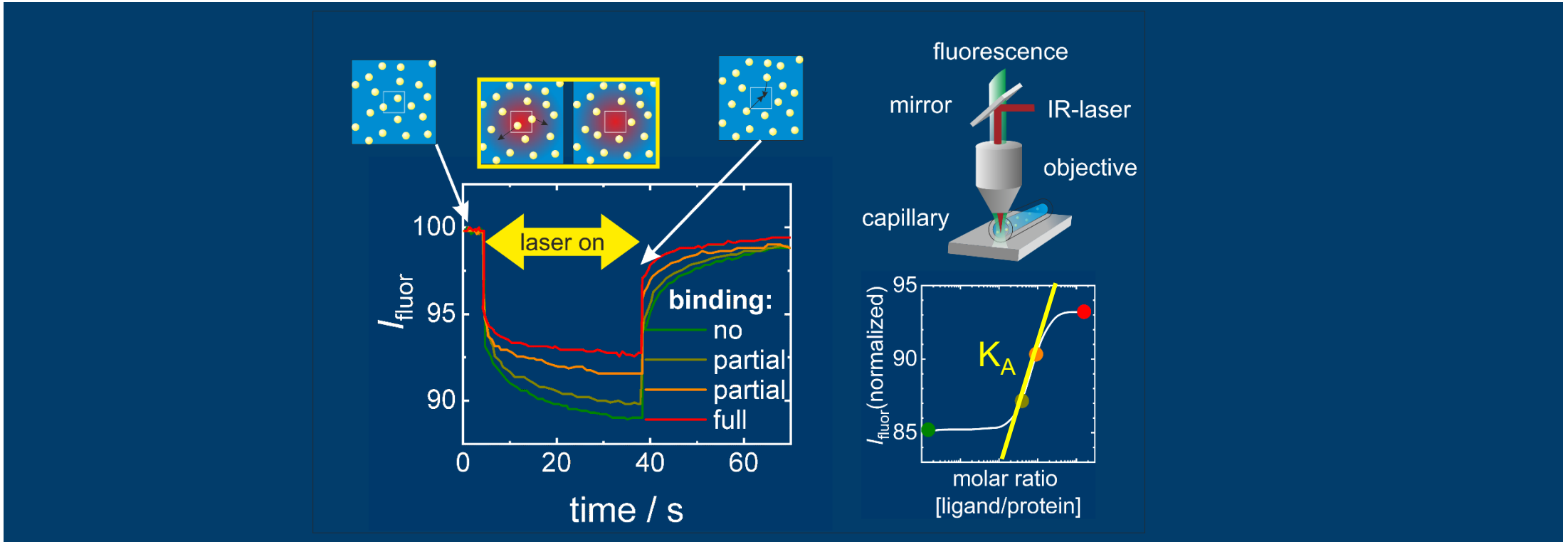
A. Königer et al. *Philos Mag* **89**, (2009)907–923.

No microscopic theory for fluids

THERMOPHORESIS

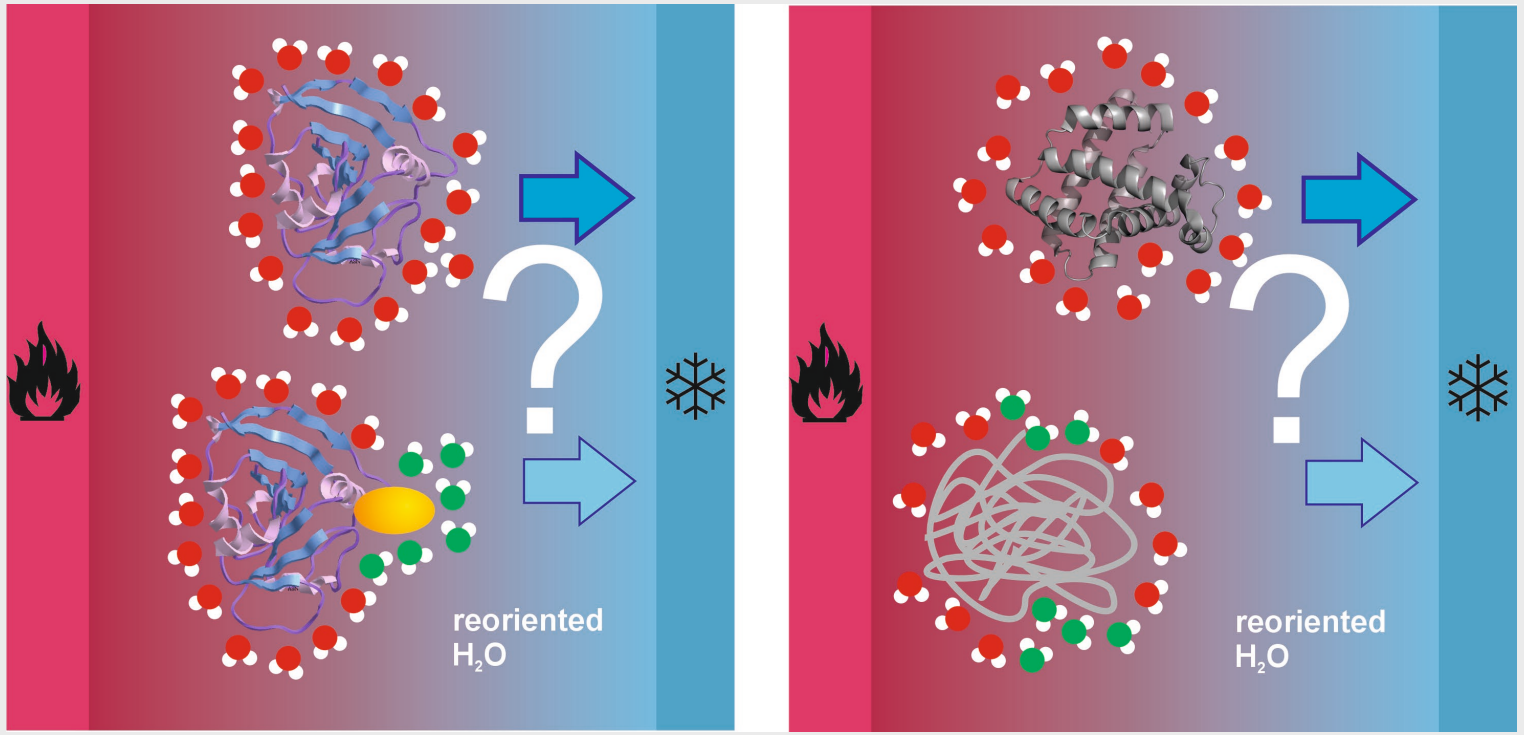
Microscale thermophoresis (MST) □binding constant K_A

