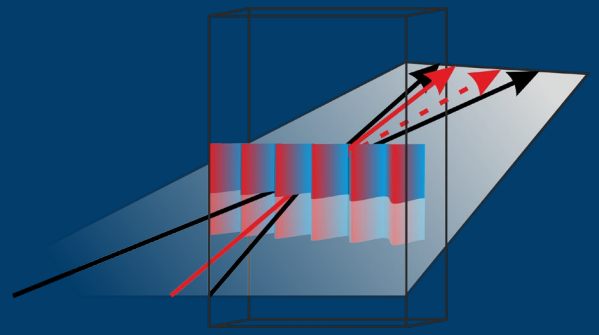
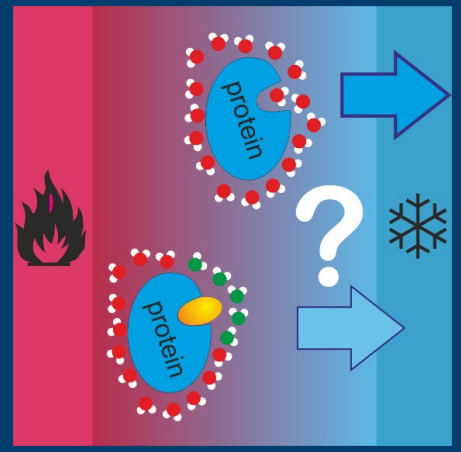
THERMOPHORESIS: THE CASE OF APOMYOGLOBIN

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

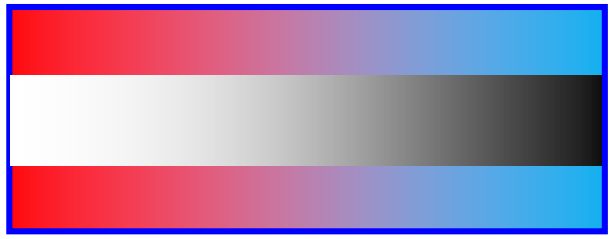




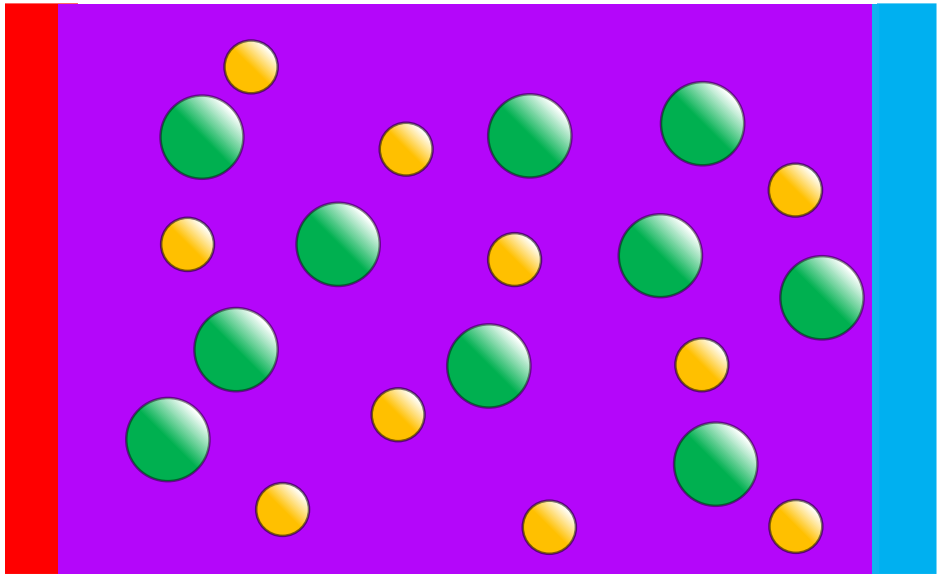
08.10.2024 I BINNY RUDANI

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

$S_T = 10^{-3}\,\mathrm{K}^{-1}$ – $1\,\mathrm{K}^{-1}$

molecules colloids

FACTORS INFLUENCING S_T

mass

moment of inertia

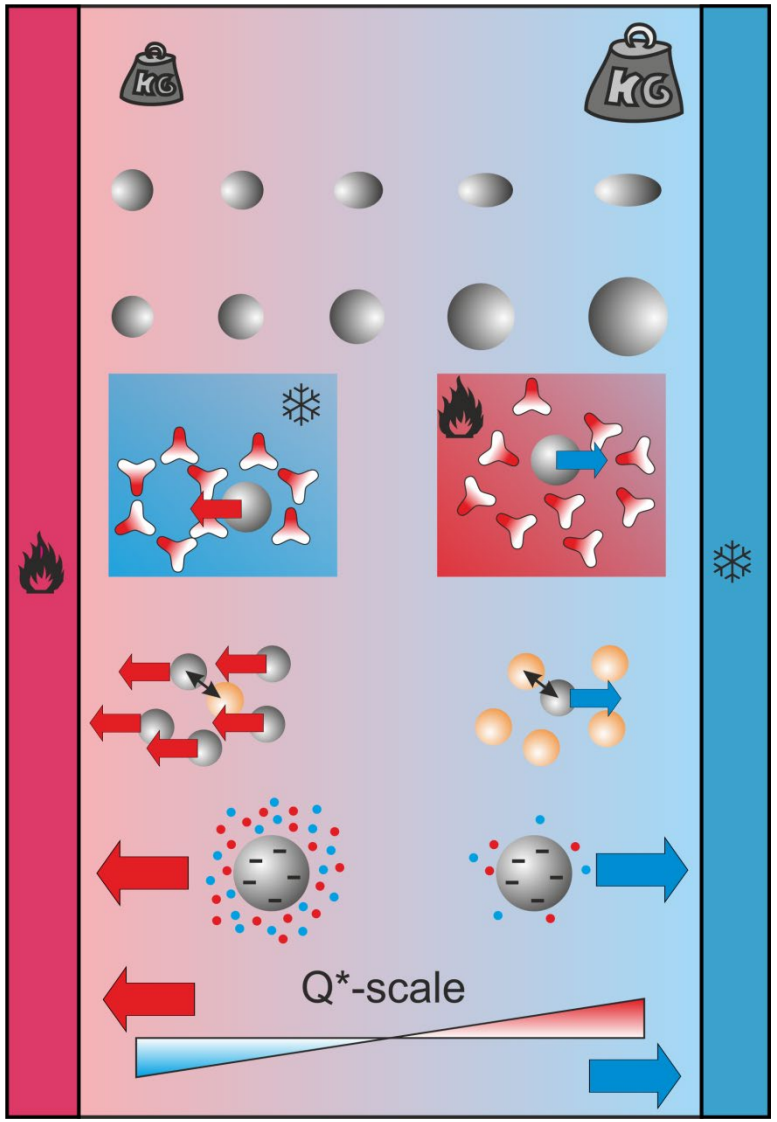
size

hydrogen bond network

strong cross interaction

ionic strength

heat of transfer



Ethanol/water

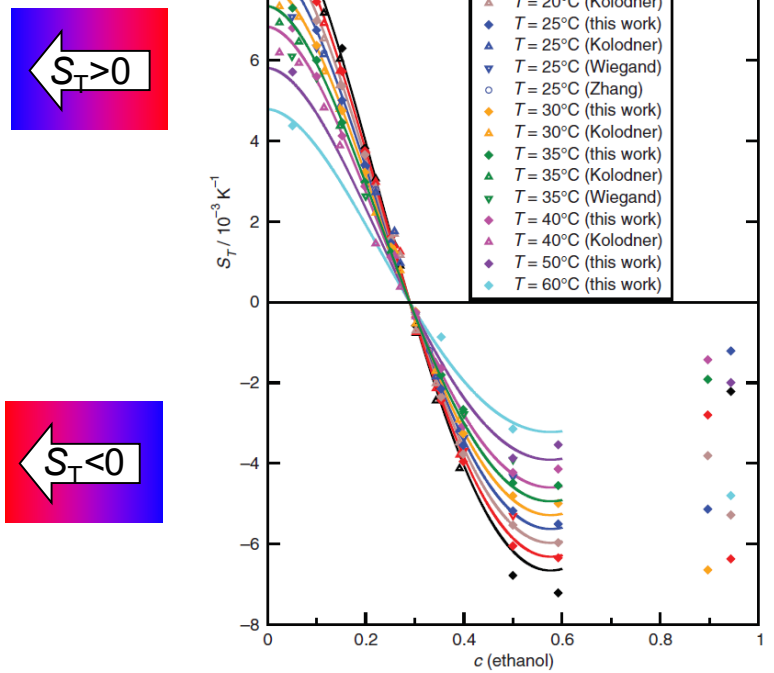


Figure 5. (Colour online). Soret coefficient S_T of ethanol–water as a function of ethanol mass fraction at various temperatures. See text for references.

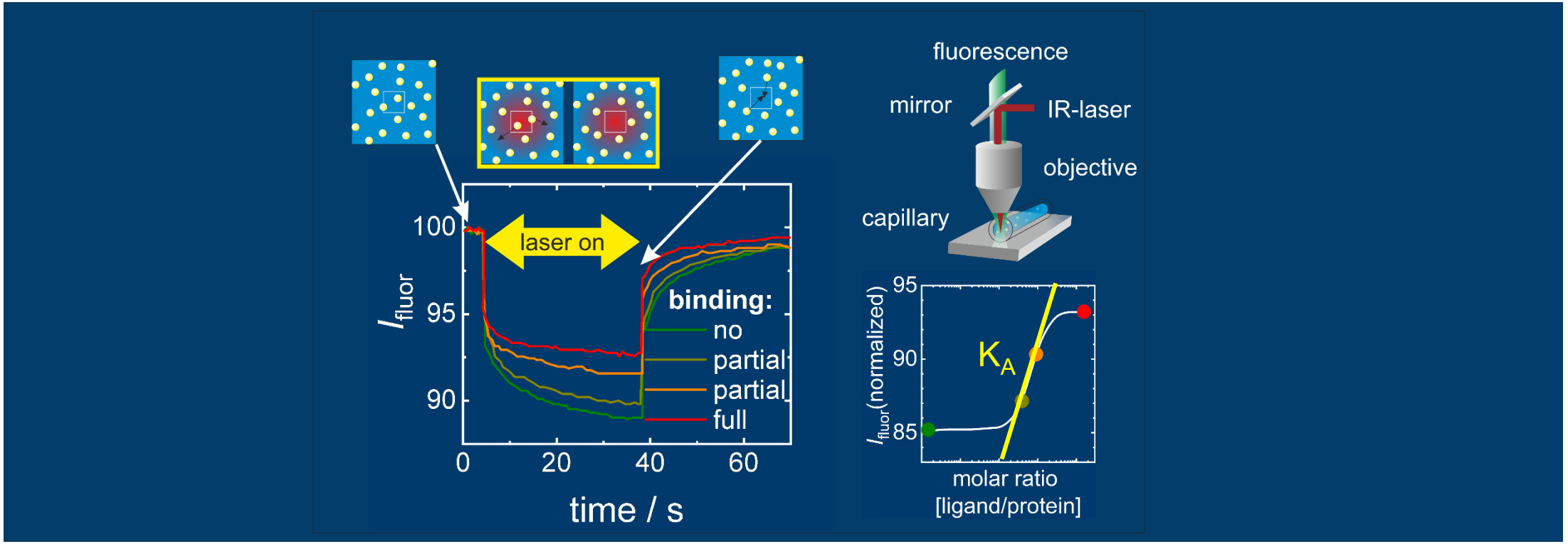
A. Königer et al. *Philos Mag* **89**, (2009)907–923.

