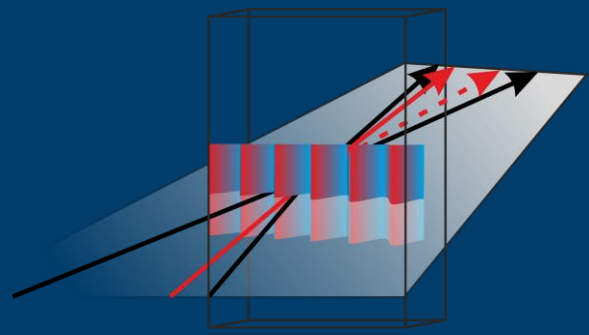
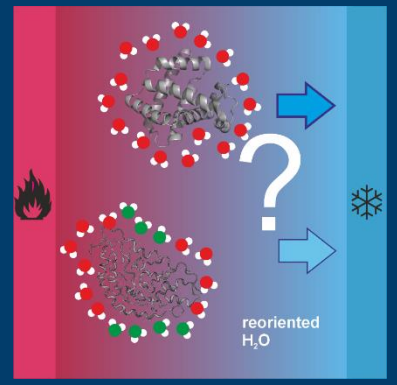
INFLUENCE OF ALPHA-HELICAL CONTENT ON THE THERMODIFFUSION OF APOMYOGLOBIN

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





23.06.2026 I BINNY RUDANI

B. A. Rudani et al., Langmuir 41, 28322 (2025).

JÜLICH
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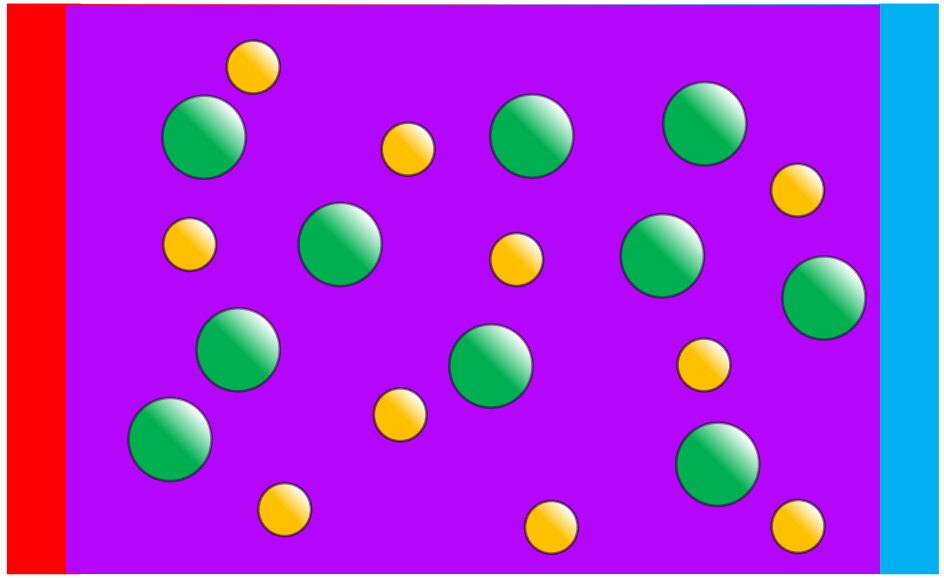
THERMODIFFUSION / THERMOPHORESIS

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



Steady state defines Soret coefficient S_T.

$$S_T \equiv \frac{D_T}{D} \propto \frac{\Delta c}{\Delta T}$$

D diffusion coefficient

D_T thermal diffusion coefficient

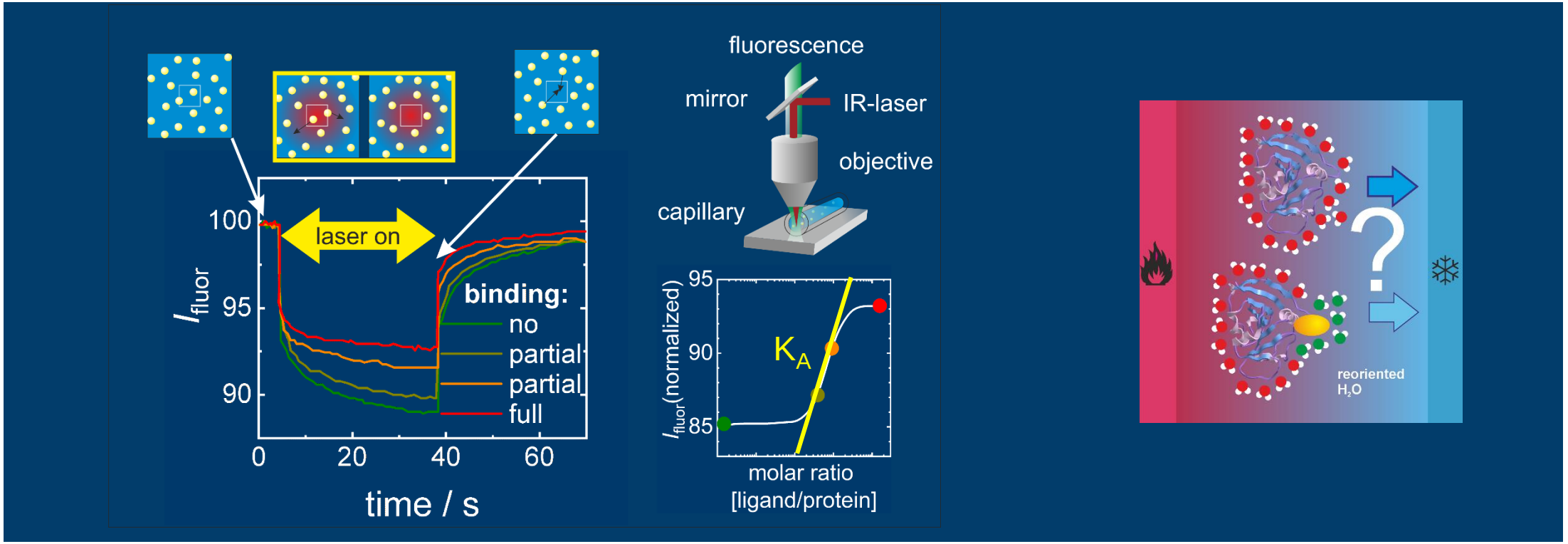
$S_T = 10^{-3} K^{-1}$ – $1 K^{-1}$

molecules colloids

THERMOPHORESIS

Microscale thermophoresis (MST) determines the binding constant K_A

- Monitors protein-ligand interaction and conformational changes



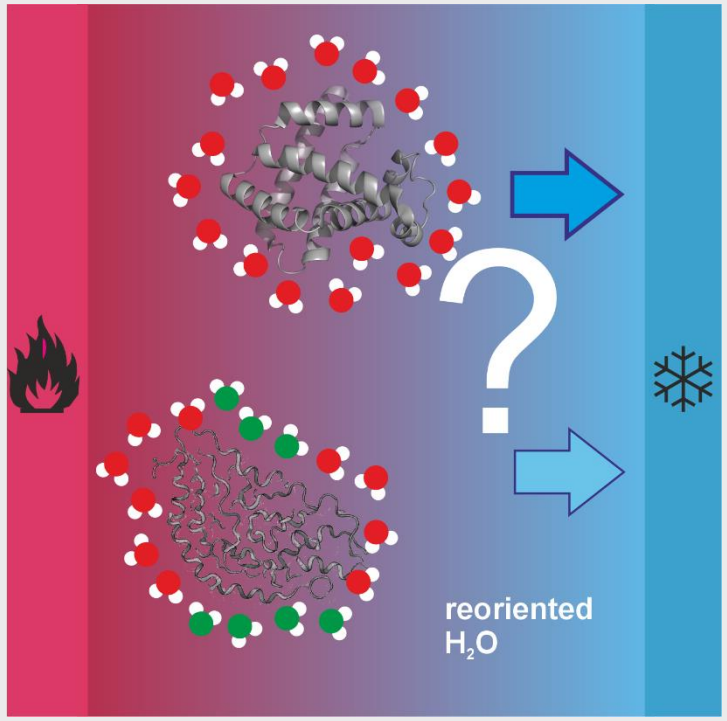
D. Niether, S. Wiegand *J. Phys. Condens. Matter* 31 (2019) 503003.