- Monitors protein-ligand interaction



OBJECTIVE

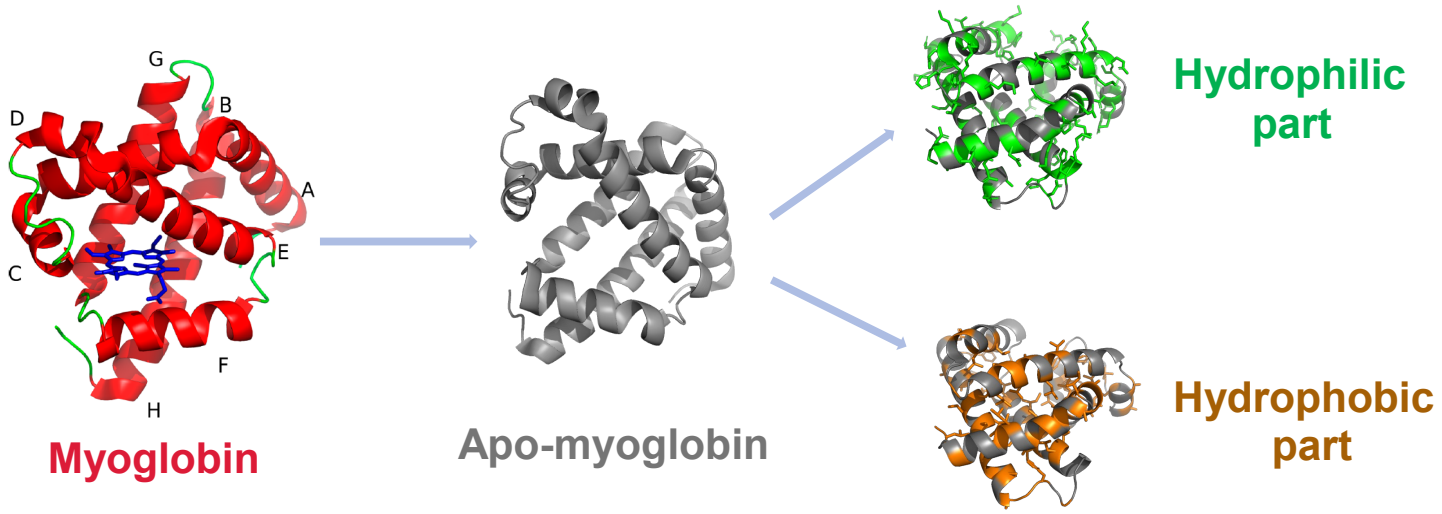
Can we quantify the relation between thermodiffusion and hydration



Hypothesis:
Movement in a temperature gradient is sensitive to changes in the hydration layer

APOMYGLOBIN

- **Apomyoglobin** (apo-Mb), myoglobin without the heme group, serves as a model system in the field of protein folding.
- It consists of 153 amino acids, which form 8 α-helices connected by loops.
- Hydrophilicity and conformation changes with pH variation.



protein	state
apo-Mb at pH 2	unfolded
apo-Mb at pH 2, 100 mM NaCl	MG
apo-Mb at pH 4	MG I1
apo-Mb pH 2, 20 mM NaTCA	MG I2
apo-Mb at pH 6	folded

Andreas M. Stadler, Michael Marek Koza, and Jörg Fitter. *The Journal of Physical Chemistry B* **2015** *119* (1), 72-82

SAMPLE PREPARATION:

Protein dissolved in water/20mM Na_2HPO_4/NaH_2PO_4 buffer

↓

Centrifugation (29000g at 10°C for 10min)