No microscopic theory for fluids

THERMOPHORESIS

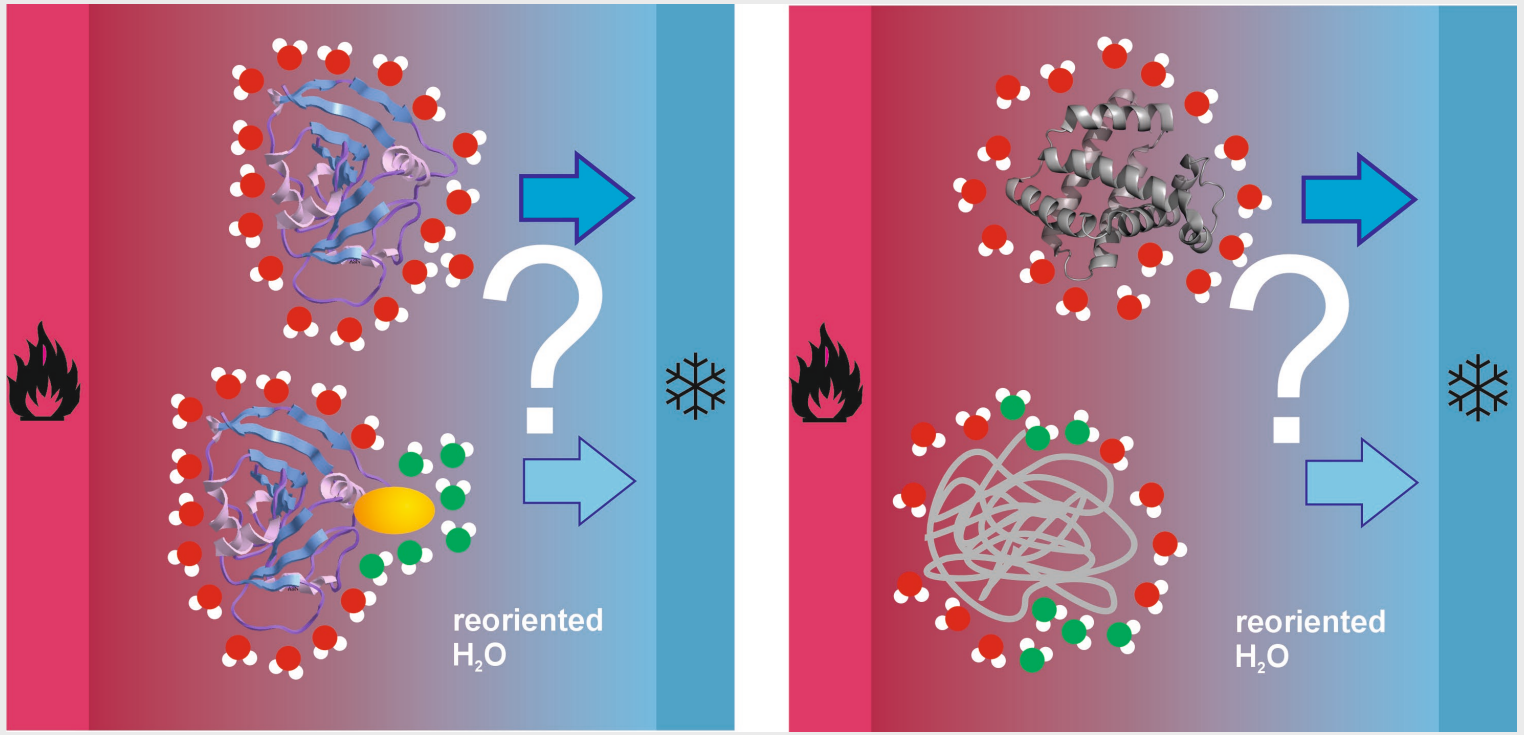
Microscale thermophoresis (MST) determines the binding constant K_A

- Monitors protein-ligand interaction



OBJECTIVE

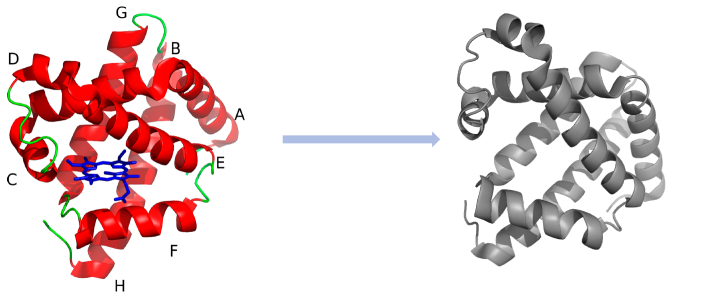
Can we quantify the relation between thermodiffusion and hydration



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

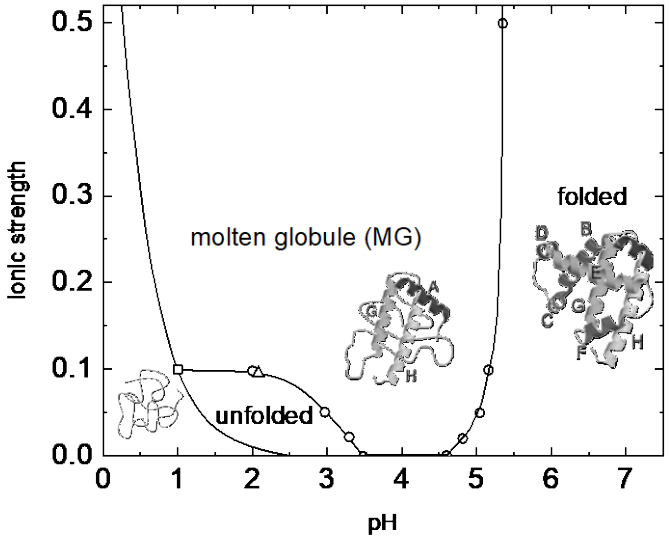
APOMYGLOBIN

- **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.



Myoglobin

Apo-myoglobin



Yuji Goto and Anthony L. Fink. *Journal of Molecular Biology*. **1990,** 214(4), 803-805.

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 or

20mM Na-phosphate (NaP) buffer or 10mM/20mM Na-Acetate (Ac) buffer

↓

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

↓

0.2µm filter

↓

pH adjustment

↓

Concentration confirmed by nanodrop

52.2µM for CD at 20°C

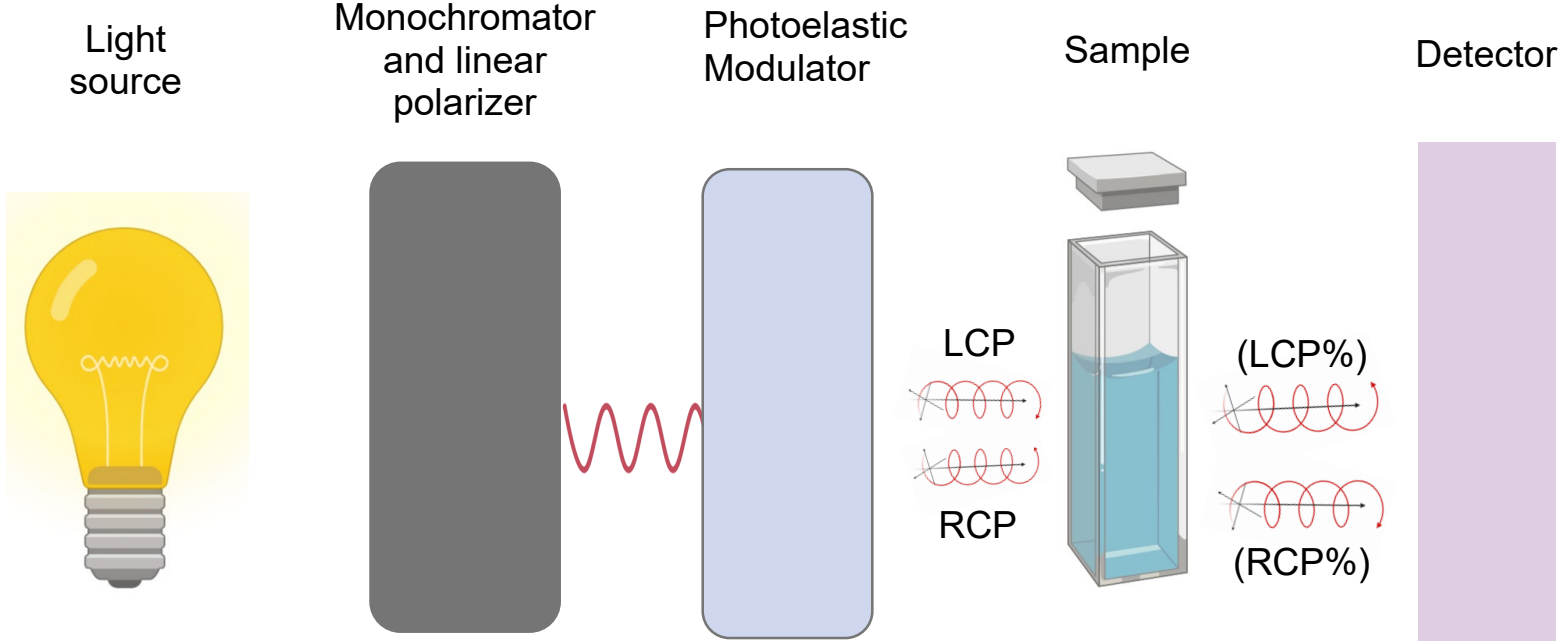
412µM for TDFRS at 15°C-45°C

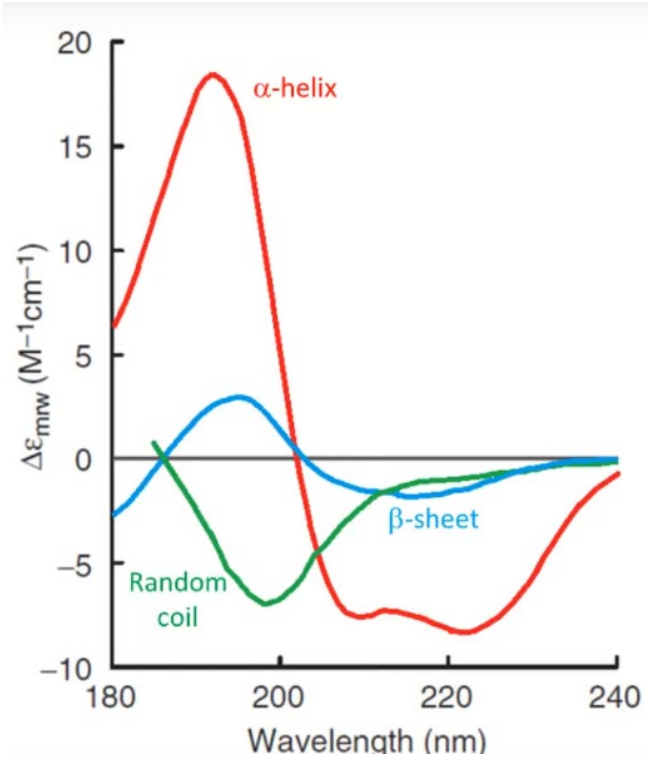
Andreas M. Stadler, Michael Marek Koza, and Jörg Fitter. *The Journal of Physical Chemistry B* **2015** *119* (1), 72-82