OBJECTIVE

Can we quantify the relation between thermodiffusion and hydration

folded

unfolded



Conformation change

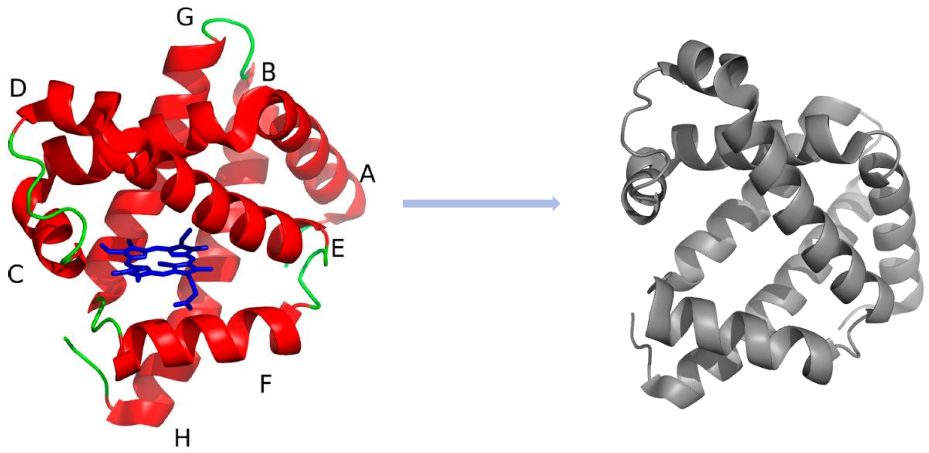
Hypothesis:

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

D. Niether, S. Wiegand *J. Phys. Condens. Matter* 31 (2019) 503003.

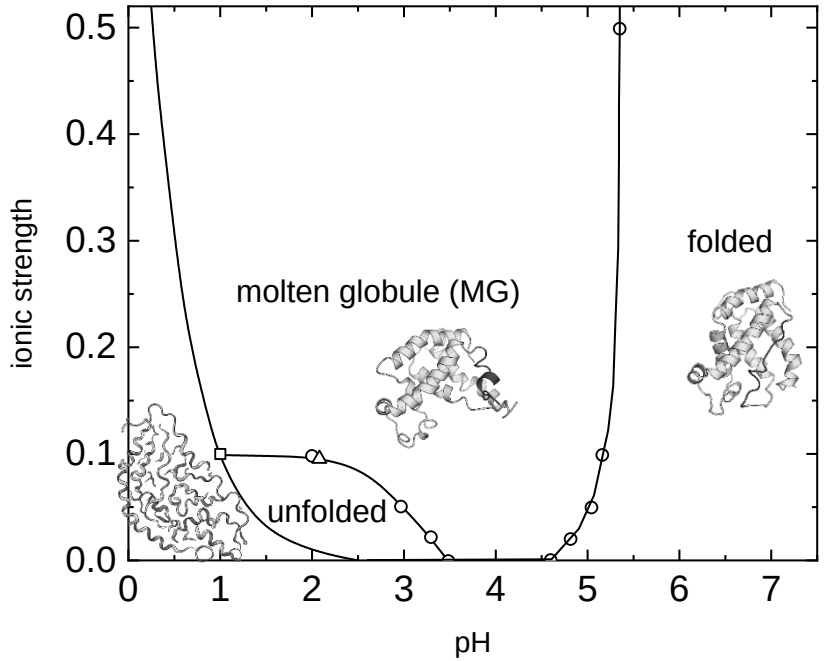
APOMYGLOBIN

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



Myoglobin (red colored)

Apo-myoglobin (transparent)

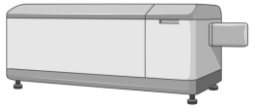


Y. Goto and A. L. Fink. *J. Mol. Biol.*. 214 (**1990)**, 803.

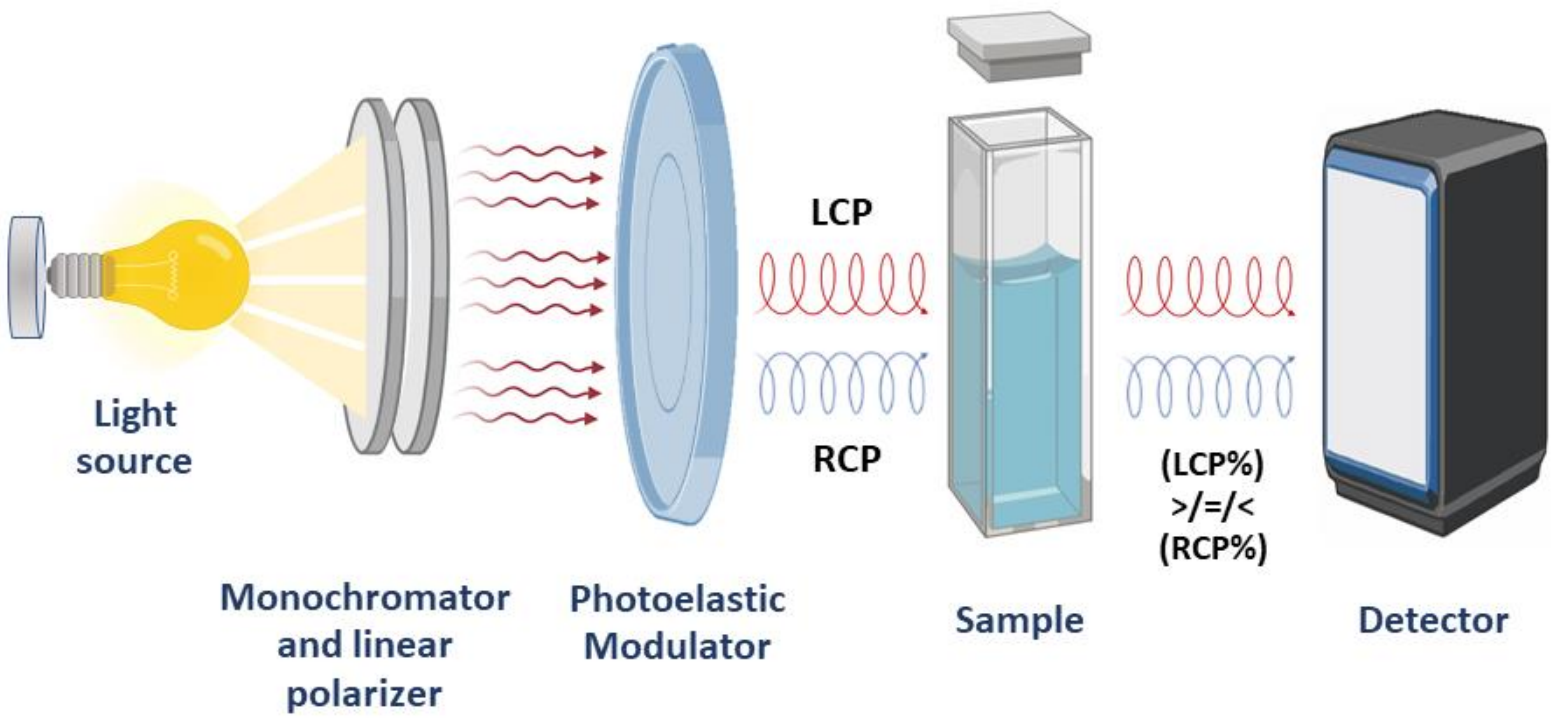
Charge increases, protein unfolds

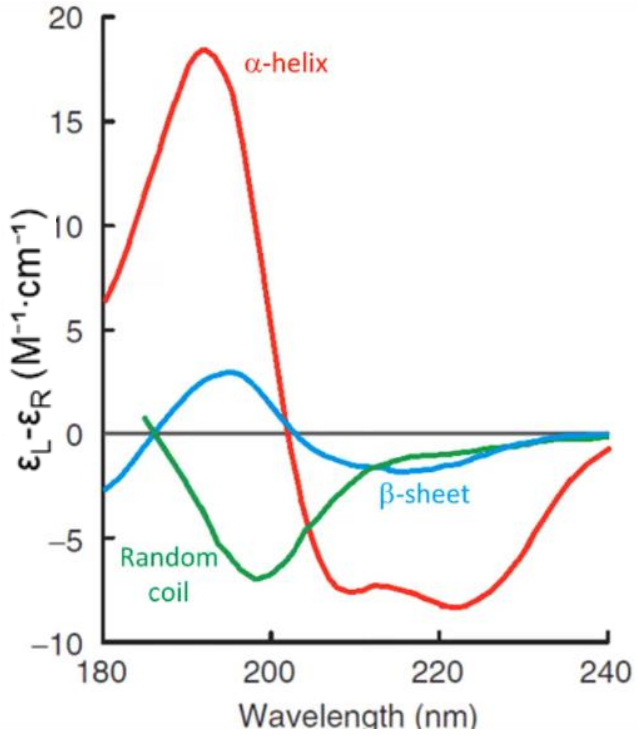
A. M. Stadler et al. *J. Phy. Chem. B 119 (*2015) 72.