↓

0.2µm filter

↓

pH adjustment

↓

Concentration confirmed by nanodrop

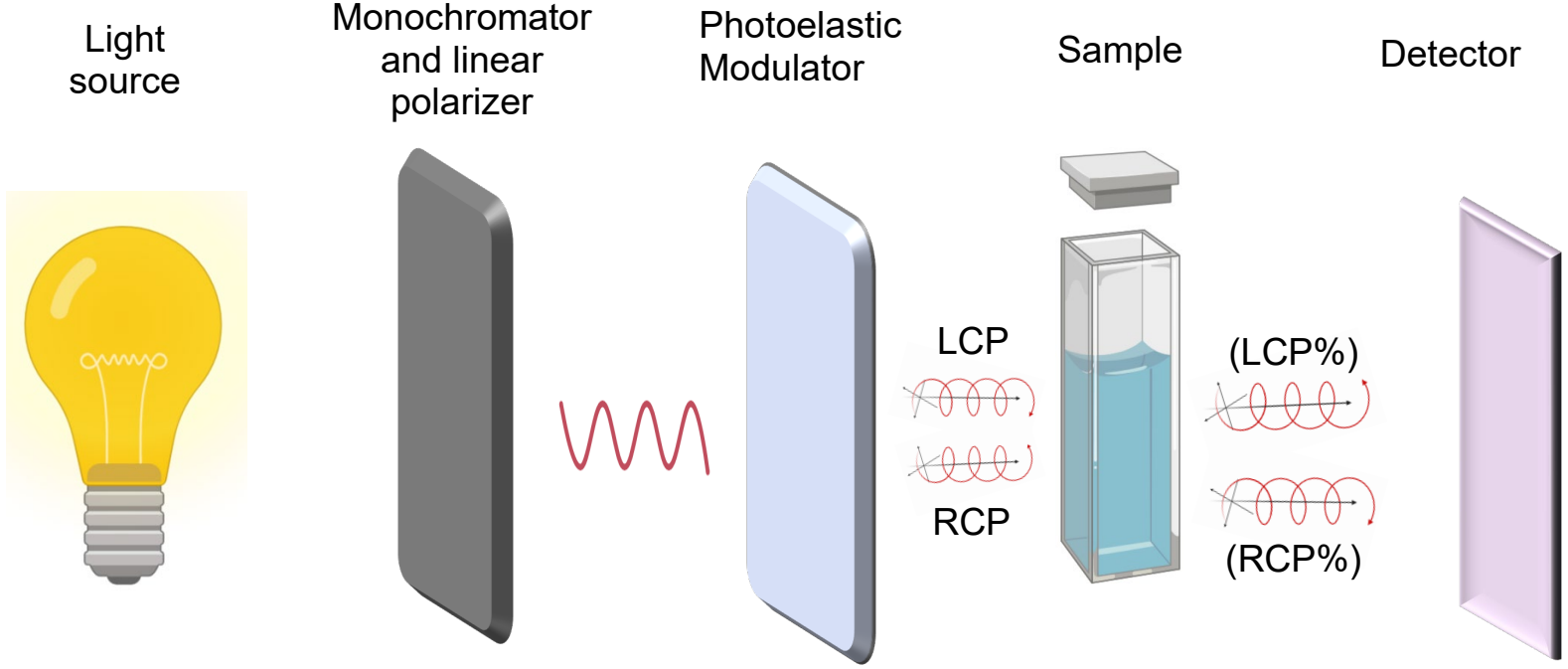
52.2µM for CD

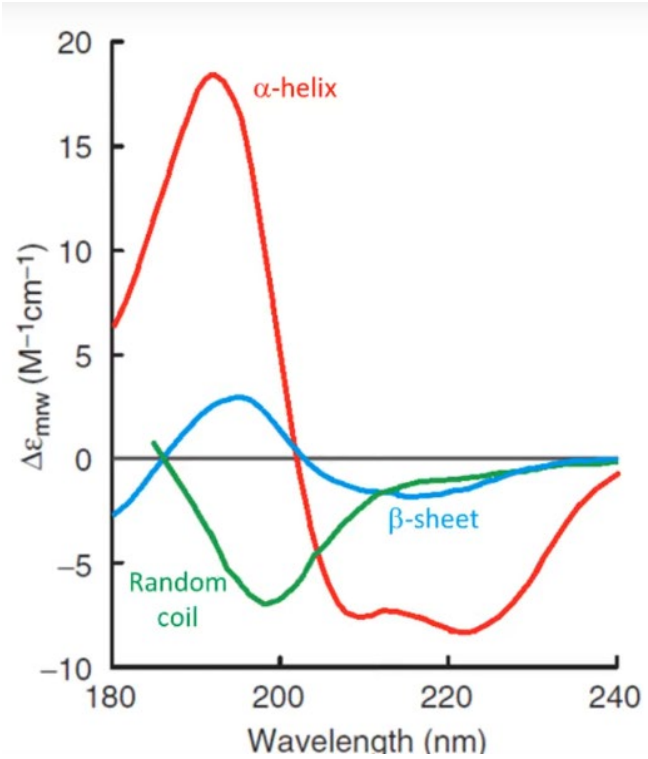
412µM for TDFRS

Andreas M. Stadler, Michael Marek Koza, and Jörg Fitter. *The Journal of Physical Chemistry B* **2015** *119* (1), 72-82

Charles Twist, Catherine Royer, and Bernard Alpert. Biochemistry ***2002*** *41 (32), 10343-10350*

CIRCULAR DICHROISM





Differential absorption: $\Delta A = A_l - A_r$

Beer–Lambert law, $\Delta A = (\varepsilon_L - \varepsilon_R)cl$

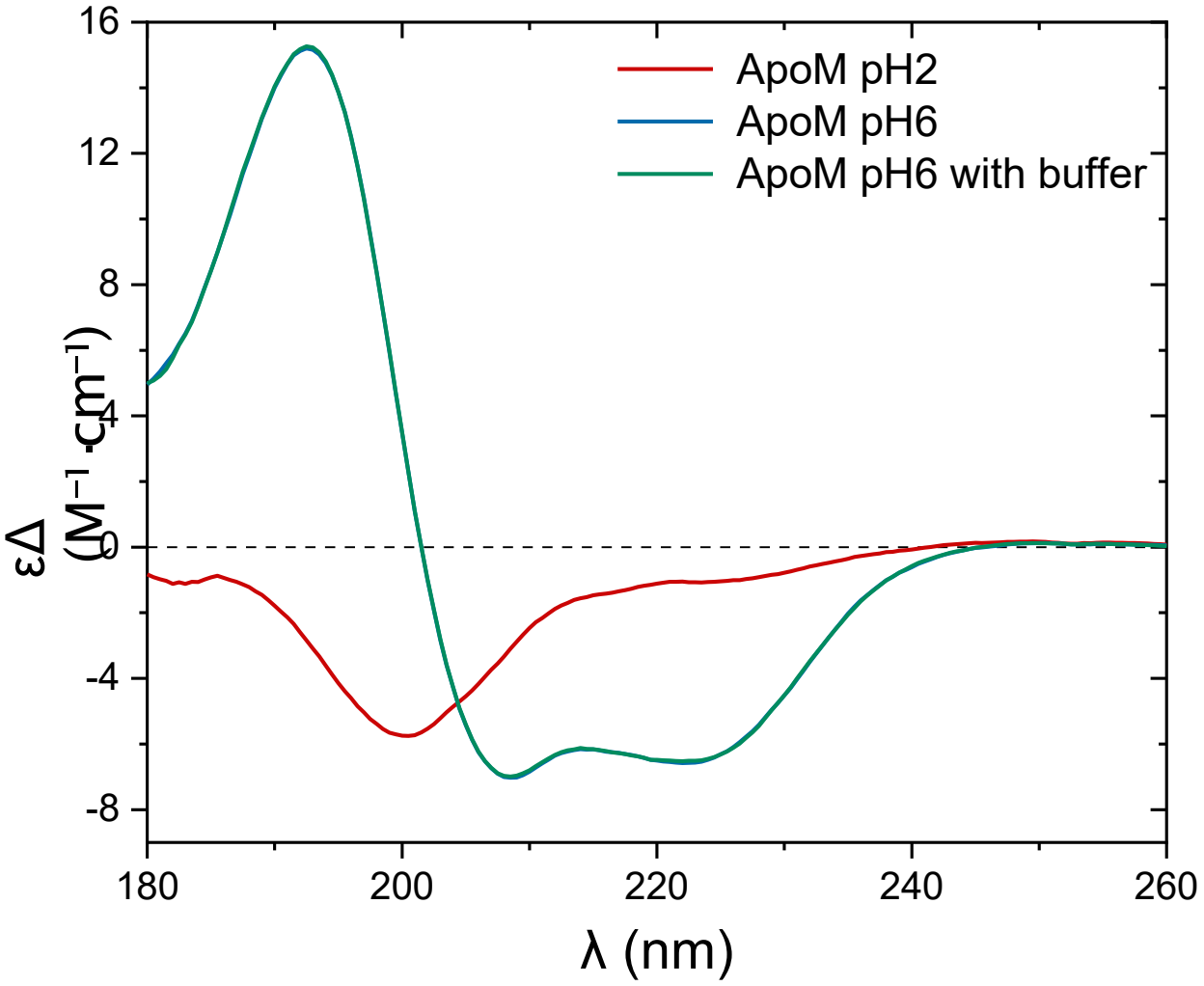
ε = molar extinction coefficient
c = molar concentration
l = path length (cm)