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

CIRCULAR DICHROISM

Principle





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

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

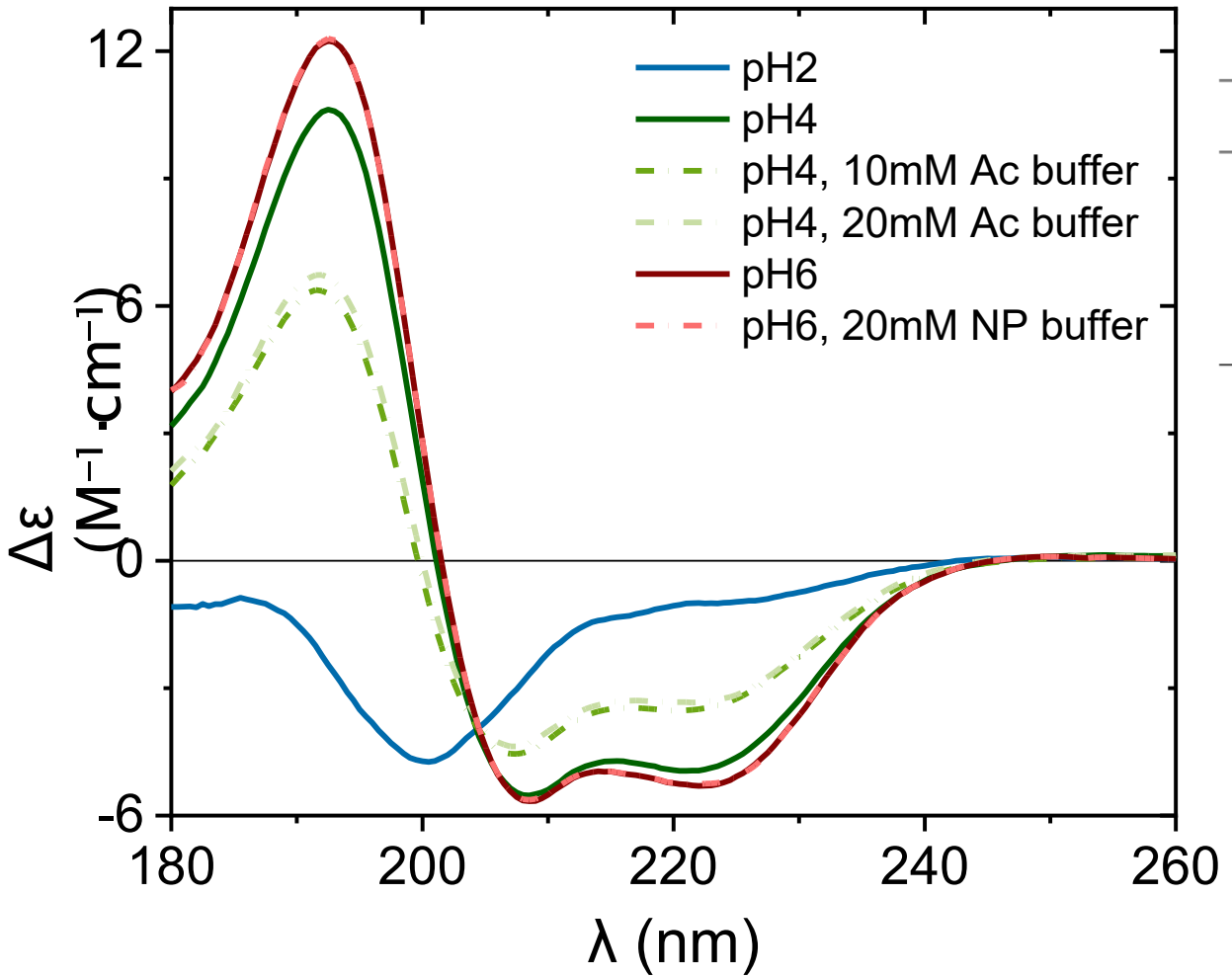
ε = molar extinction coefficient
c = molar concentration
l = path length (cm)

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



α-helical content: **pH6** > **pH4** > **pH2**

52.2µM ApoM at 20°C

protein	state	α-helical content (%)* this work	α-helical content (%)/ Reference
apo-Mb at pII2	unfolded	6	5
apo-Mb at pH4	MG I2	42.9	43 (Ref. 14)
apo-Mb at pH4, 10mM Ac buffer	MG I1	34.7	-
apo-Mb at pH4, 20mM Ac buffer	MG I1	34.2	-
apo-Mb at pH6	folded	51.9	49
apo-Mb at pH6, 20mM NaP buffer	folded	51.2	55

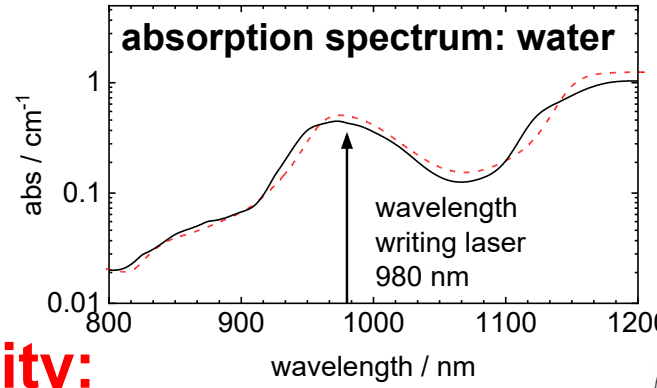
- **pH6**: same α-helical content with and without buffer
- **pH4:** buffer graph deviates significantly from normal acidic solution
- **pH2:** minimum α-helical content

Uzawa *et al. Biophysics and Computational Biology* **2004** 101(5), 1171-1176

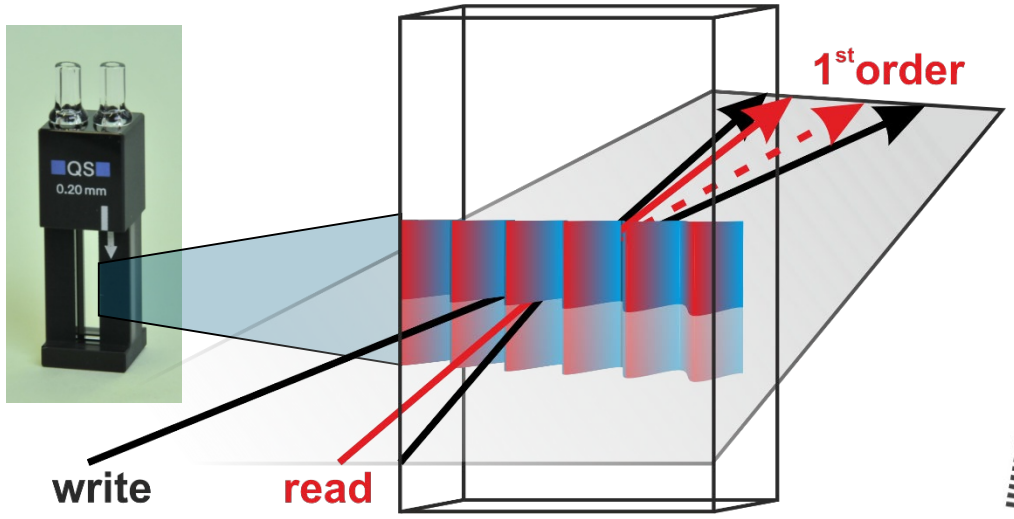
Andreas M. Stadler, Michael Marek Koza, and Jörg Fitter. *The Journal of Physical Chemistry B* **2015** *119* (1), 72-82

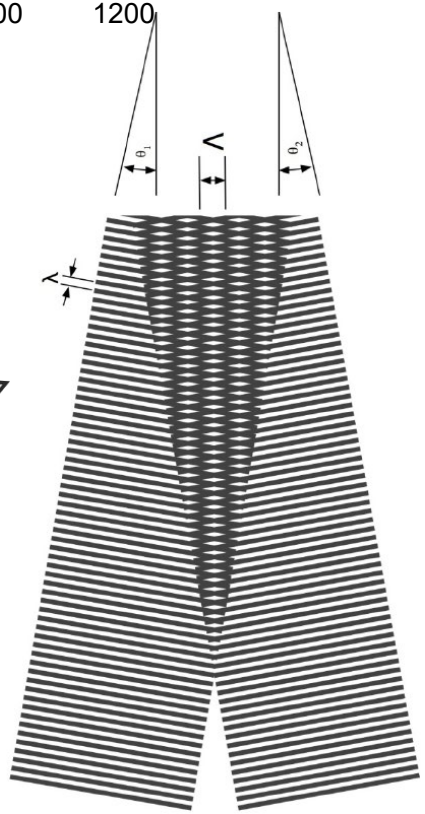
TDFRS: HOW DO WE MEASURE?

Refractive index grating

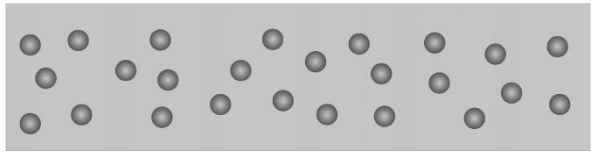


Measured quantity: intensity of diffracted reading beam

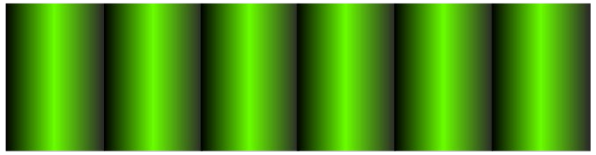




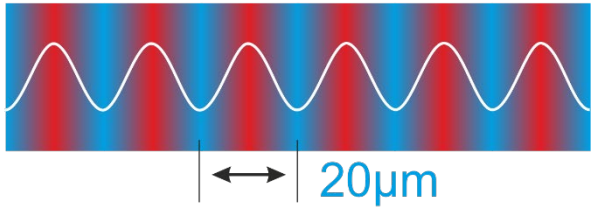
homogen



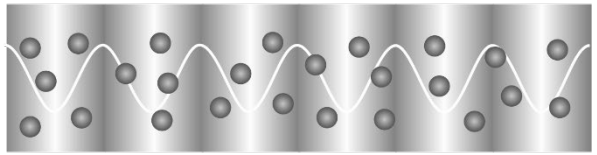
laser grating



temperature grating (20-100 µK)

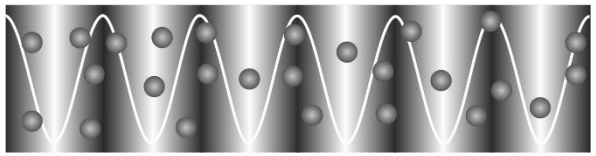


refractive index grating (temperature)



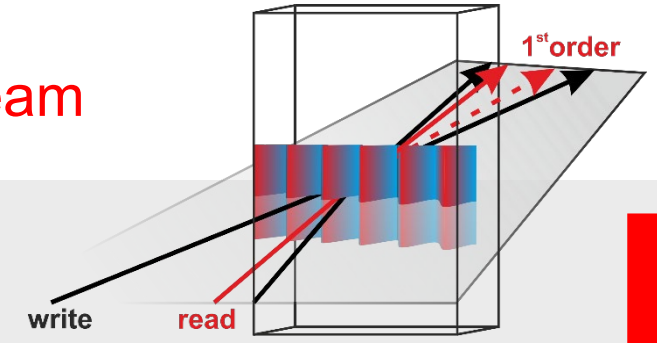
Thermodiffusion

refractive index grating (concentration)