CIRCULAR DICHROISM



Principle





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

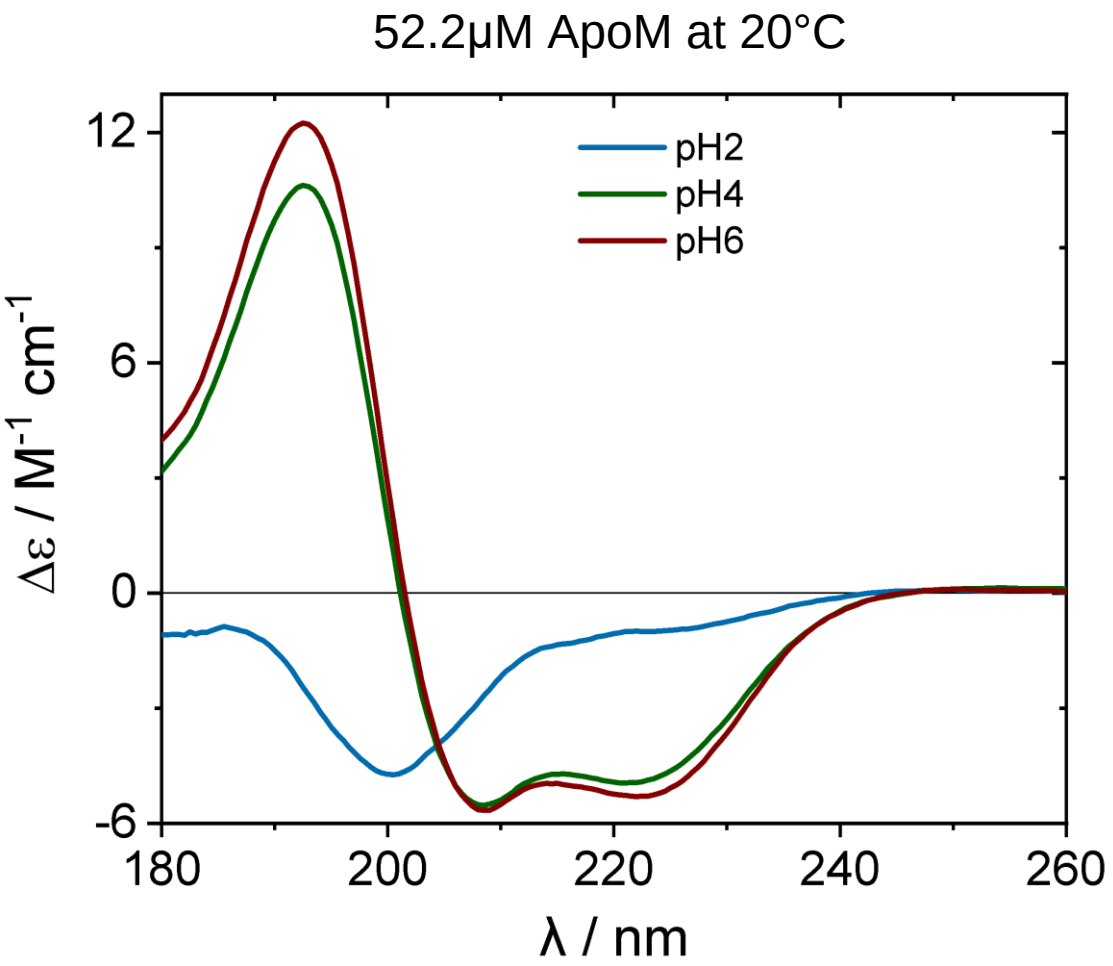
Beer–Lambert law, $\Delta A = (\varepsilon_L - \varepsilon_R)cl$

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

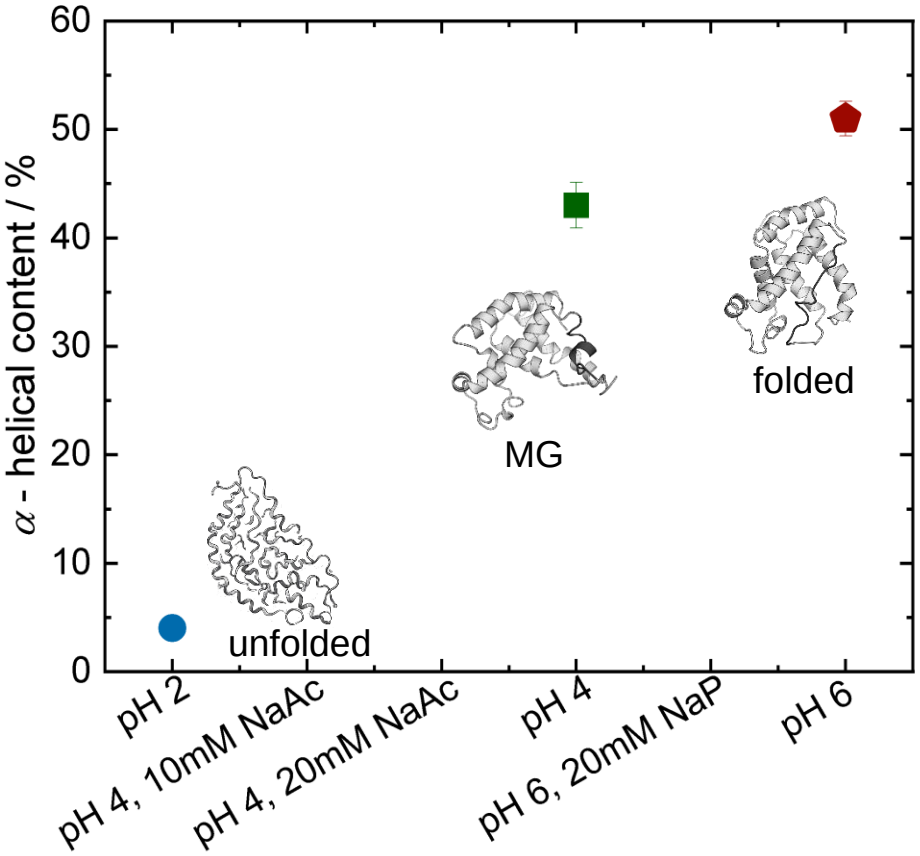
Subadini et. al.. In: Sahoo, H. (eds) *Optical Spectroscopic and Microscopic Techniques*. Springer, Singapore (2022).