JÜLICH Forschungszentrum

Subadini et. al. (2022). Circular Dichroism Spectroscopy: Principle and Application. In: Sahoo, H. (eds) Optical Spectroscopic and Microscopic Techniques. Springer, Singapore.

CIRCULAR DICHROISM

Results

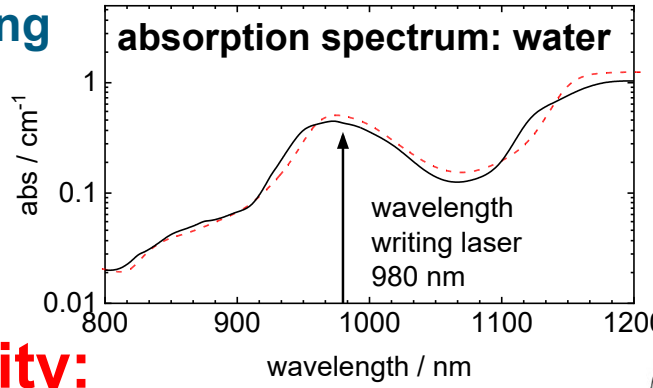


52.2µM ApoM at 20°C

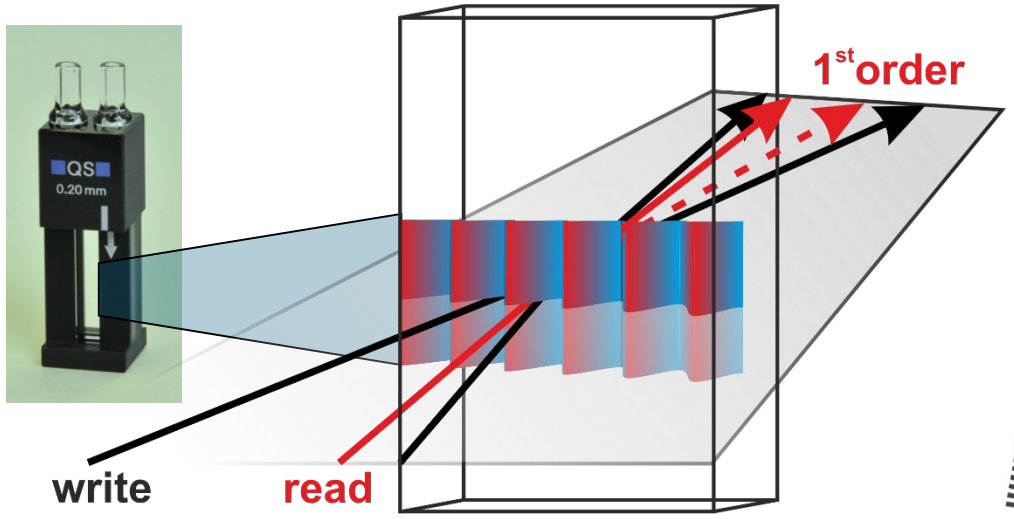
Protein	α-helical content (%)
pH2	7.1
pH6	61
pH6 in buffer	60.4

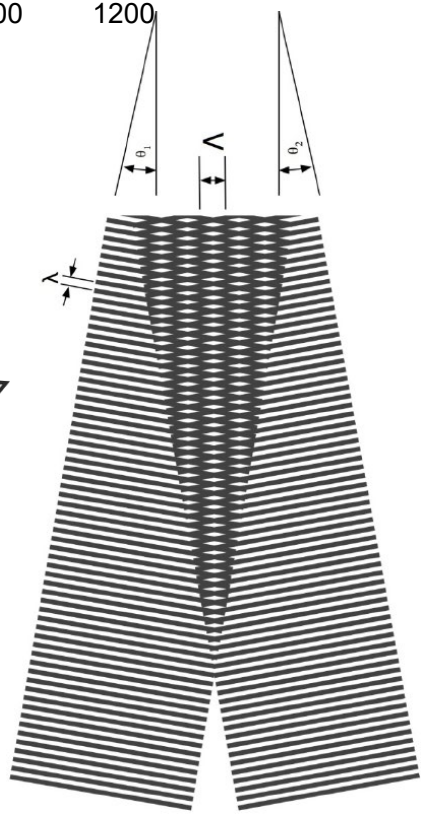
TDFRS: HOW DO WE MEASURE?

