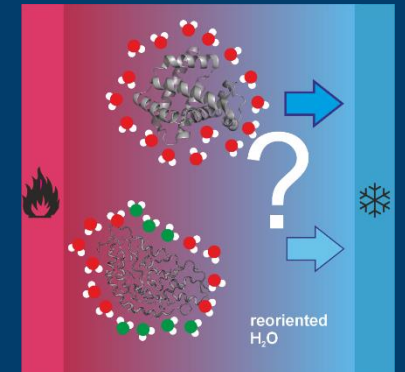
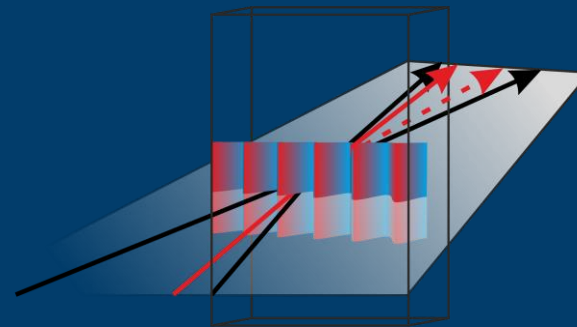


INFLUENCE OF ALPHA-HELICAL CONTENT ON THE THERMODIFFUSION OF APOMYOGLOBIN

INSTITUTE OF BIOLOGICAL INFORMATION (IBI)