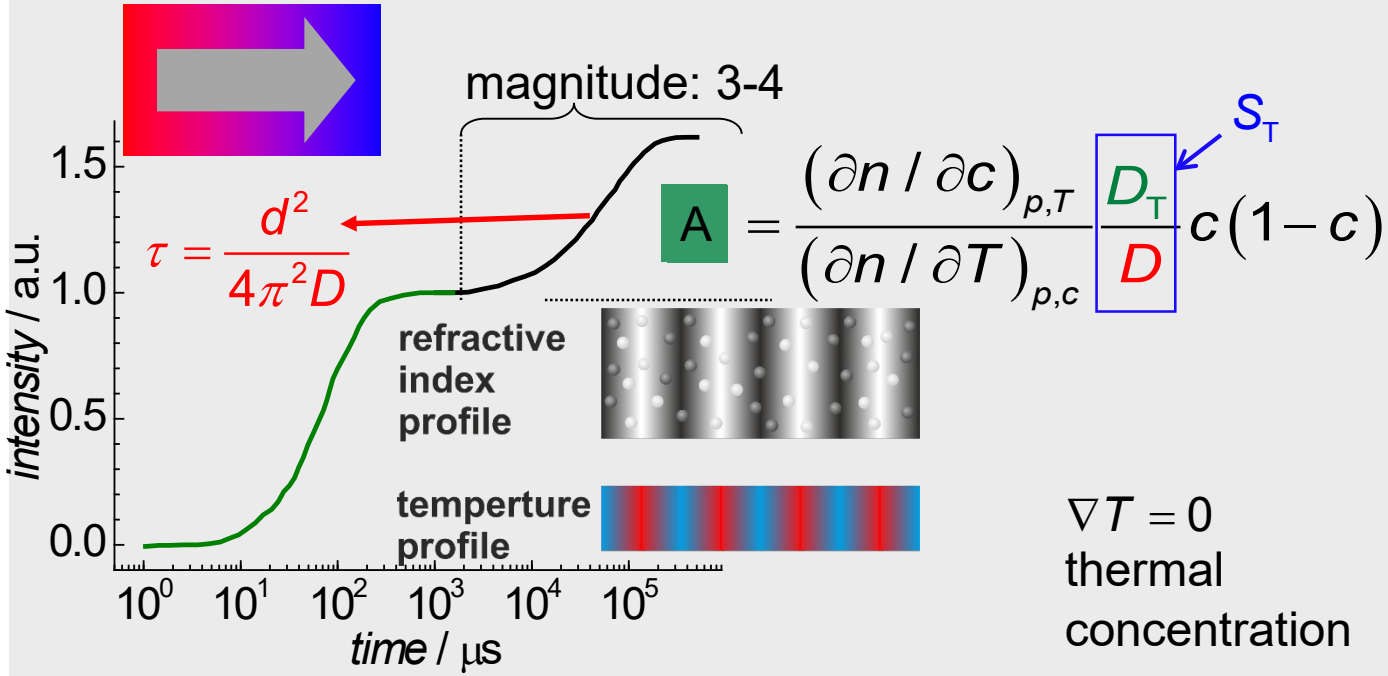


IR-TDFRS: MEASUREMENT SIGNAL

- Intensity of the read-out beam



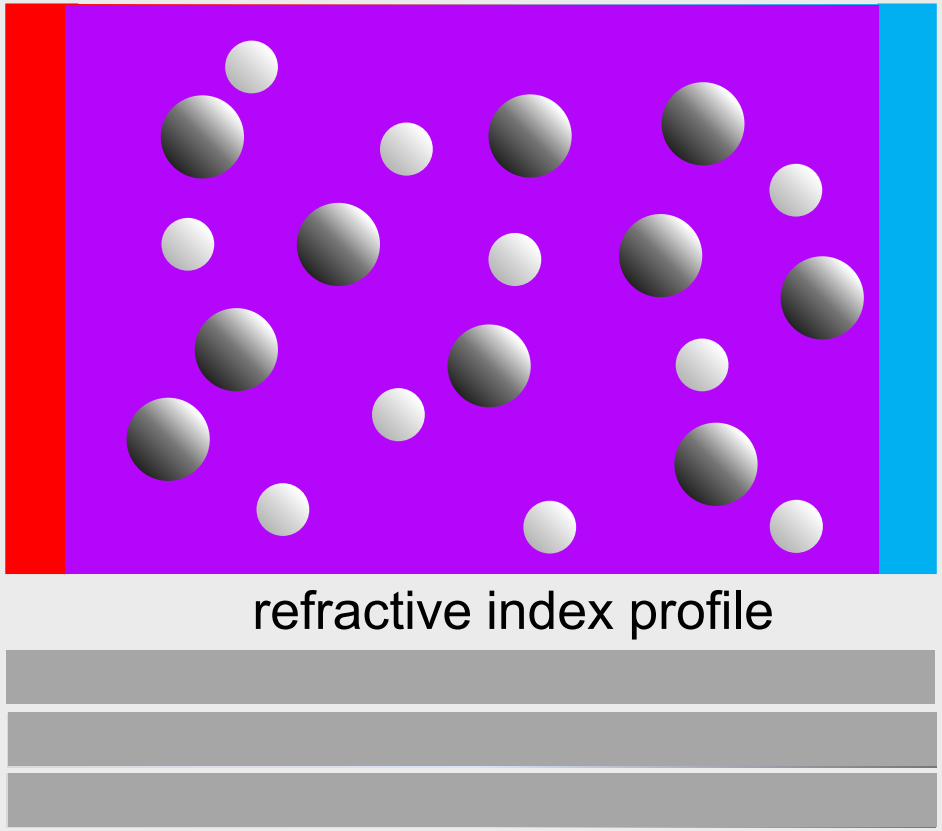
Molecules/colloids with higher refractive index move to cold side