CIRCULAR DICHROISM

Results



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

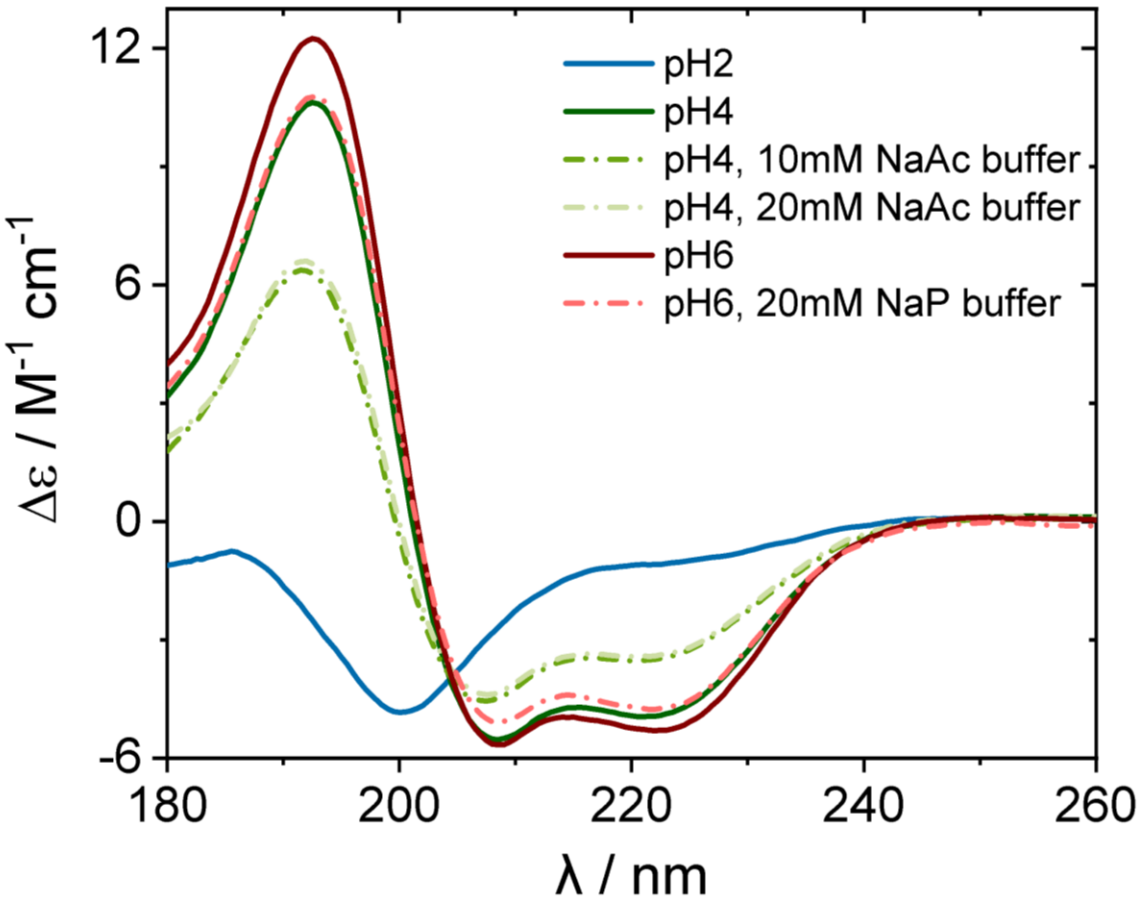


B. A. Rudani et al., Langmuir 41, 28322 (2025).
A. M. Stadler et al. *J. Phy. Chem. B 119 (*2015) 72.

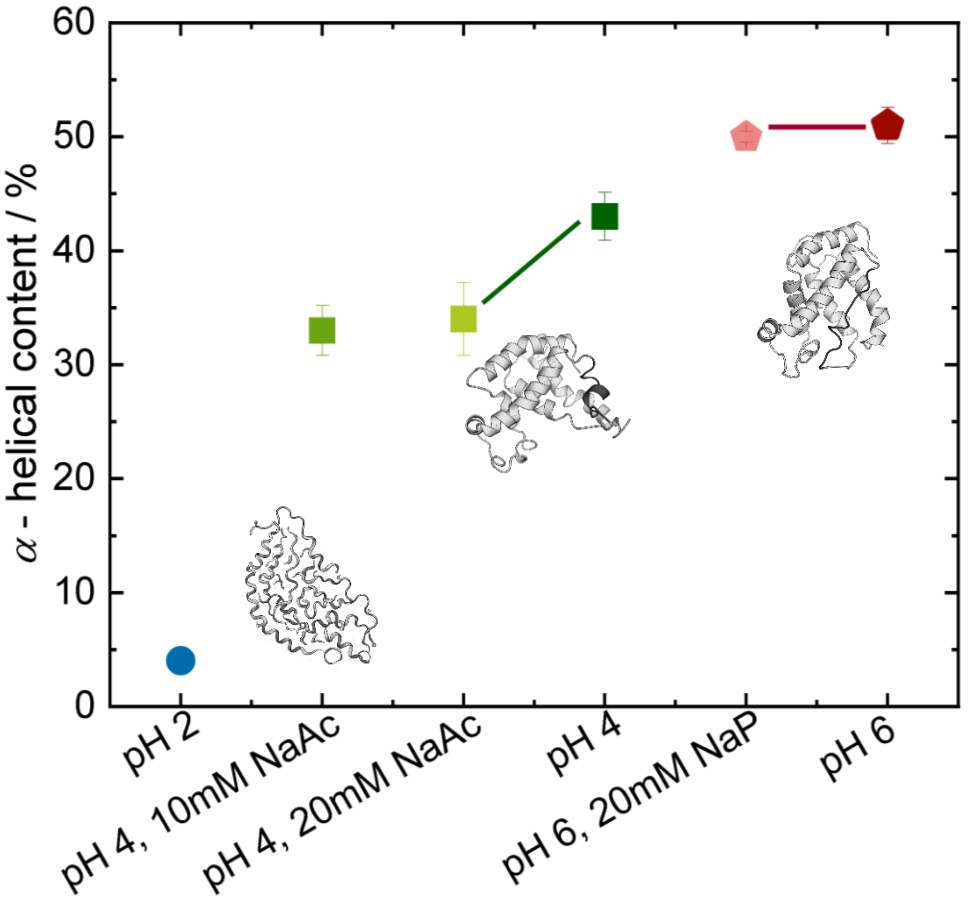
CIRCULAR DICHROISM

Results

52.2µM ApoM at 20°C



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

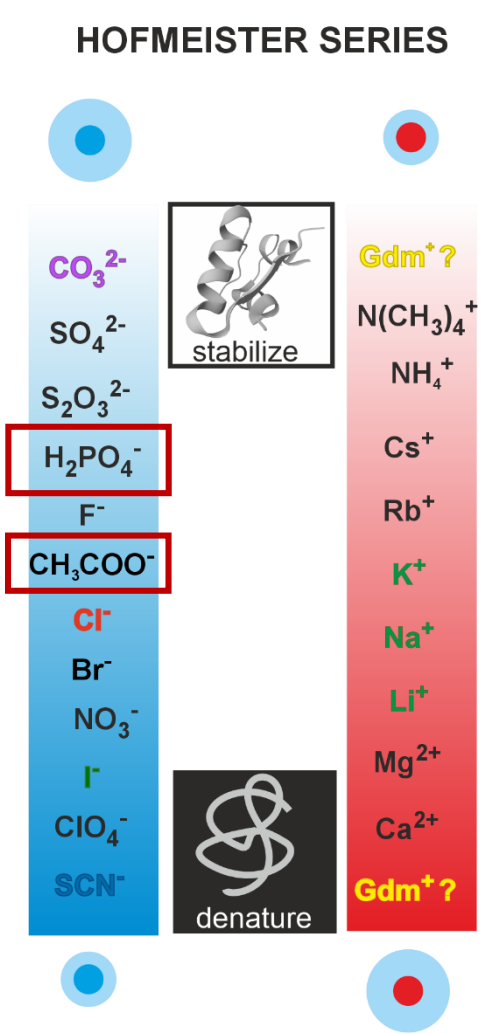


- **pH6**: same α-helical content with and without buffer
- **pH4:** buffer graph deviates significantly from normal acidic solution
- **pH2:** minimum α-helical content

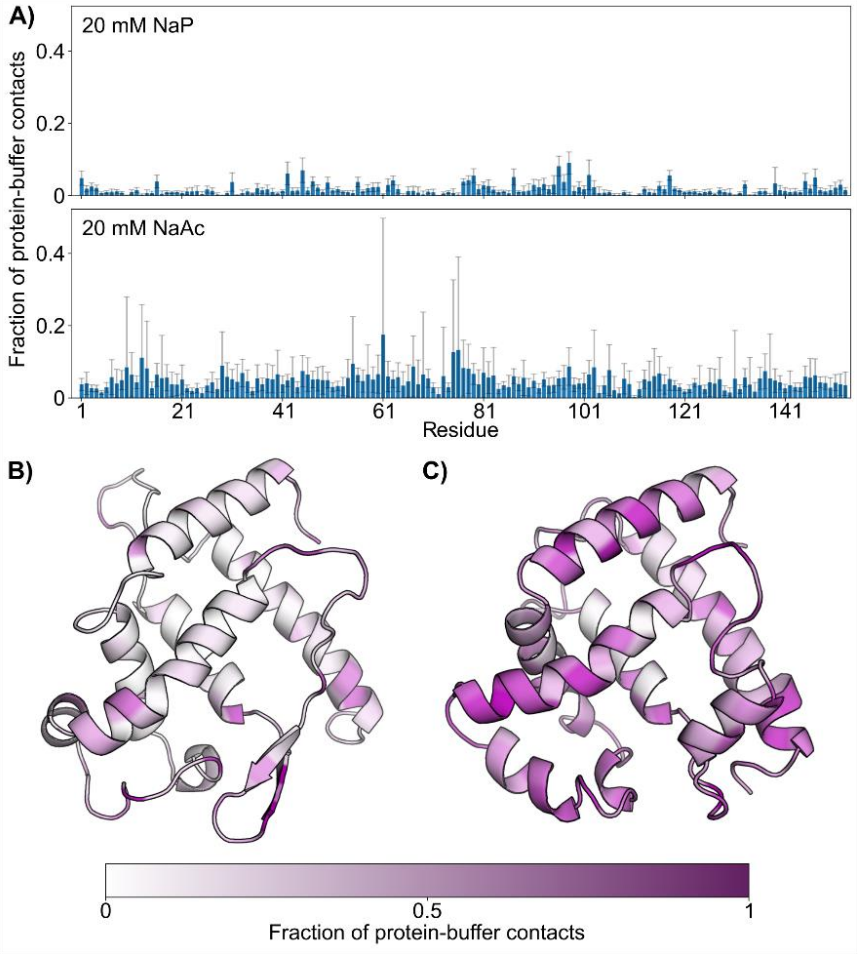
B. A. Rudani et al., Langmuir 41, 28322 (2025).
A. M. Stadler et al. *J. Phy. Chem. B 119 (*2015) 72.