IBI-4:BIOMACROMOLECULAR SYSTEMS AND PROCESSES

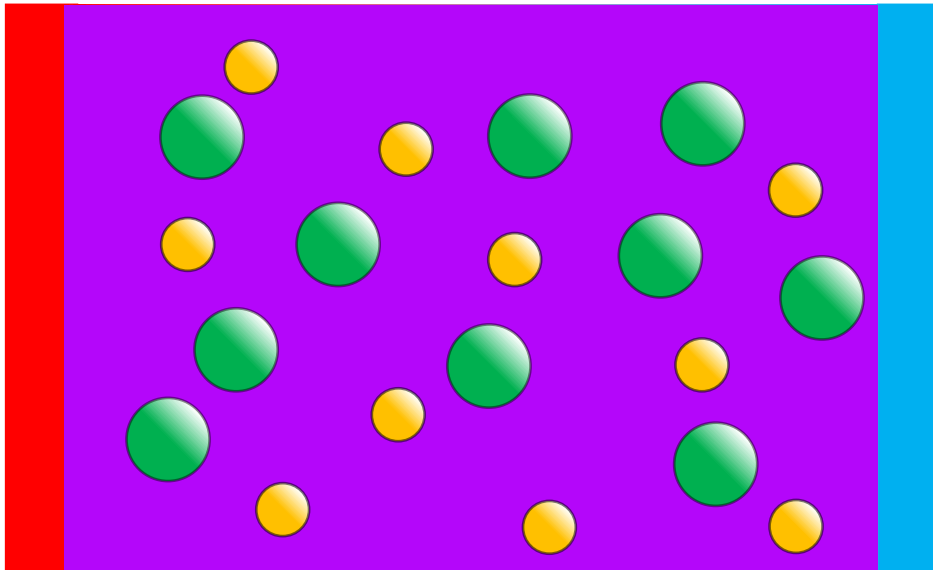


16.06.2026 | BINNY RUDANI

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

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

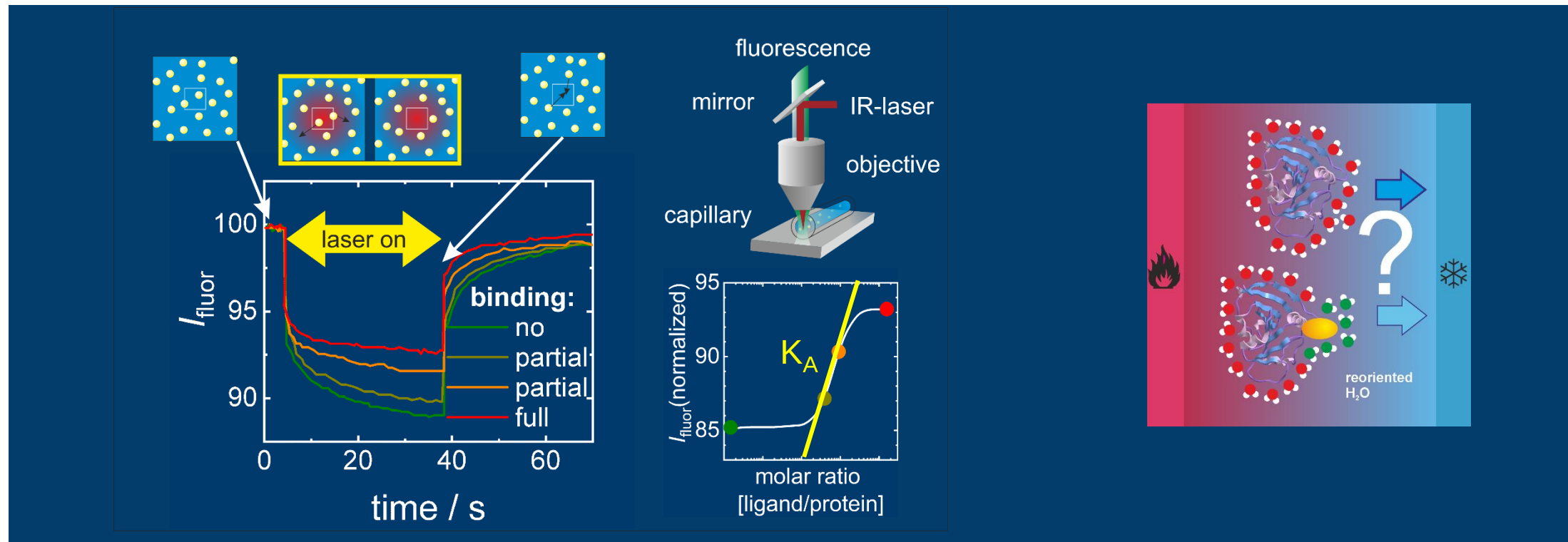
$$S_T = 10^{-3} \text{K}^{-1} \quad - \quad 1 \text{K}^{-1}$$

molecules colloids

THERMOPHORESIS

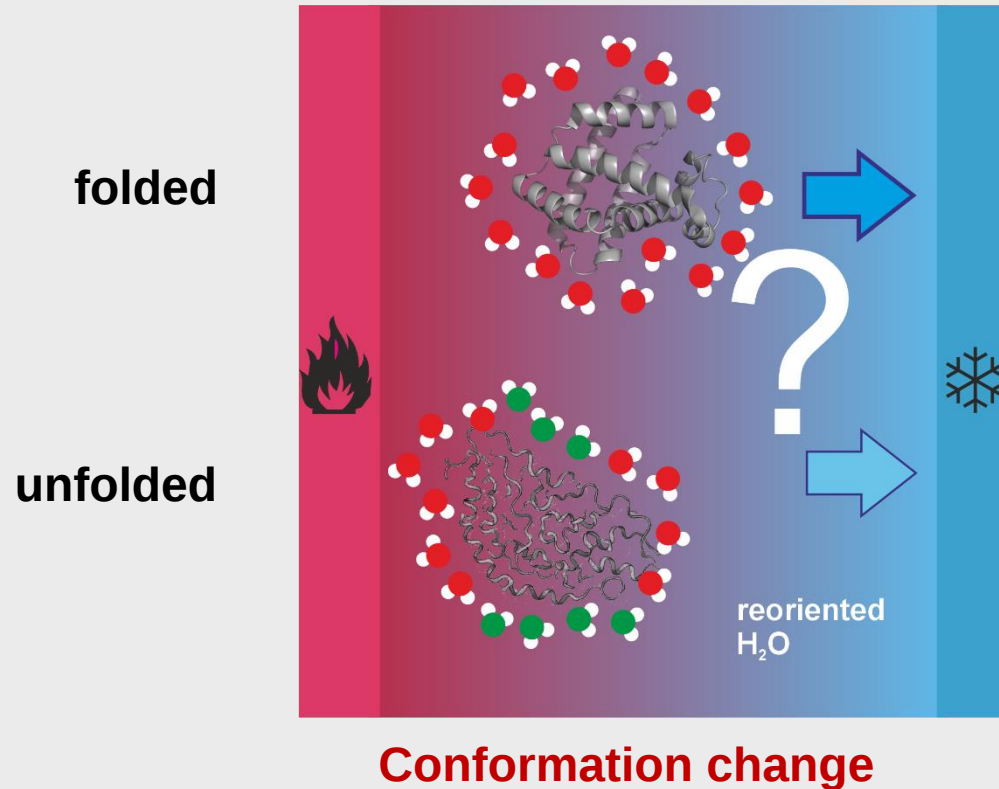
Microscale thermophoresis (MST) determines the binding constant K_A