Refractive index grating



Measured quantity: intensity of diffracted reading beam





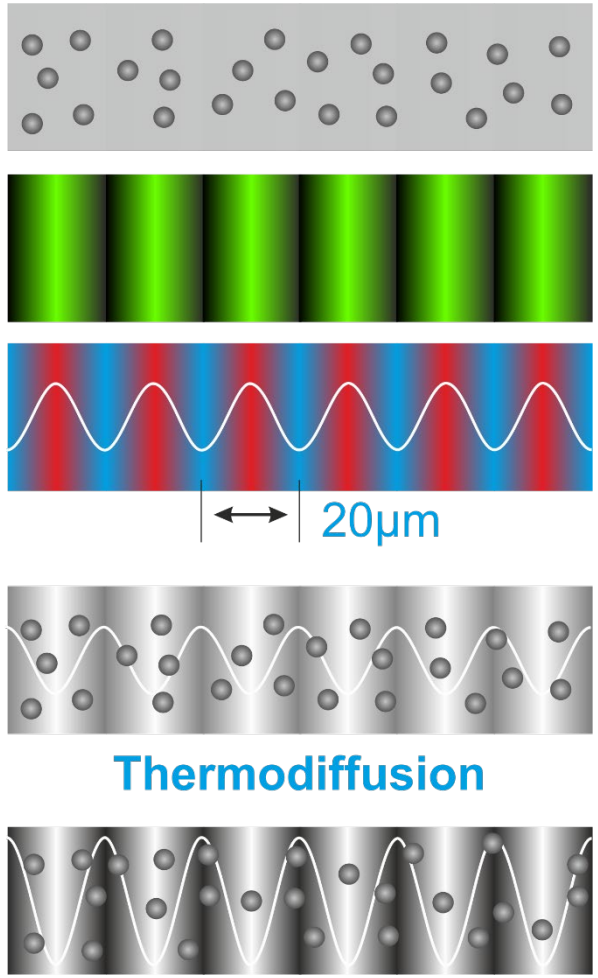
homogen

laser grating

temperature grating (20-100 µK)

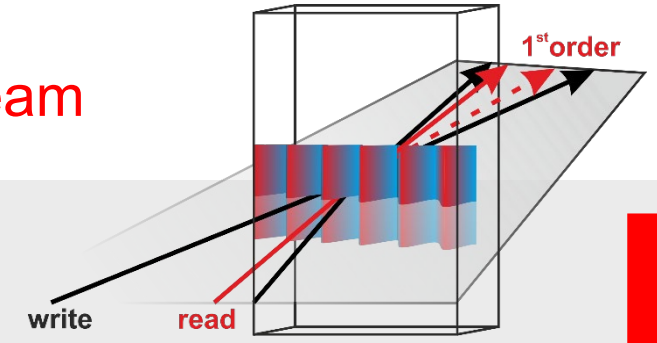
refractive index grating (temperature)

refractive index grating (concentration)