PHOSPHATE VS ACETATE BUFFER

(Simulation – IBG-4)



B. A. Rudani et al. JCP **160**, 214502 (2024).



20 mM NaP **20 mM NaAc**

B. A. Rudani et al., Langmuir 41, 28322 (2025).

Steffen Docter

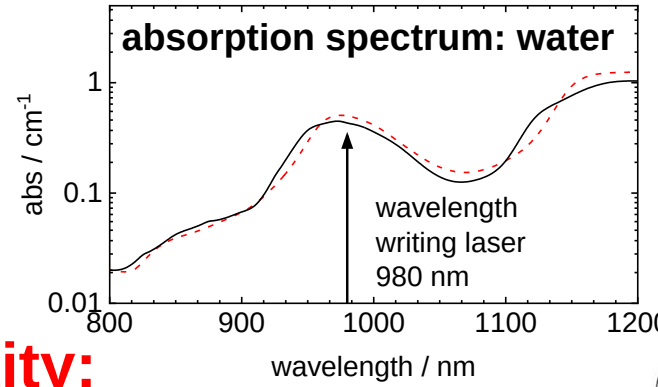
Stephan Schott-Verdugo

Holger Gohlke

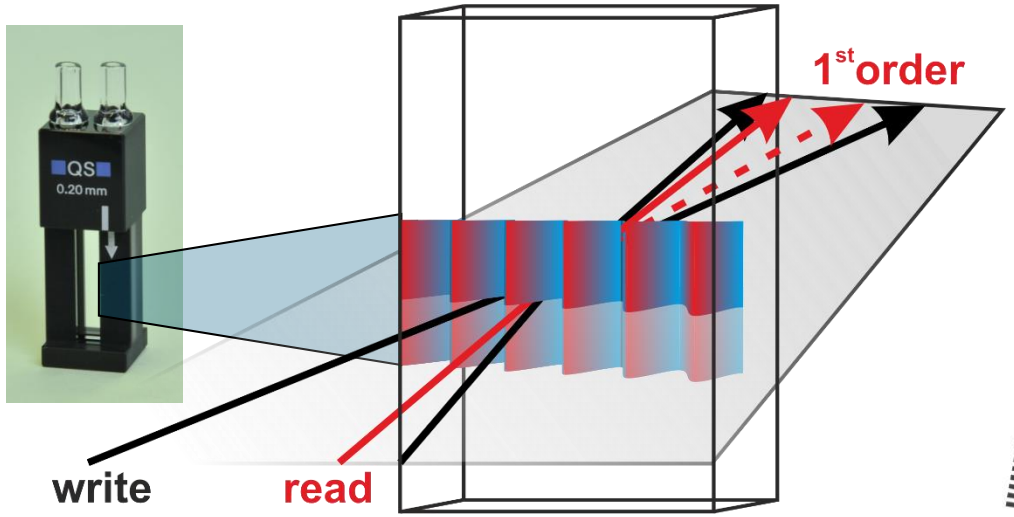
Acetate buffer interaction with protein is high in comparison to phosphate buffer

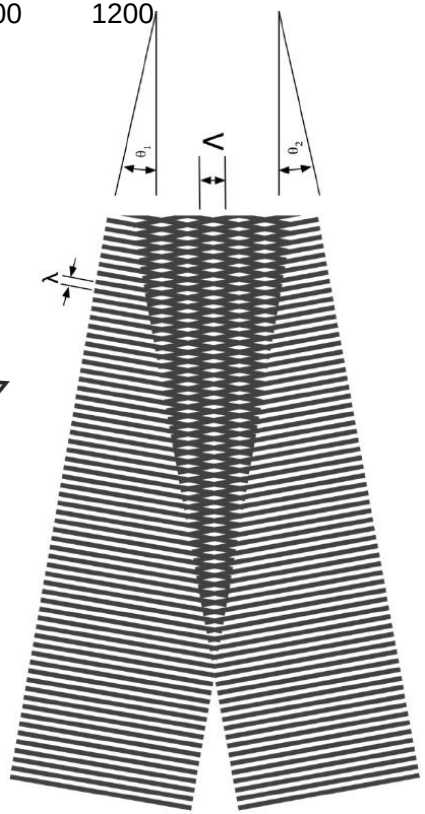
TDFRS: HOW DO WE MEASURE?

Refractive index grating



Measured quantity: intensity of diffracted reading beam





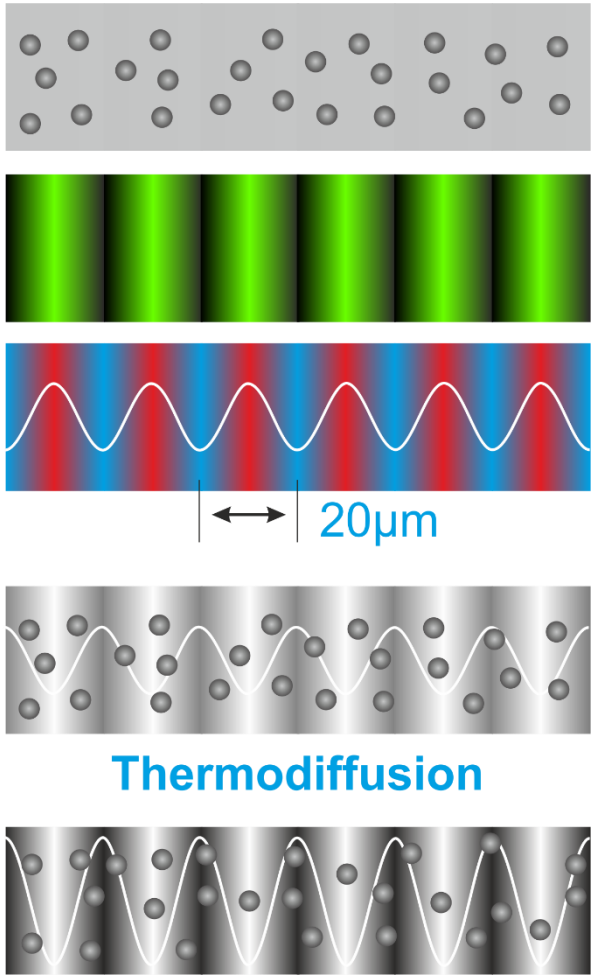
homogen

laser grating

temperature grating (20-100 µK)

refractive index grating (temperature)

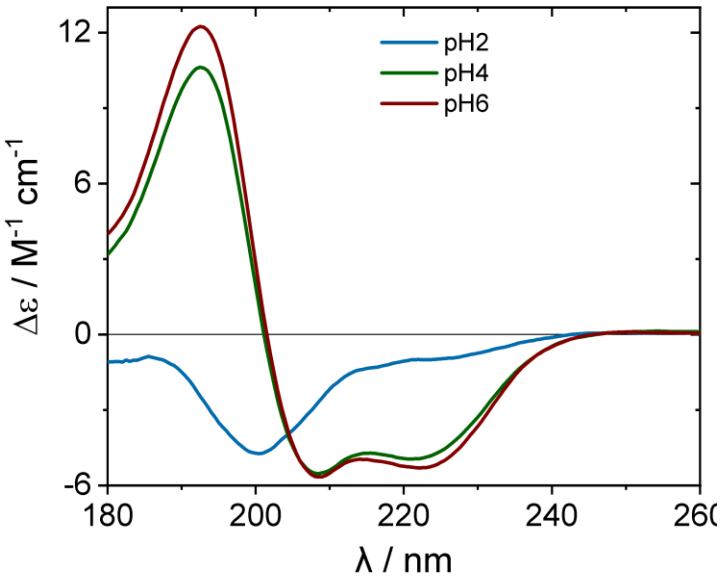
refractive index grating (concentration)