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

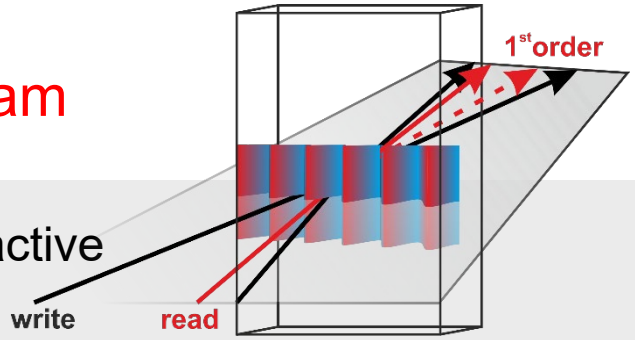
S_T

$\nabla T = 0$
thermal concentration

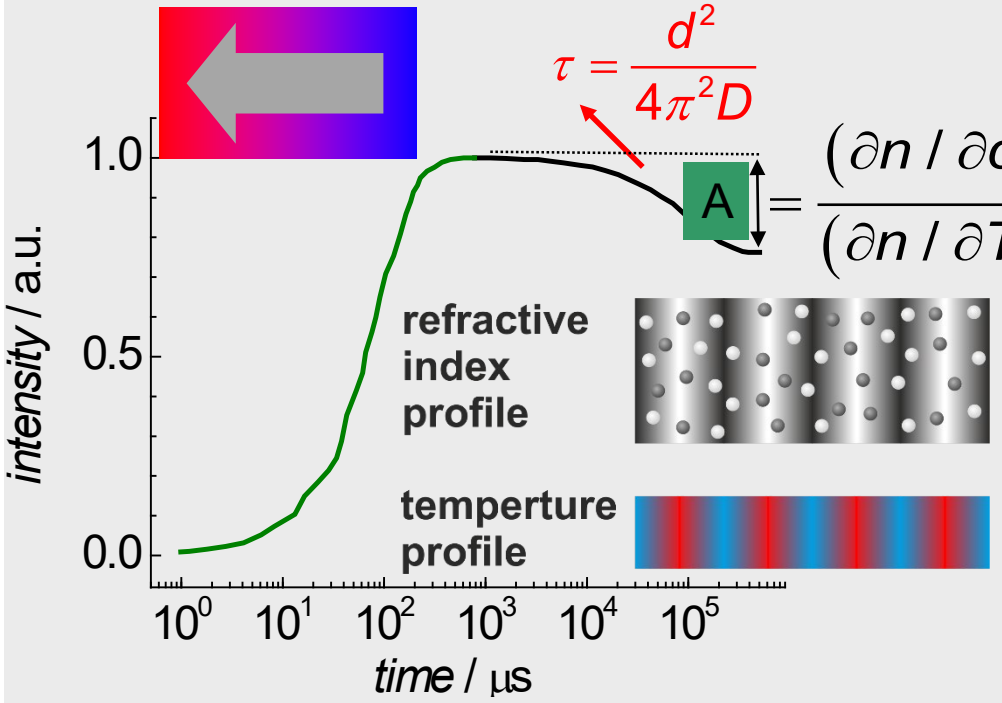


IR-TDFRS: MEASUREMENT SIGNAL

- Intensity of the read-out beam



Molecules/colloids with higher refractive index move to warm side

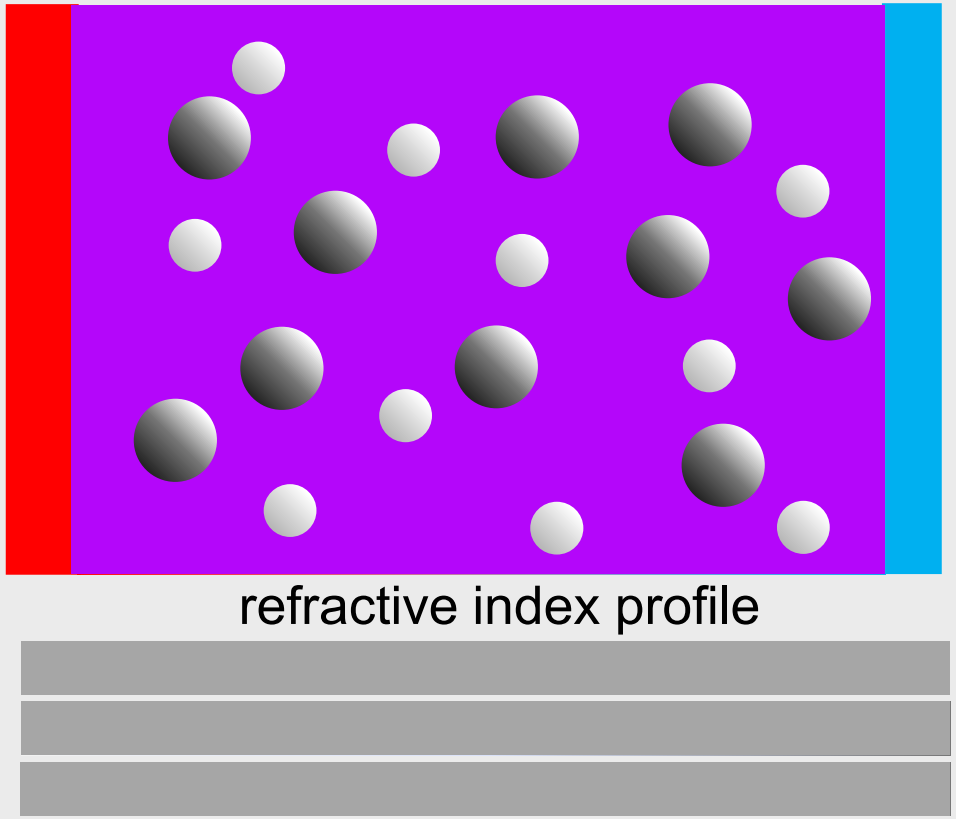


$$\tau = \frac{d^2}{4\pi^2 D}$$

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

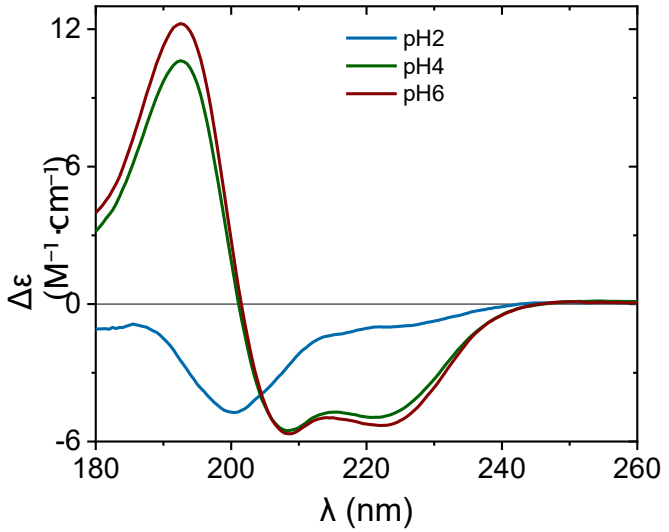
S_T

$\nabla T = 0$
thermal
concentration



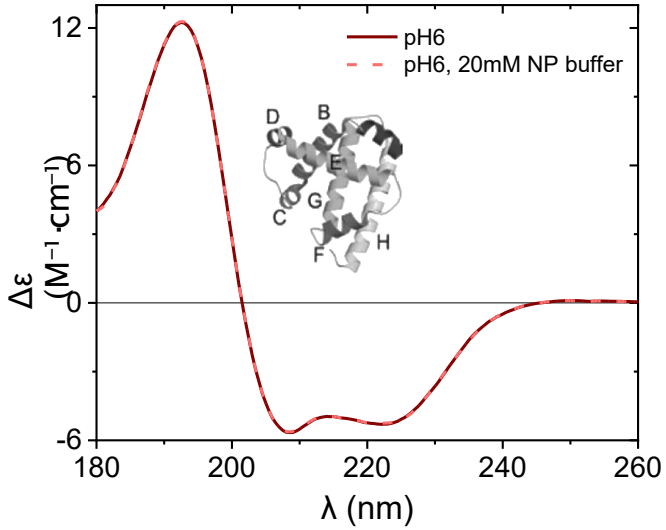
DISCUSSION OF TDFRS RESULTS IN STEPS

No buffer

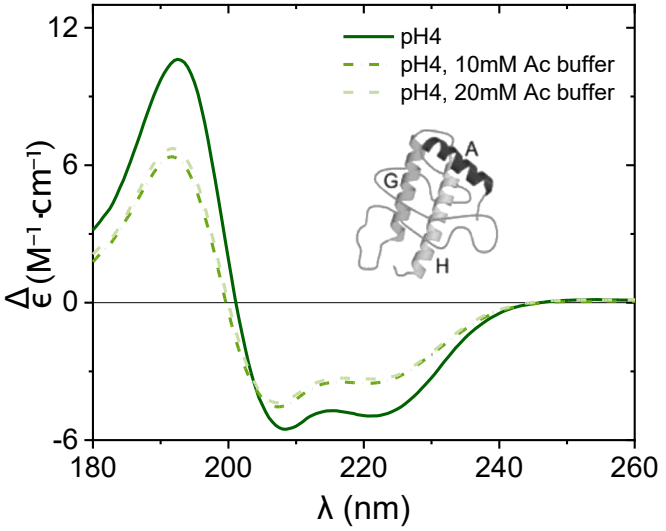


(1)

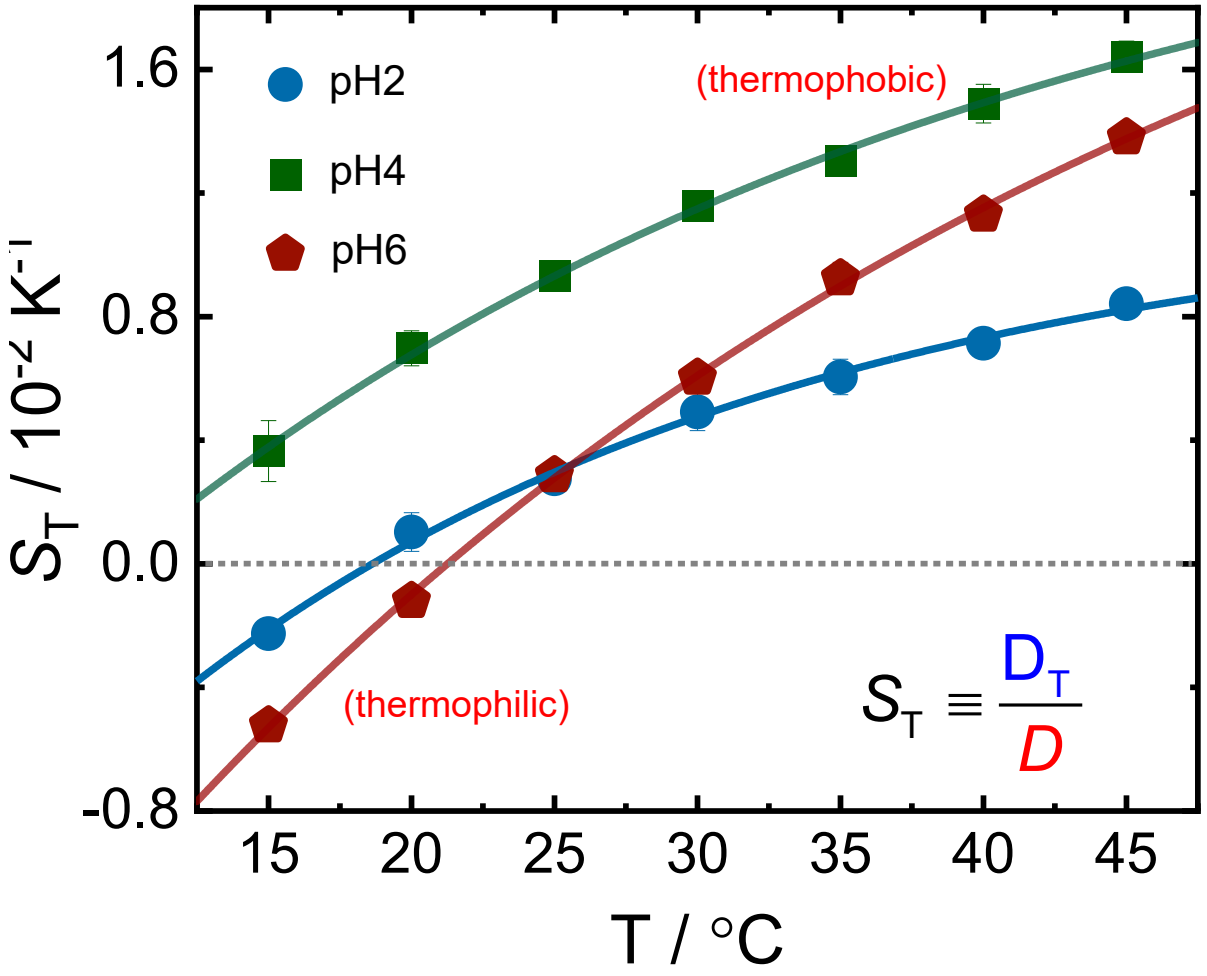
NaP buffer no influence on CD spectrum



Ac buffer influences CD spectrum



SORET COEFFICIENT (S_T) – NO BUFFER



- Sign change of S_T for pH2 and pH6
- Positive S_T for pH4

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

S. Iacopini, R. Rusconi, and R. Piazza, *Eur. Phys. J. E,* **19**, 59–67 (2006)

- **What can we learn from it?**

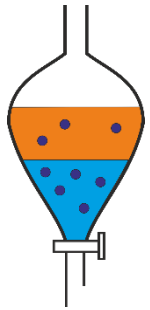
TEMPERATURE SENSITIVITY OF S_T CORRELATES WITH LOG *P*

For non-ionic solutes

ΔS_T is measure for temperature sensitivity

$$\Delta S_T = S_T(T_{high}) - S_T(T_{low})$$

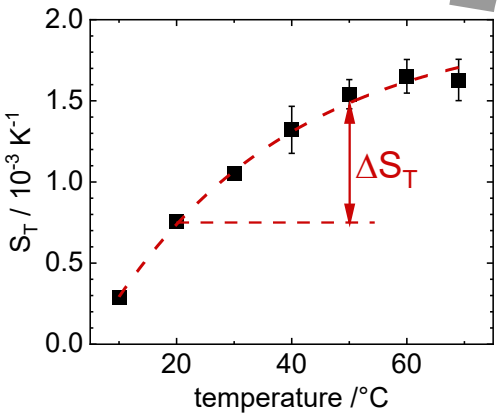
log *P* is a measure for hydrophilicity

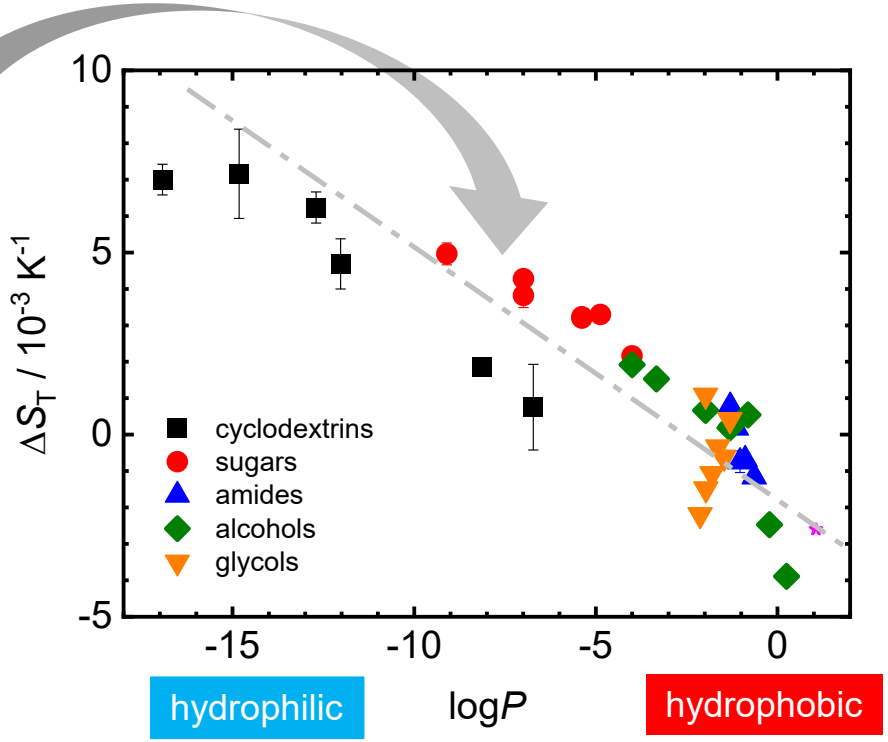


organic phase: c_{org}

water: c_{aq}

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





ΔS_T correlates with hydrophilicity (log *P*)

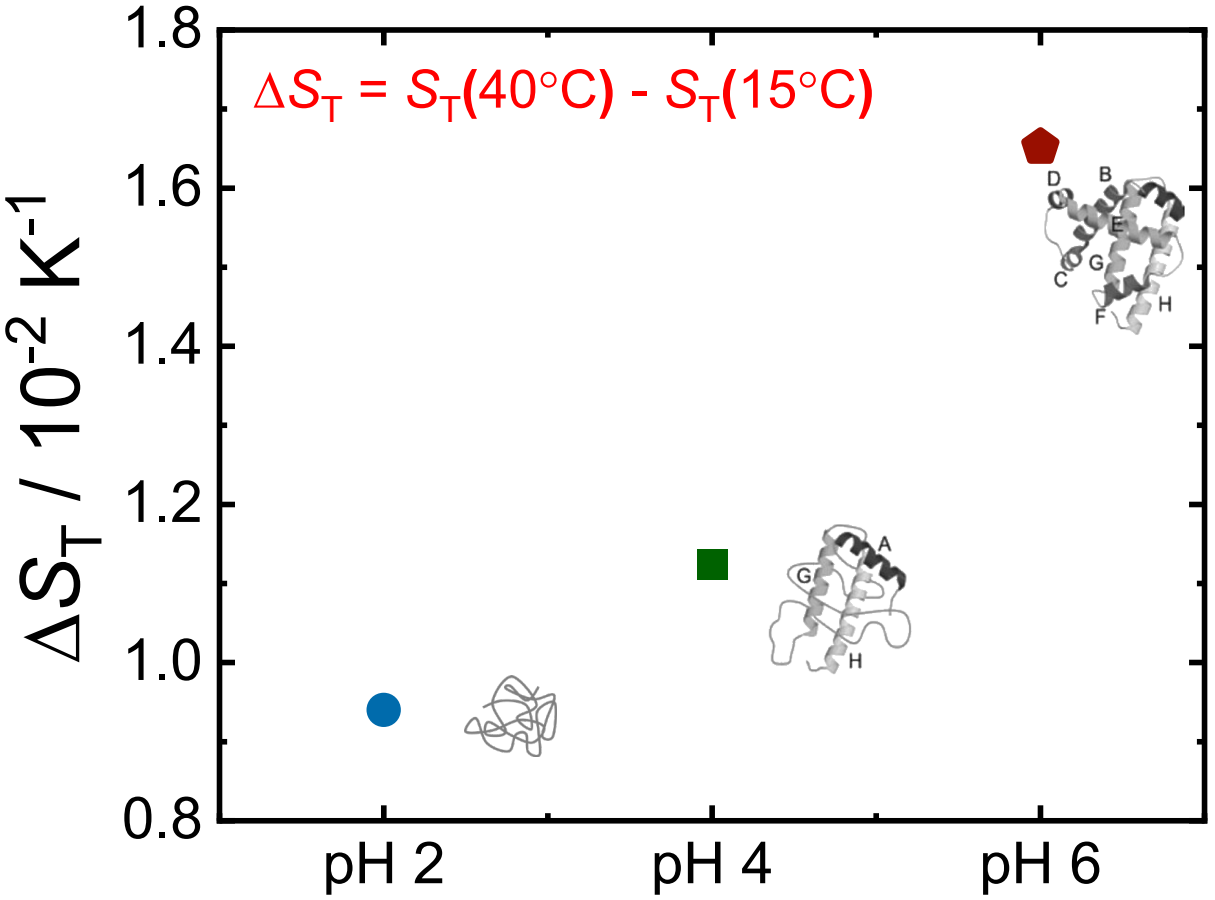
hydrophilicity and thermodiffusion are connected