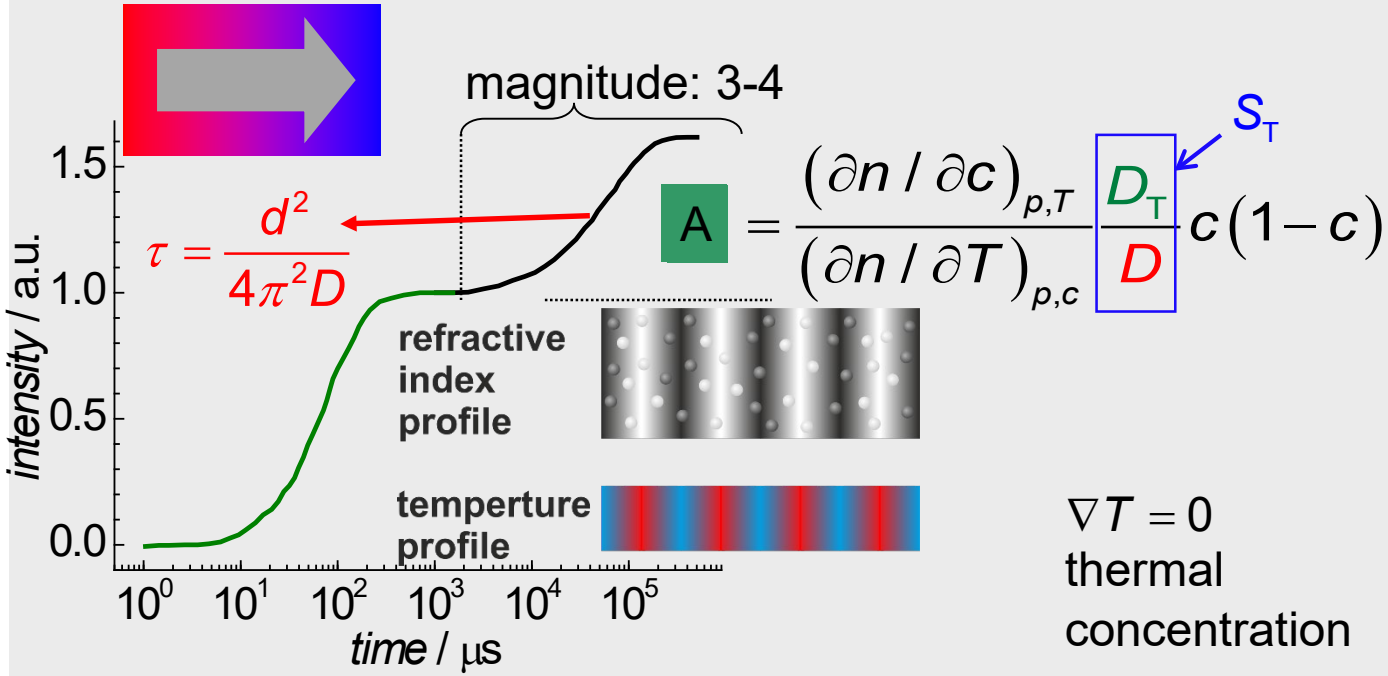


IR-TDFRS: MEASUREMENT SIGNAL

- Intensity of the read-out beam



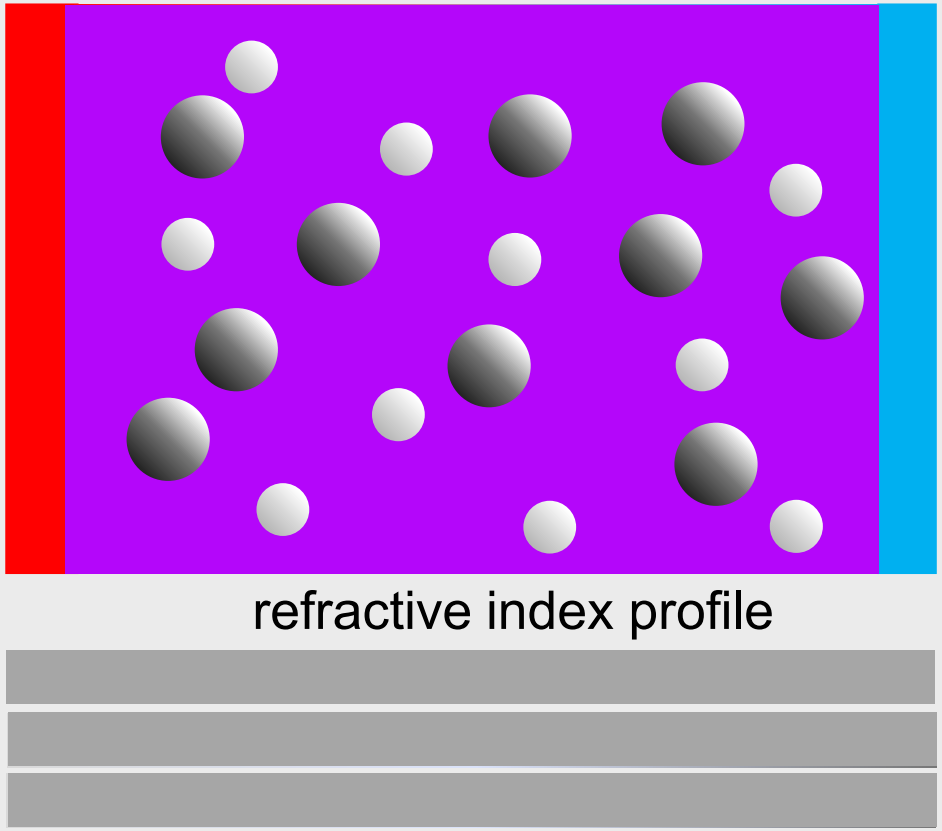
Molecules/colloids with higher refractive index move to cold side



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

S_T

$\nabla T = 0$
thermal concentration

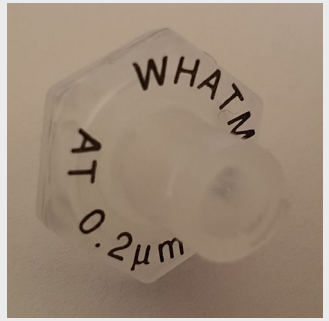


SAMPLE REQUIREMENTS

Aqueous solutions

∂n/∂c-measurement
~200 µL per
temperature and
concentration

- transparent solutions
- solvent with sufficient absorption at 980 nm
- sample amount: 1-2 mL
- typical concentrations 0.5mg/mL (fd-virus) 0.5mol/kg (salts)
- dustfree (solutions need to be filtered)
- no aggregation
- no sedimentation