Thermodiffusion

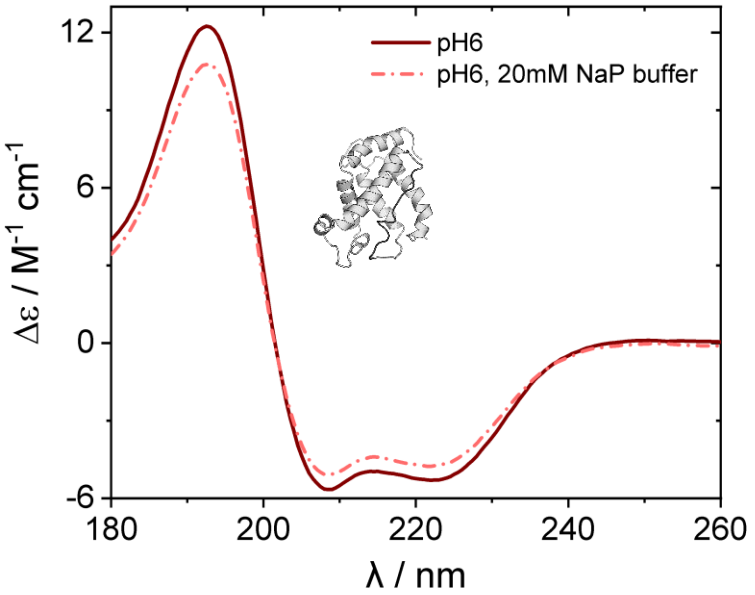
DISCUSSION OF TDFRS RESULTS IN STEPS

No buffer



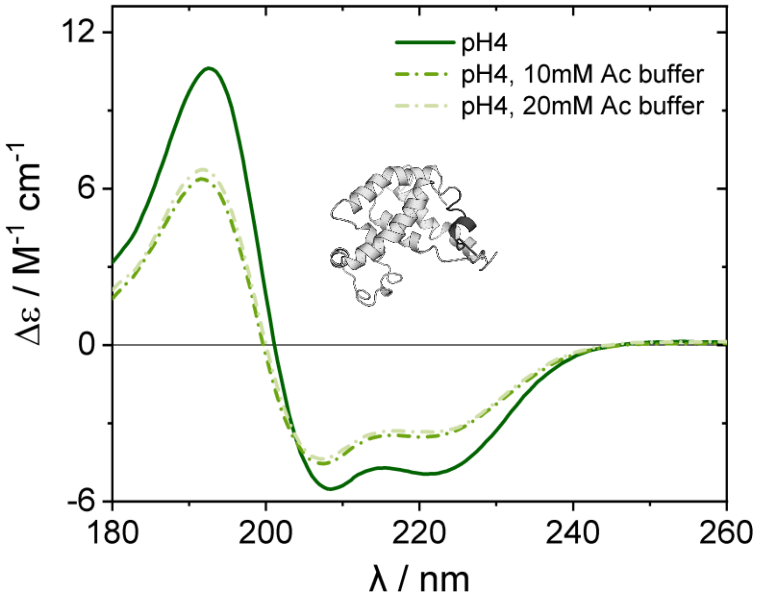
(1)

NaP buffer no influence on
CD spectrum at **pH 6**



(2)

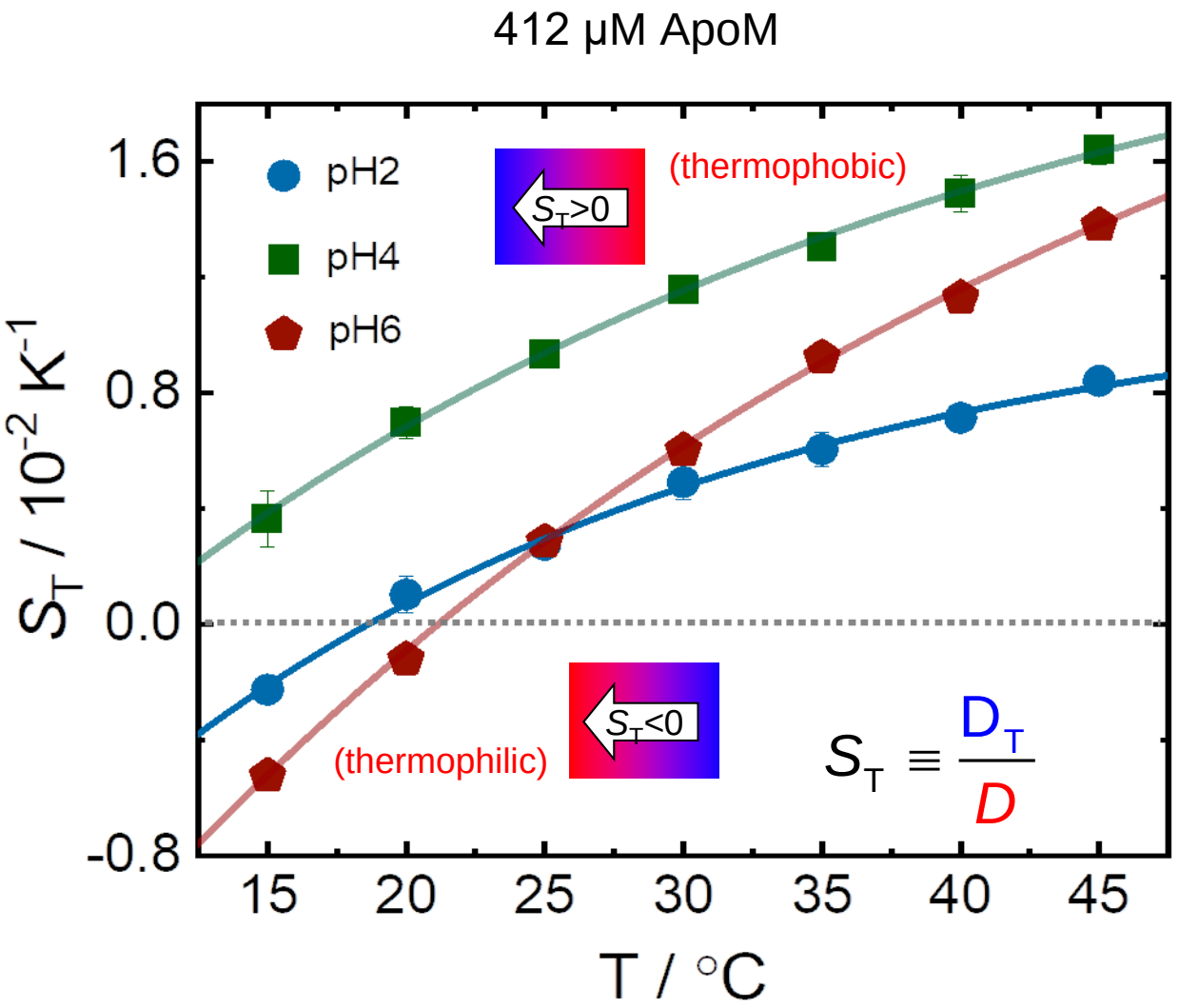
Ac buffer influences
CD spectrum at **pH 4**



(3)

B. A. Rudani et al., Langmuir 41, 28322 (2025).

1. SORET COEFFICIENT (S_T) – NO BUFFER



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

$$S_T(T) = S_T^{\infty} + A \cdot \exp(-T/T_0)$$

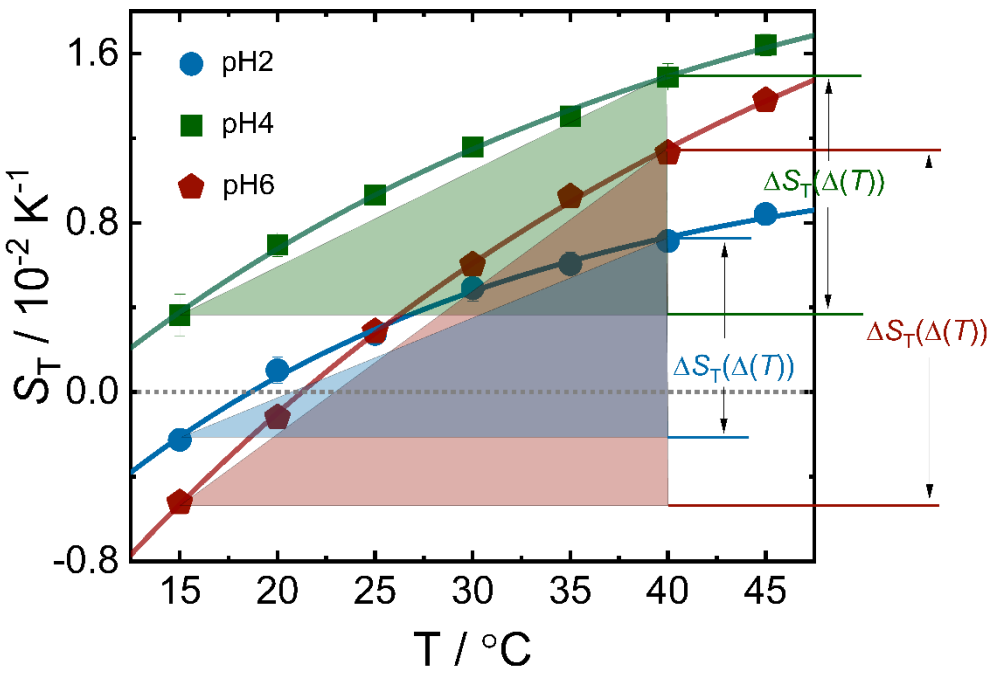
S. Iacopini, R. Rusconi, and R. Piazza, *Eur. Phys. J. E,* **19**, 59–67 (2006)