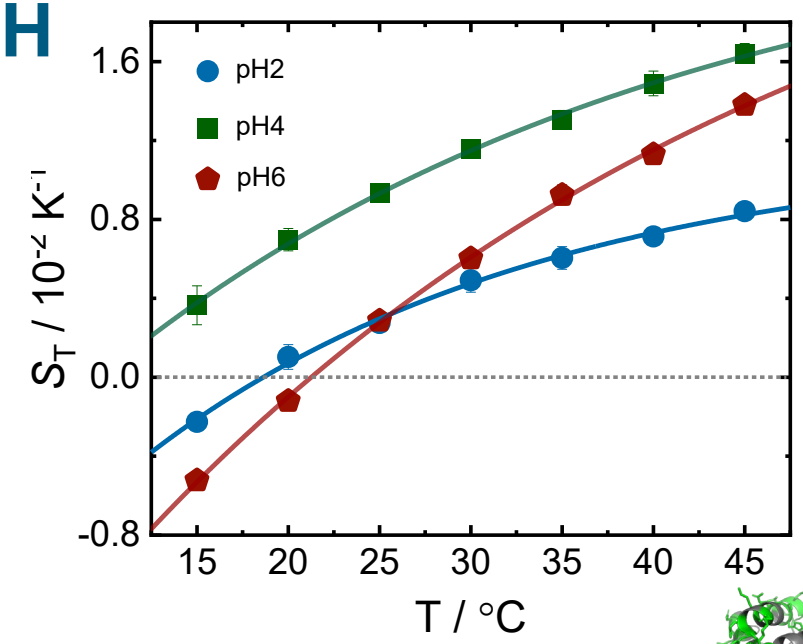
saccharides: P. Blanco et al., J. Phys. Chem. B (2010)
cyclodextrins: K. Eguchi et al., Eur. Phys. J. E (2016)
urea: D. Niether et al., PCCP. (2018)
formamide: D. Niether et al., PNAS (2016)

Log P works only for small molecules
For proteins, we need a different measure

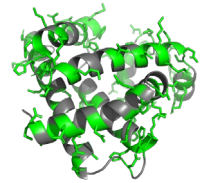
Temperature sensitivity of S_T at different pH

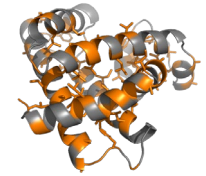
No buffer





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





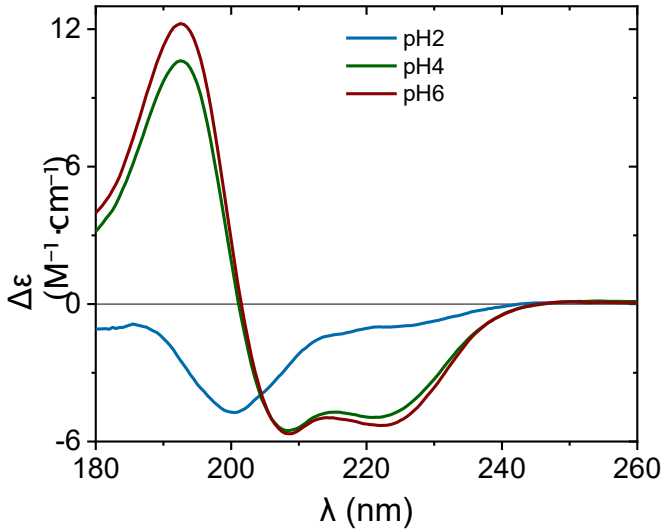
- FOLDED state @ pH 6: **ratio=2.7**
- UNFOLDED state @ pH2:**ratio=1.2**

The folded protein is more hydrophilic than the unfolded

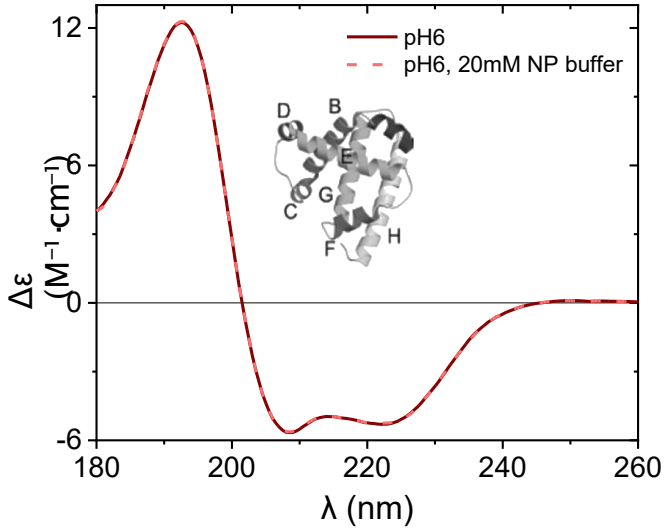
(Calculation using PyMOL)

DISCUSSION OF TDFRS RESULTS IN STEPS

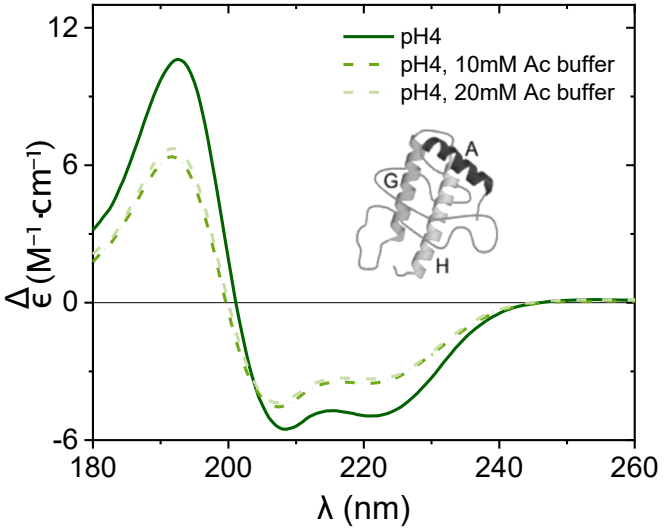
No buffer



NaP buffer no influence on CD spectrum

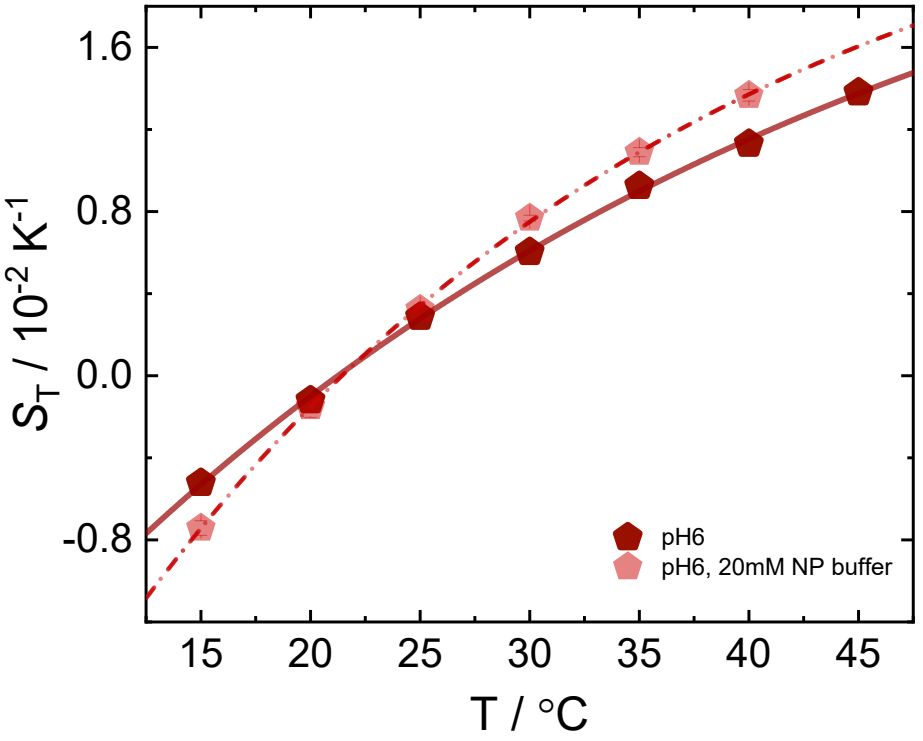


Ac buffer influences CD spectrum

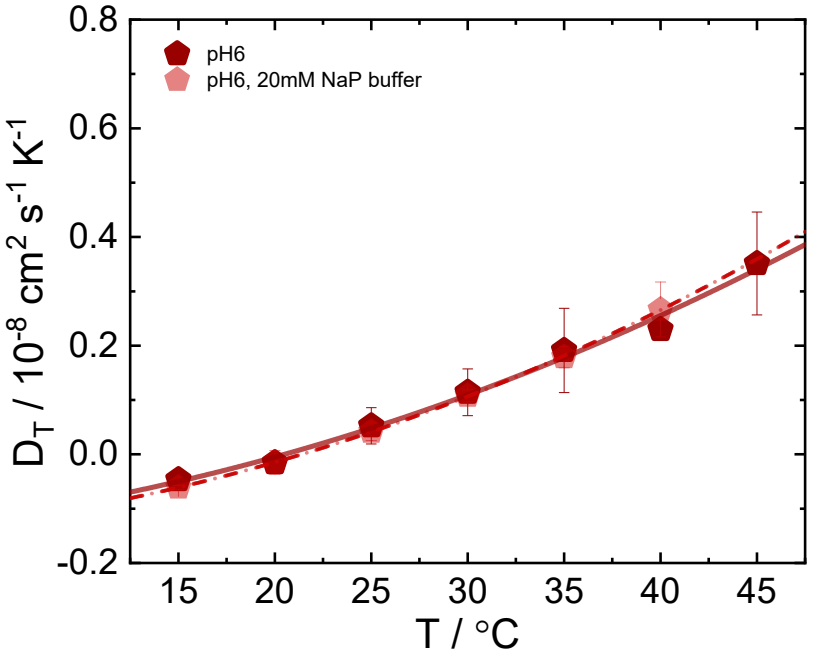


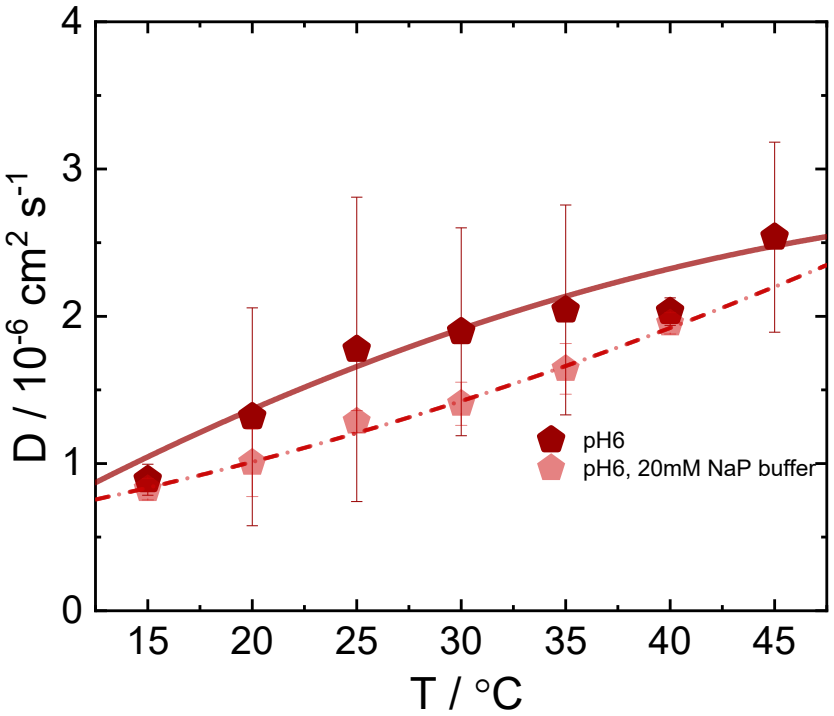
(2)