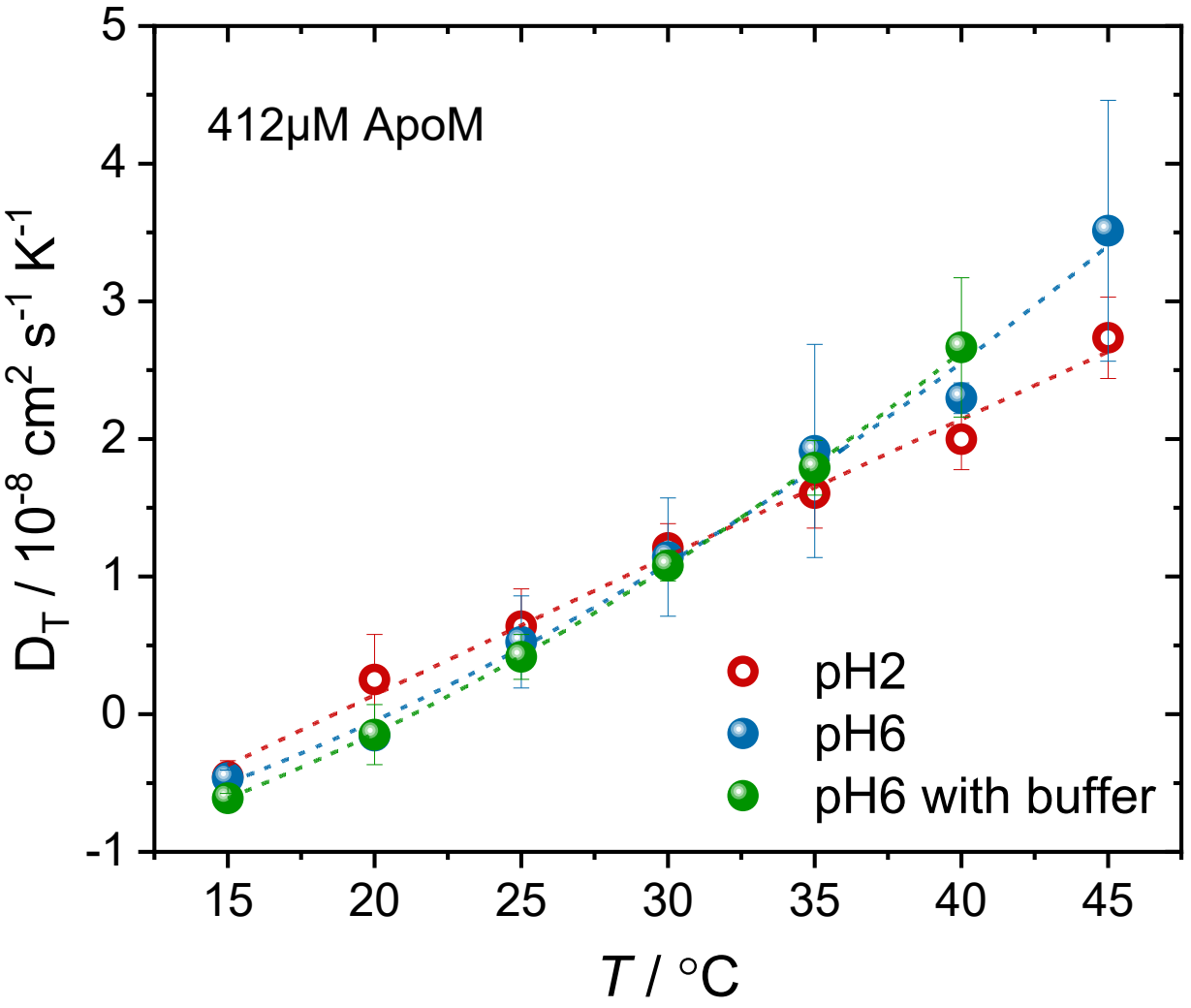


∂n/∂T-cell

~100 µL

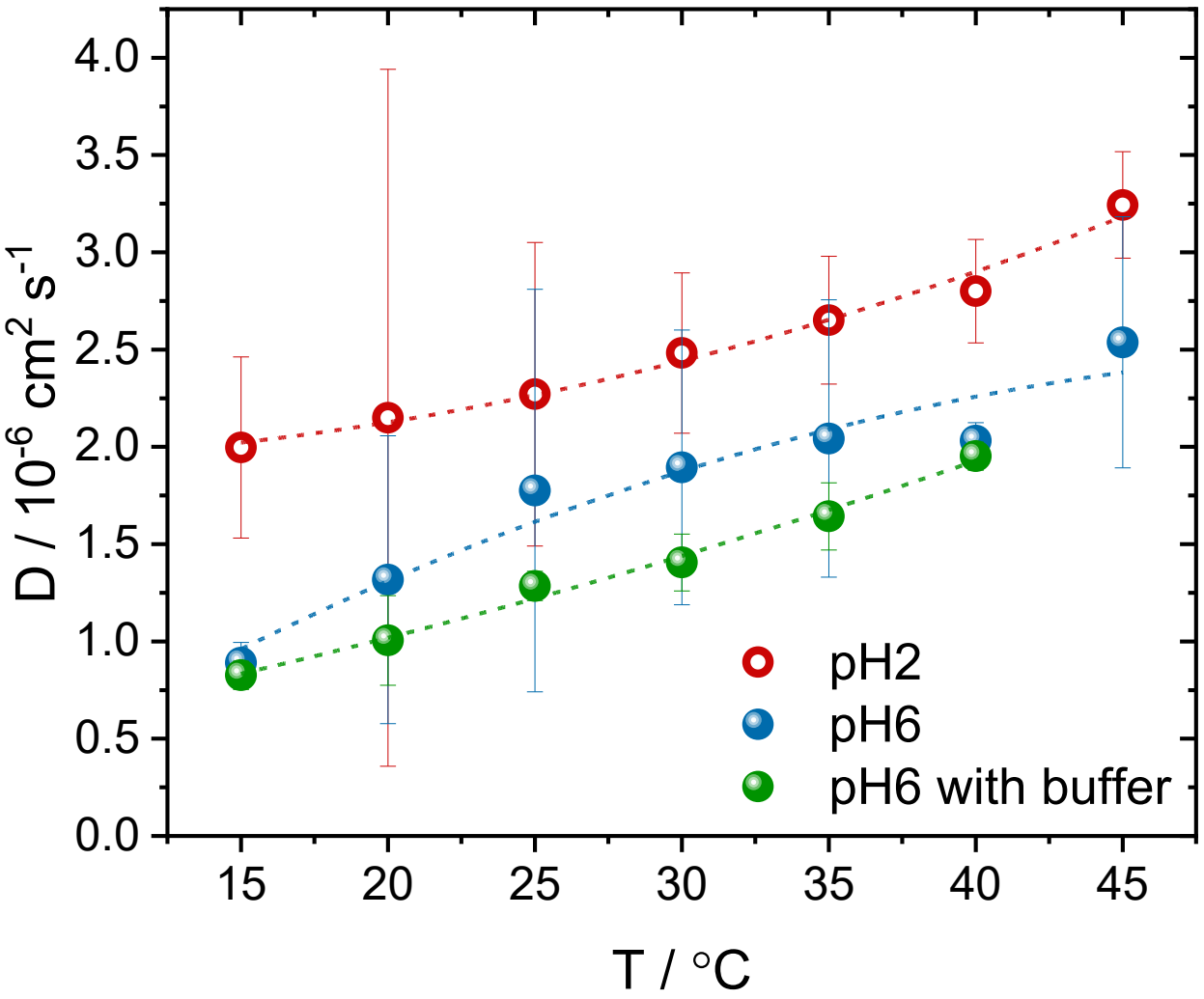
~13 µL

Thermal Diffusion Coefficient (D_T)