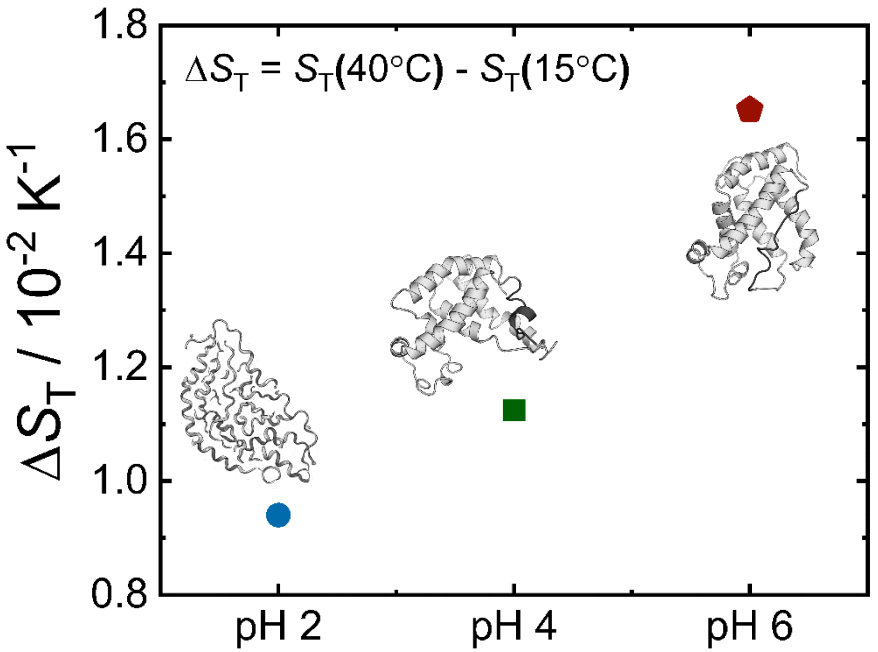
- **What can we learn from it?**

B. A. Rudani et al., Langmuir 41, 28322 (2025).

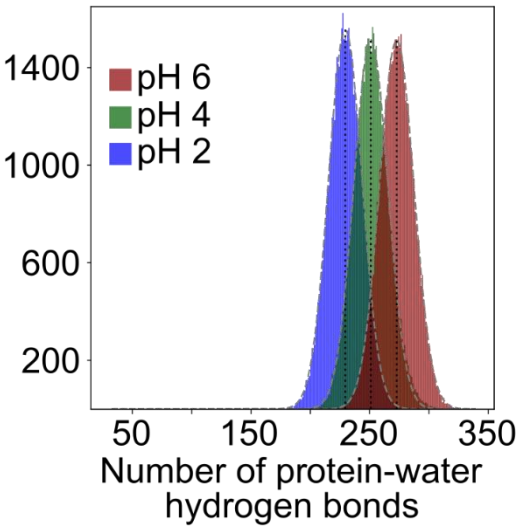
Temperature sensitivity of S_T at different pH

No buffer





Simulation – IBG-4

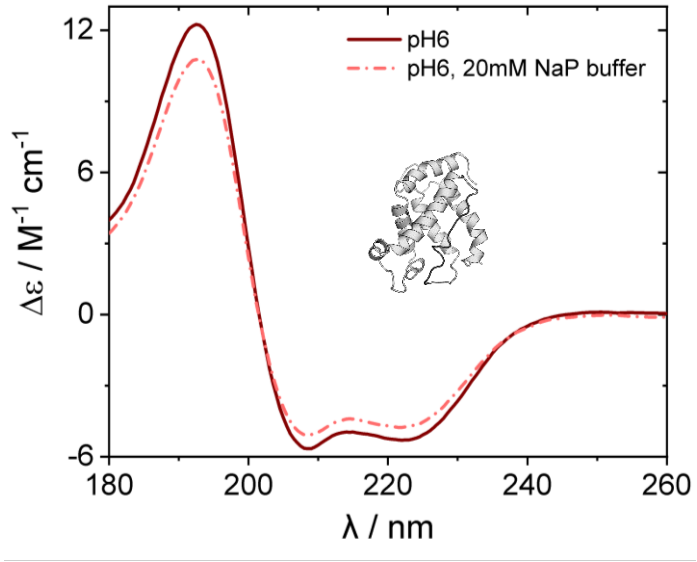


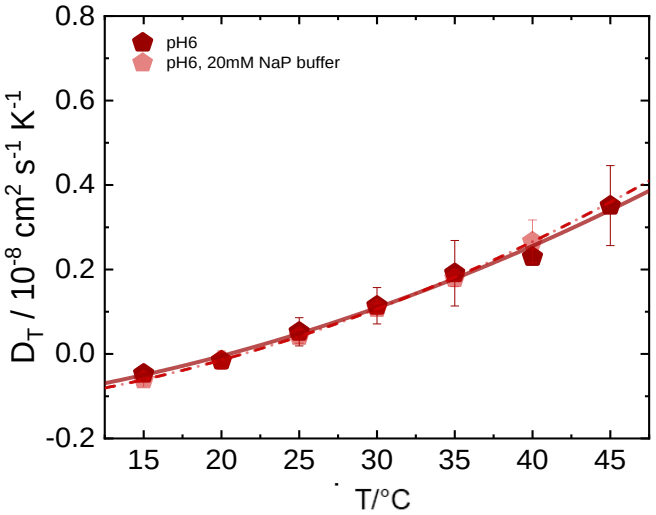
The folded protein is more hydrophilic than the unfolded

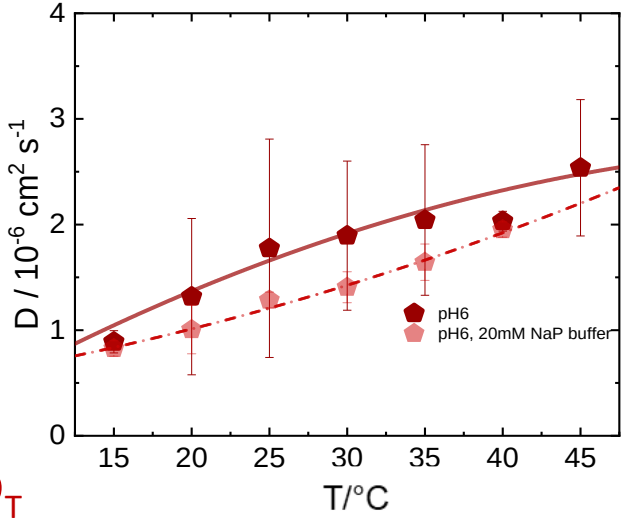
B. A. Rudani et al., Langmuir 41, 28322 (2025).

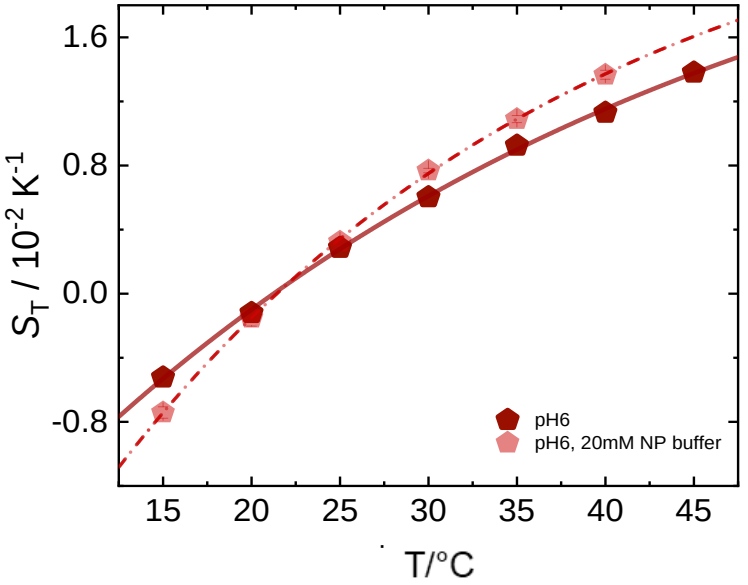
2. Comparison of S_T at pH6 with NaP buffer