Comparison of S_T at pH6



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





- pH6: Use of buffer does not change D_T
- But leads to stronger aggregation, reflected also in a lower diffusion coefficient

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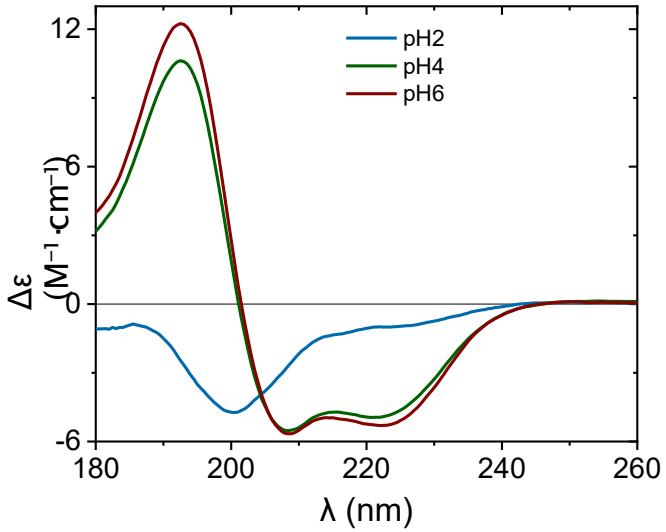
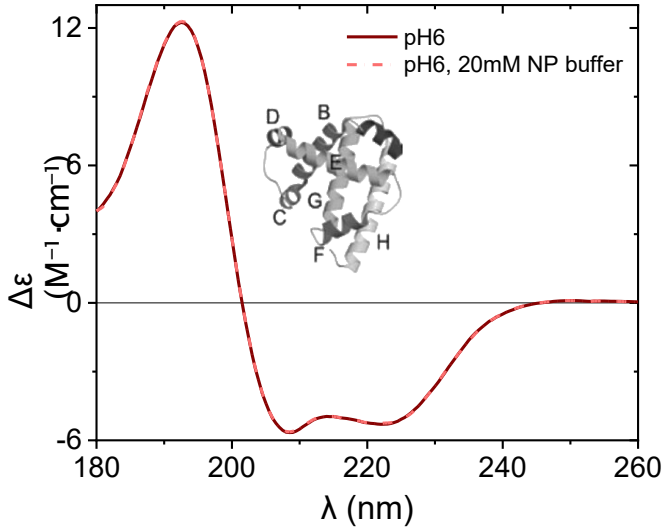
DISCUSSION OF TDFRS RESULTS IN STEPS

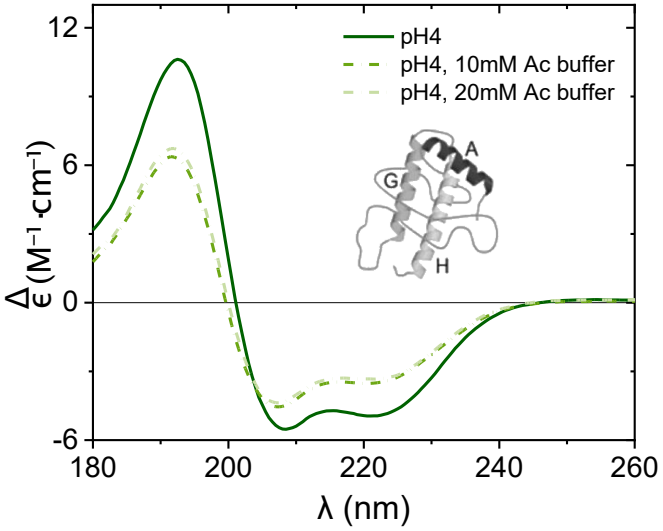
No buffer



NaP buffer no influence on CD spectrum

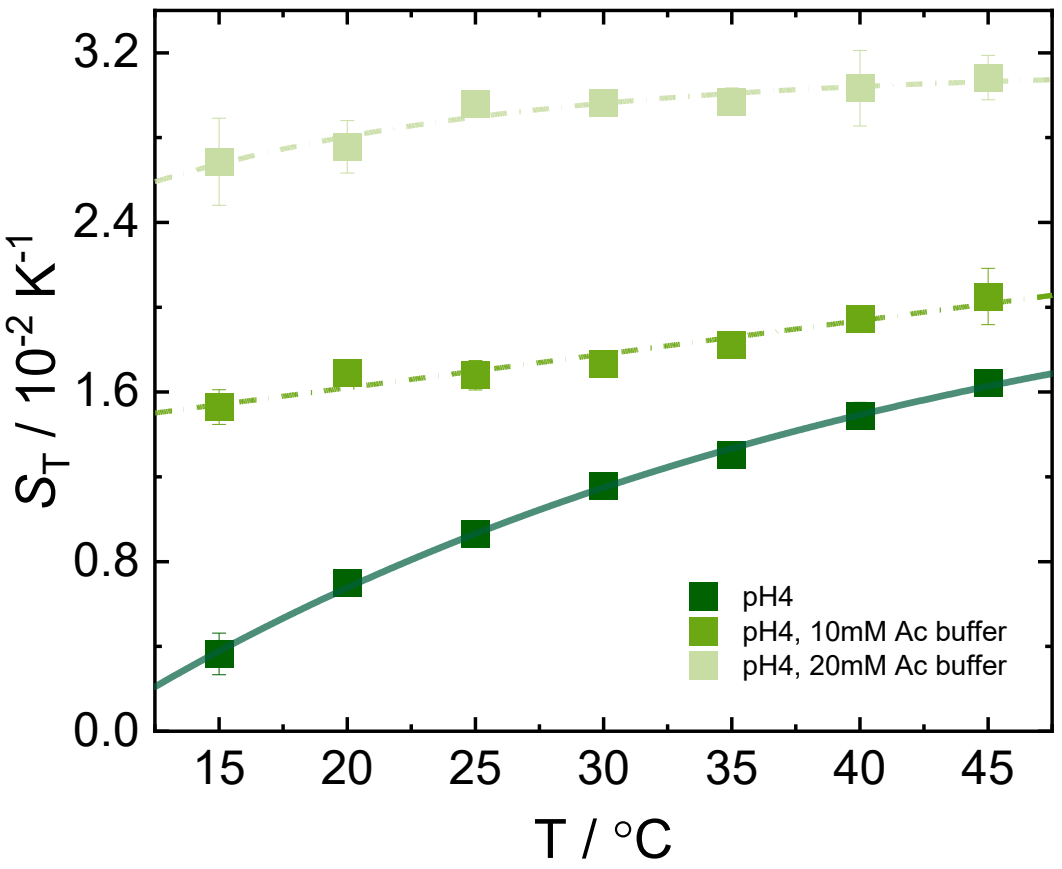


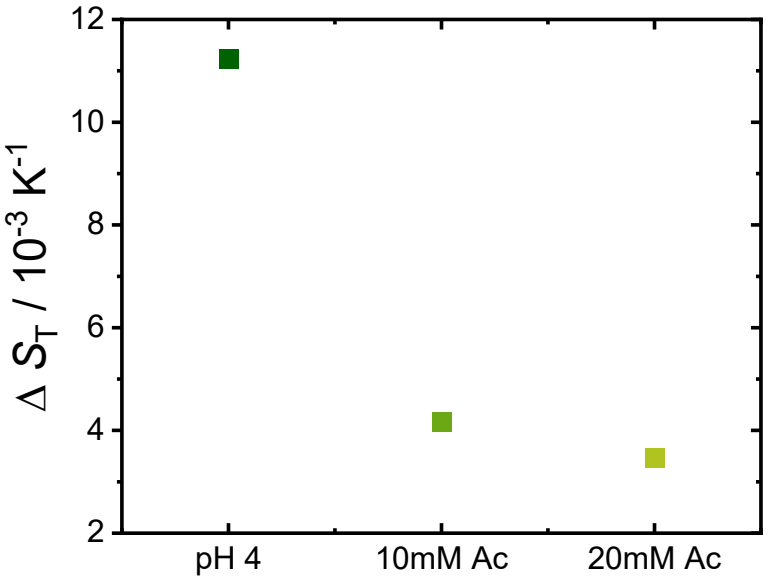
Ac buffer influences CD spectrum



(3)

Comparison of S_T at pH4

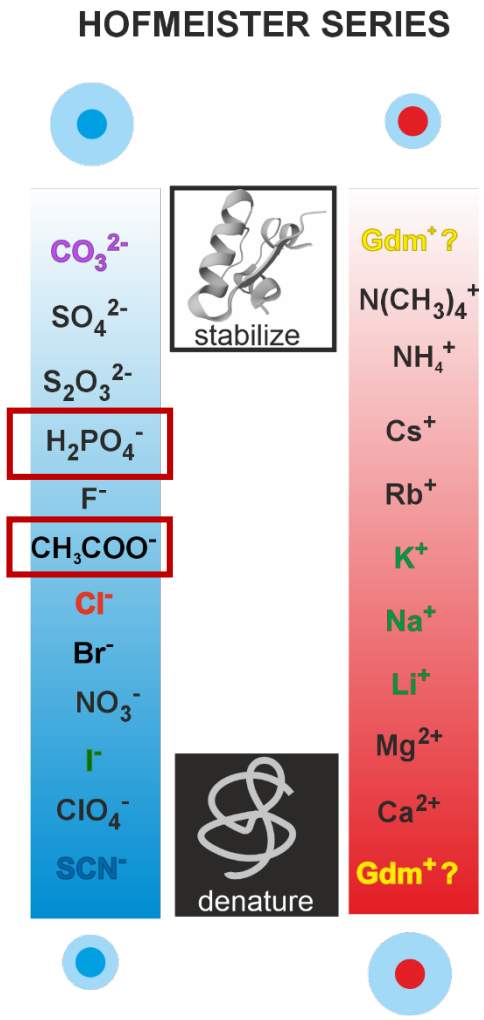




Acetate buffer reduces the temperature sensitivity of S_T of the protein

Can we understand this?