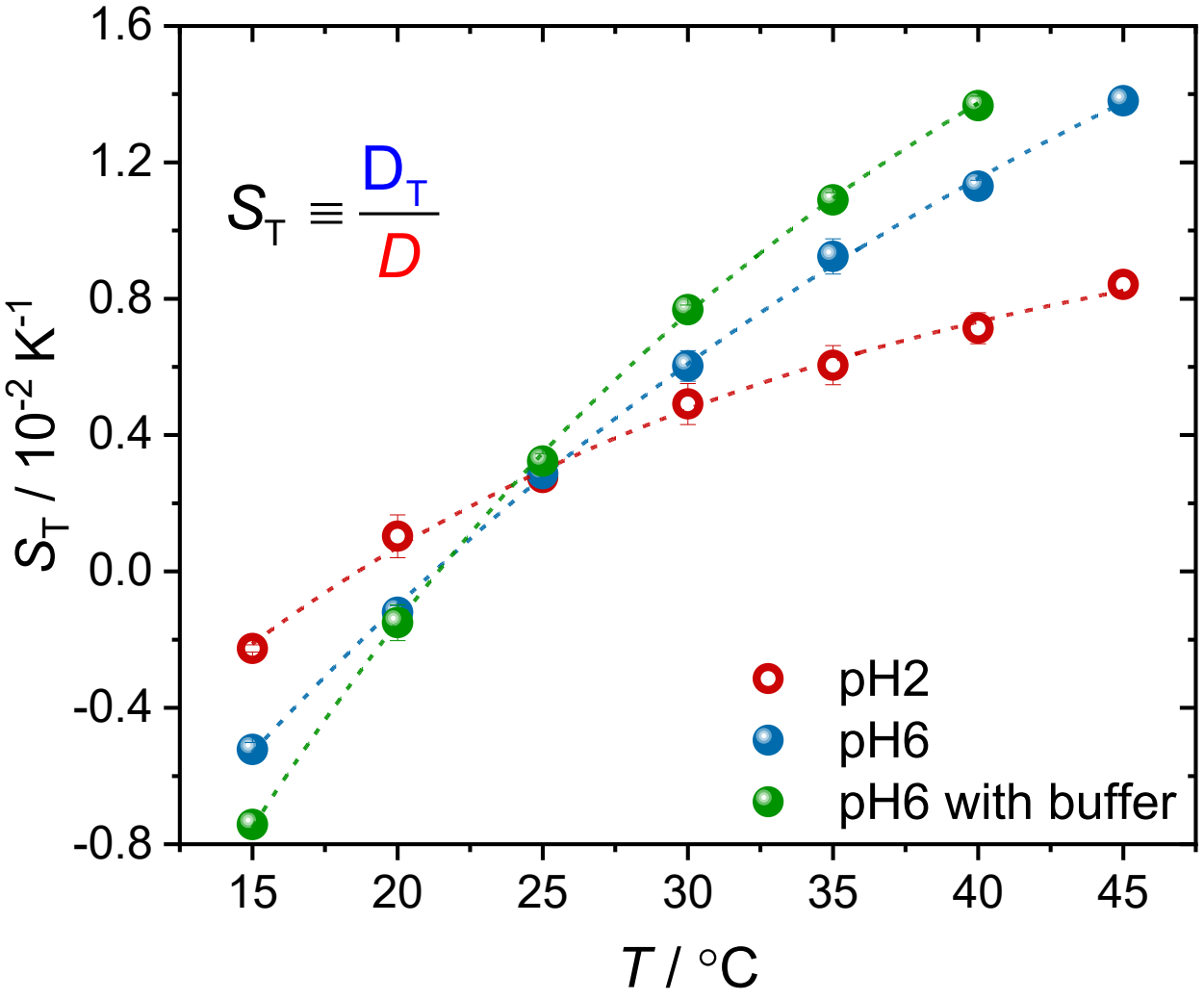
- **D_T at pH6 ≈ pH6 (in buffer)**
- **D_T at pH2**
- **Temperature dependence steeper at pH 6**

Diffusion Coefficient (D)



- **D: pH2 > pH6 > pH6 (in buffer)**
- **Majorly influences S_T**

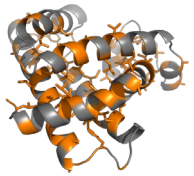
Soret Coefficient (S_T)



$$S_T\ (T) = S_T^{\infty} + Aexp(-\frac{T}{T_0})$$

Protein	Amplitude
pH2	-0.027
pH6	-0.052
pH6 in buffer	-0.061

$$\text{ratio} = \frac{AREA_{hydrophilic}}{AREA_{hydrophobic}}$$



FOLDED state @ pH 6: Based on water accessible area: ratio=2.7

UNFOLDED state @ pH2
Based on molecular area: ratio=1.2

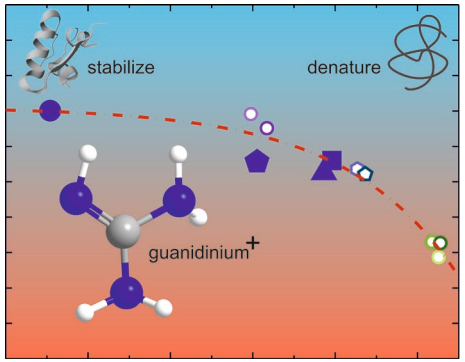
(Calculation using PyMOL)

CONCLUSION

- Folded protein (pH 6) is more hydrophilic and shows a stronger temperature dependence than the unfolded state (pH 2).
- Use of buffer leads to stronger aggregation, reflected also in a lower diffusion coefficient

FINISHED AND FUTURE PROJECTS

- Guanidinium salts:
  [Rudani, B. A., Jakubowski, A., Kriegs, H., Wiegand, S. Deciphering the guanidinium cation: Insights into thermal diffusion. The Journal of chemical physics 2024,160,214502.]

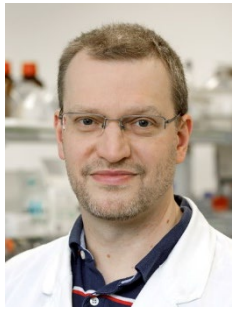


- Continuation of apomyglobin (pH 4)

protein	state
apo-Mb at pH 2	unfolded
apo-Mb at pH 2, 100 mM NaCl	MG
apo-Mb at pH 4	MG I1
apo-Mb pH 2, 20 mM NaTCA	MG I2
apo-Mb at pH 6	folded

- Ammonia salts (collaboration Holger Gohlke, IBG 4)
- Chelating agents (combination of ITC and TDFRS)
- Protein ligand binding (apomyoglobin binds with ligand e.g. protoporphrin IX, sodium tetradecysulfate)

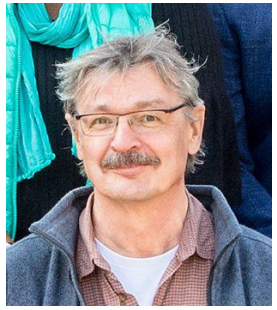
ACKNOWLEDGEMENT



Andreas Stadler (protein, JCNS-1)

Johan Buitenhuis (CD-measure-ments)

Simone Wiegand



Hartmut Kriegs (technical support)

Wim Briels (theory of thermo-diffusion of salts)

Peter Lang (head IBI-4)

Group of IBI-4

BioSoft
Biophysics and Soft Matter

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THANK YOU