CD spectrum





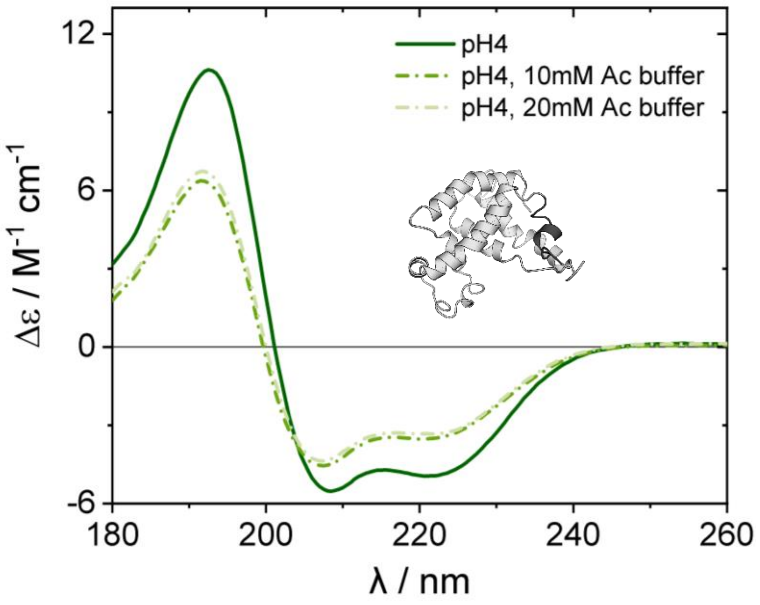




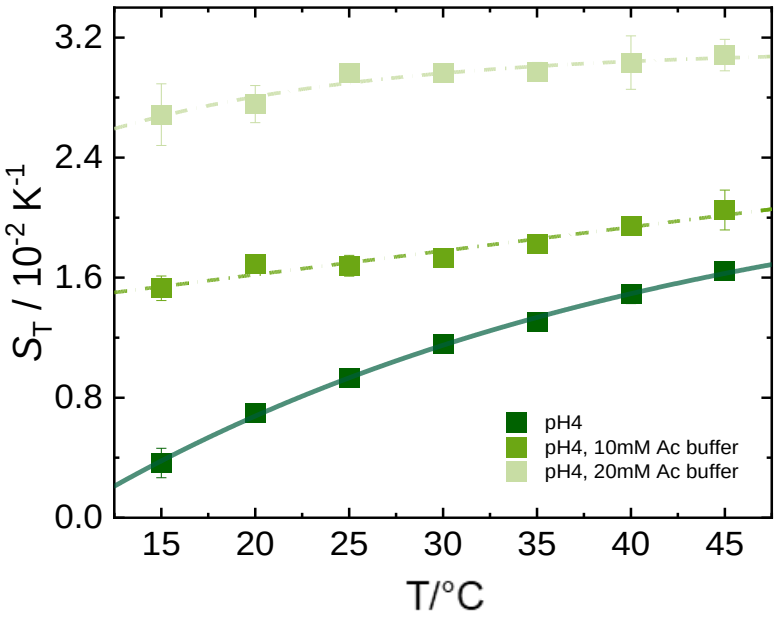
- pH6: Use of buffer does not change D_T
- Buffer leads to stronger aggregation, reflected in a lower diffusion coefficient

3. Comparison of S_T at pH4 with NaAc buffer

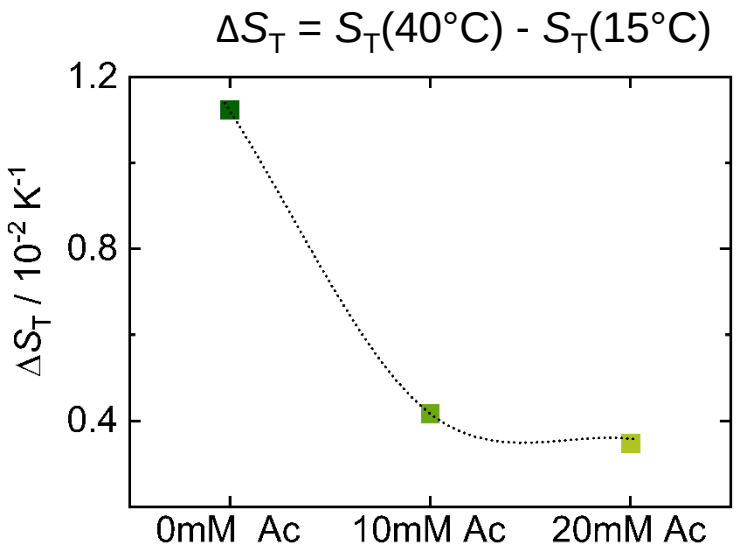
CD spectrum



Soret coefficient



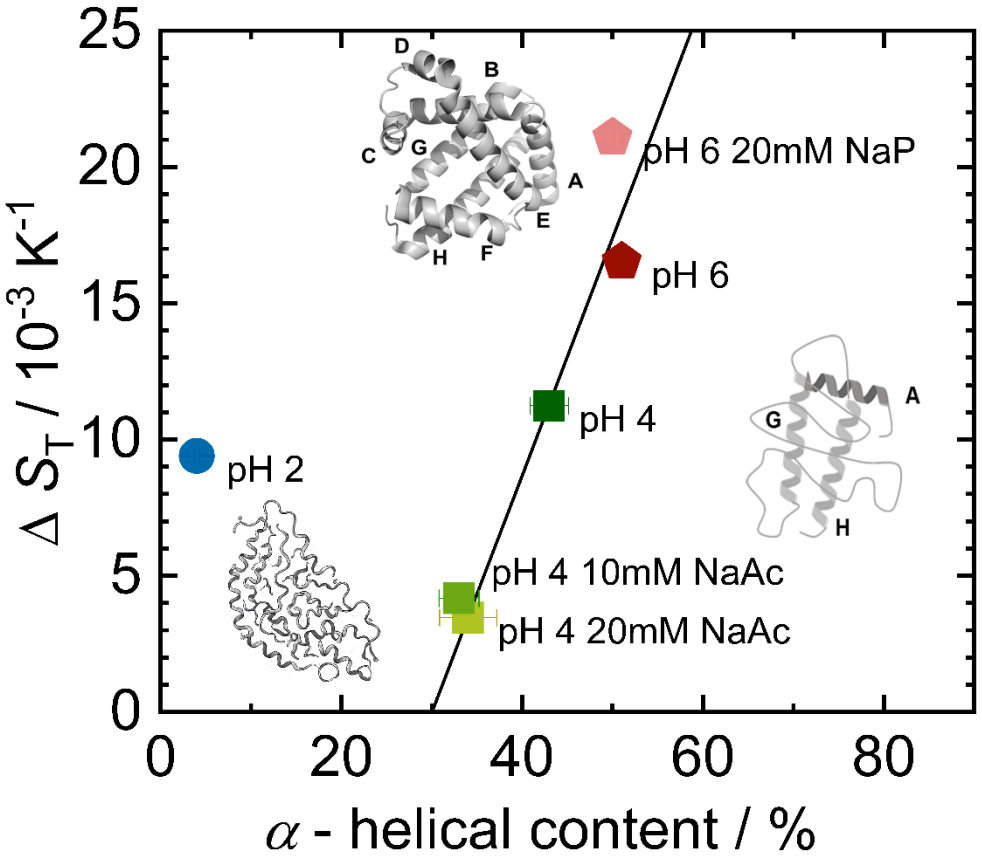
Temperature sensitivity ΔS_T



Acetate buffer leads to partial unfolding and reduces the temperature sensitivity of S_T of the protein

Conclusion

Correlation between thermodiffusion and α –helical content



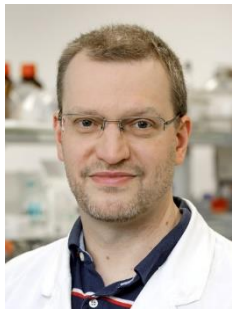
α-helical content is proportional to

- **hydrophilicity**
- **temperature sensitivity of S_T**

- Phosphate buffer leads to stronger aggregation at pH6, reflected in a lower diffusion coefficient.
- Acetate buffer promotes solubilization of protein.
- ΔS_T decreases with an increasing concentration of acetate buffer

ACKNOWLEDGEMENT

Simone Wiegand (Supervisor)



Andreas Stadler (protein, JCNS-1)

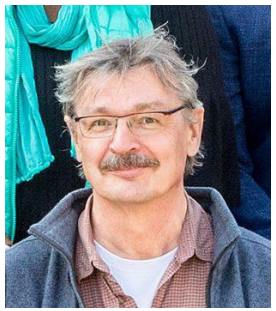
Johan Buitenhuis (CD measure-ments)

(Simulation – IBG-4)

Steffen Docter

Stephan Schott-Verdugo

Holger Gohlke



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