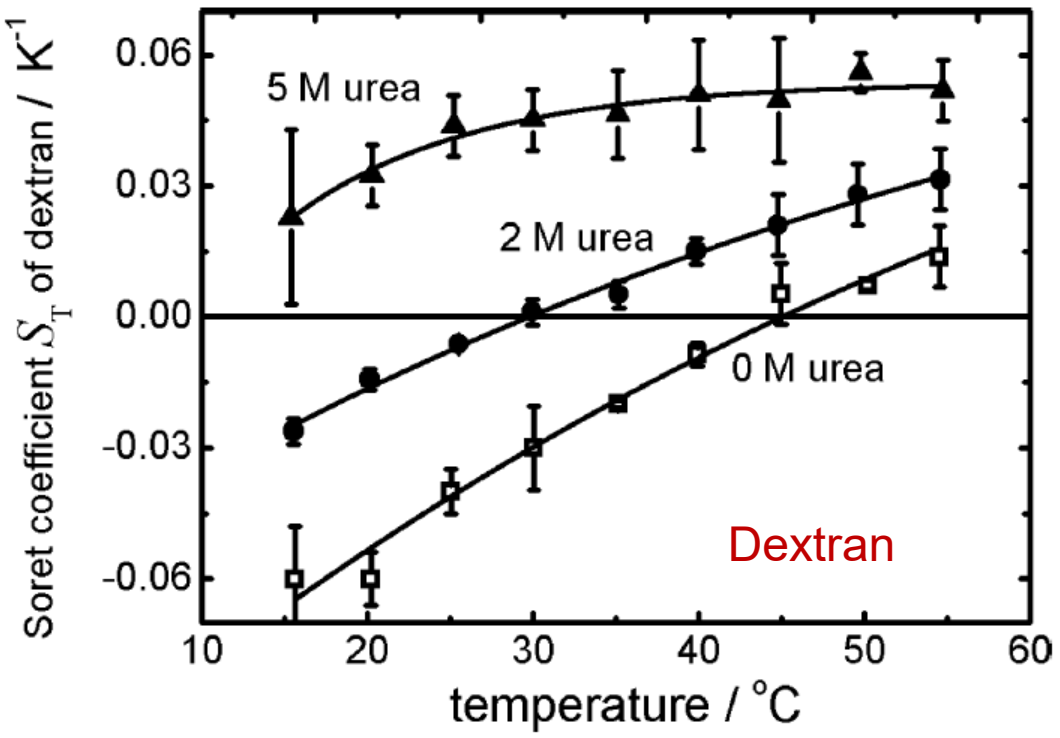
Phosphate vs Acetate buffer



G.V. Barnett et al. *Journal of Pharmaceutical Sciences* **2016**, 105,1066e1073

COMPARISON OF S_T WITH DEXTRAN-UREA

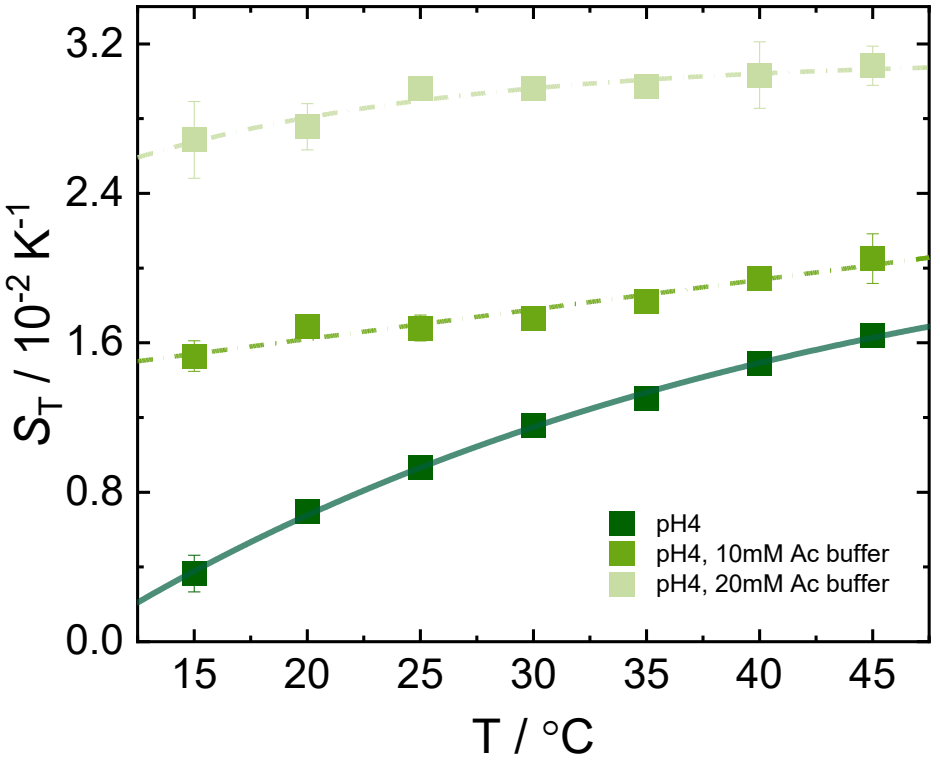
Non-ionic polymer: dextran



urea = water structure breaker

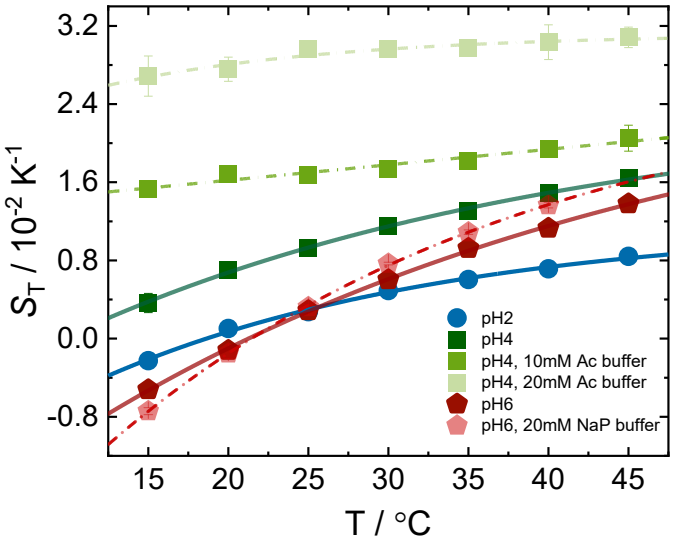
R. Sugaya, B. A. Wolf, and R. Kita. Biomacromolecules, **7** (2006) 435

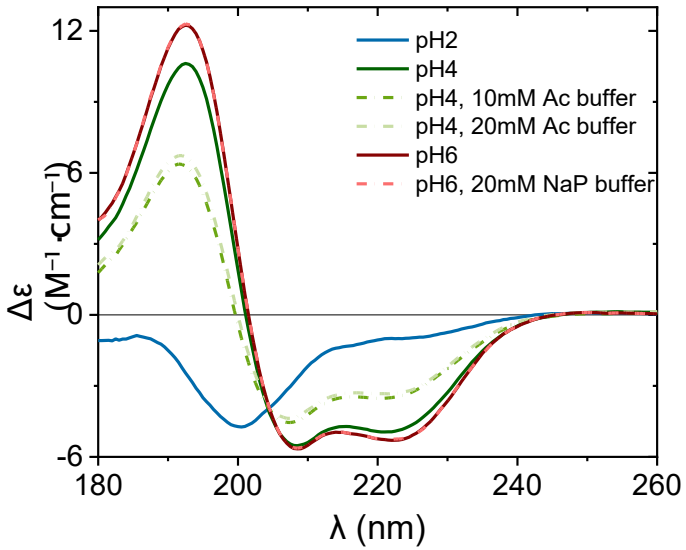
Charged protein in a molten globule state

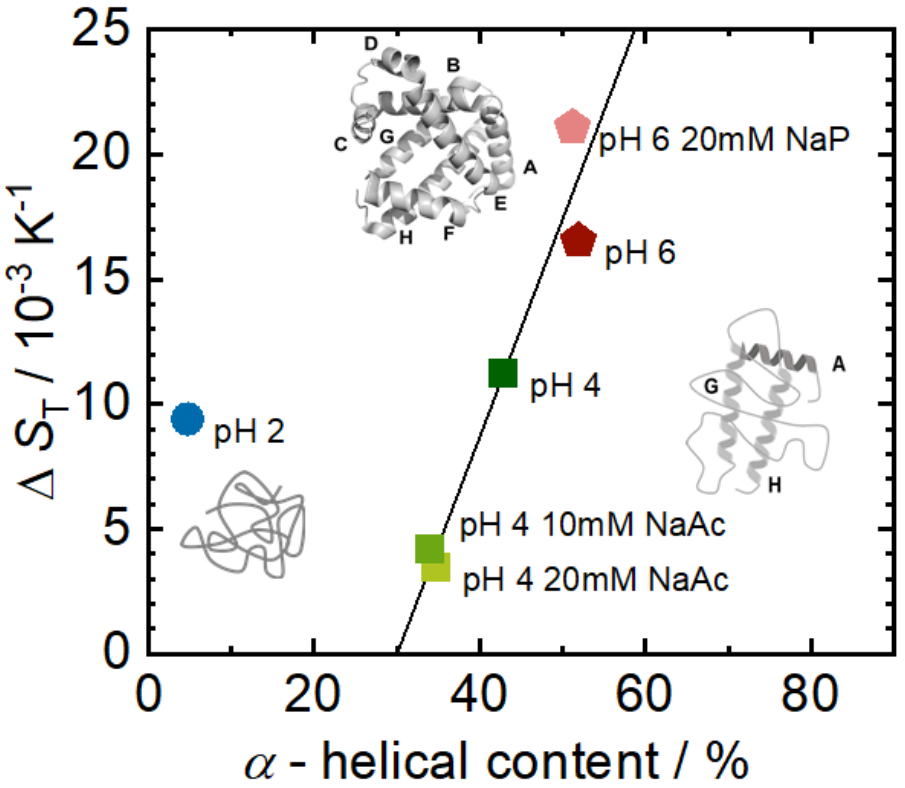


Increasing the concentration of hydrophilic compound – Promotes disruption of hydrogen bonds

Correlation between Circular Dichroism and TDFRS







For Apo-Mb the α-helical content is a good measure for hydrophilicity

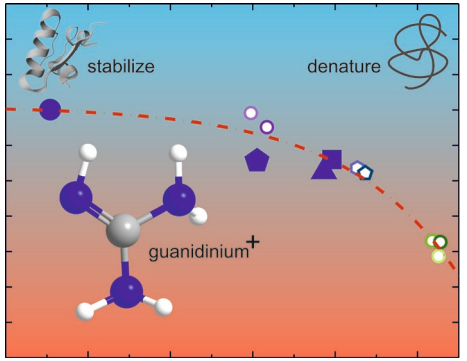
α-helical content ∝ hydrophilicity and temperature sensitivity of S_T

CONCLUSION

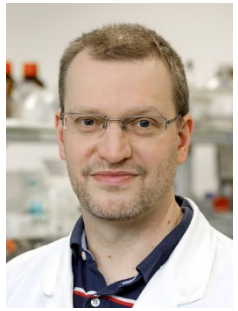
- α-helical content and ΔS_T follow the trend : **pH6 > pH4 > pH2**
- Hydrophilicity (**pH6 > pH4 > pH2**) and temperature sensitivity of S_T (ΔS_T **(pH6)** > ΔS_T **(pH4)** > ΔS_T **(pH2)**) decrease with pH:
- Phosphate buffer leads to stronger aggregation at pH6, reflected in a lower diffusion coefficient. Acetate buffer promotes solubilization of protein.
- Temperature sensitivity of S_T decreases with increase in concentration of acetate buffer at pH4.
- We observe a correlation between ΔS_T and the α-helical content.

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.]
- Interpreting the results and writing a manuscript :
  Thermophoresis: The case of Apomyoglobin
- 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

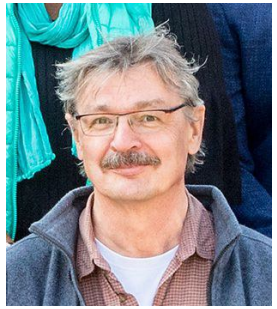


Andreas Stadler (protein, JCNS-1)

Johan Buitenhuis (CD-measure-ments)

Holger Gohlke

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

JÜLICH
Forschungszentrum

THANK YOU