- Monitors protein-ligand interaction and conformational changes



OBJECTIVE

Can we quantify the relation between thermodiffusion and hydration

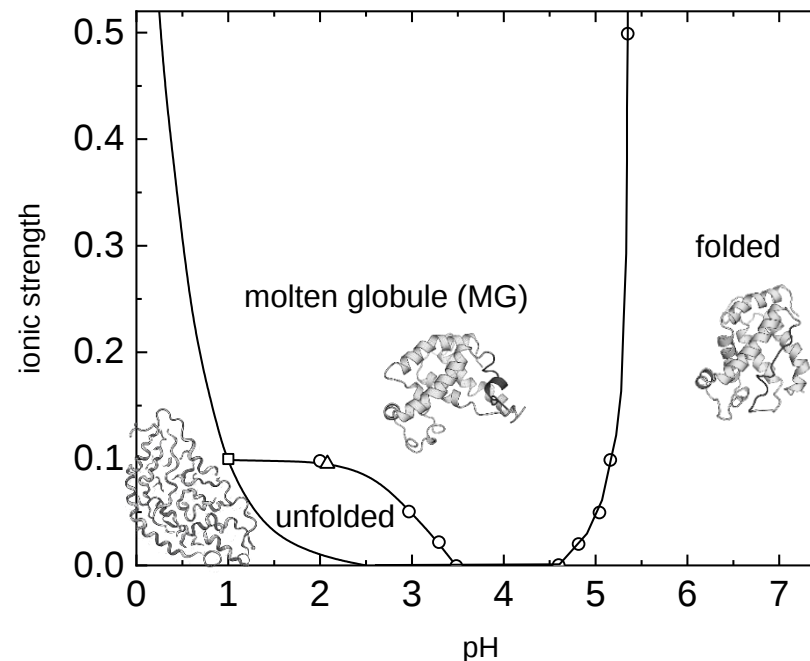
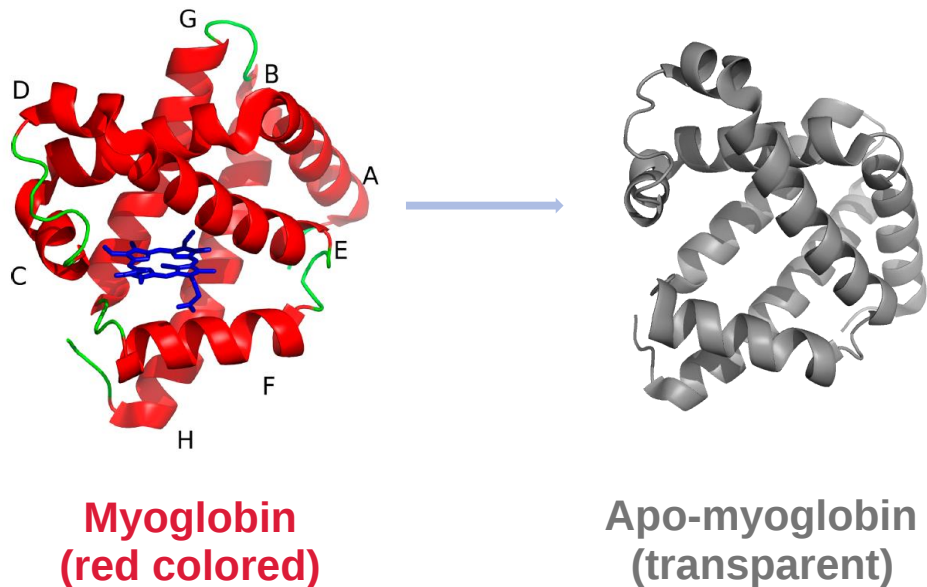


Hypothesis:

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

APOMYOGLOBIN

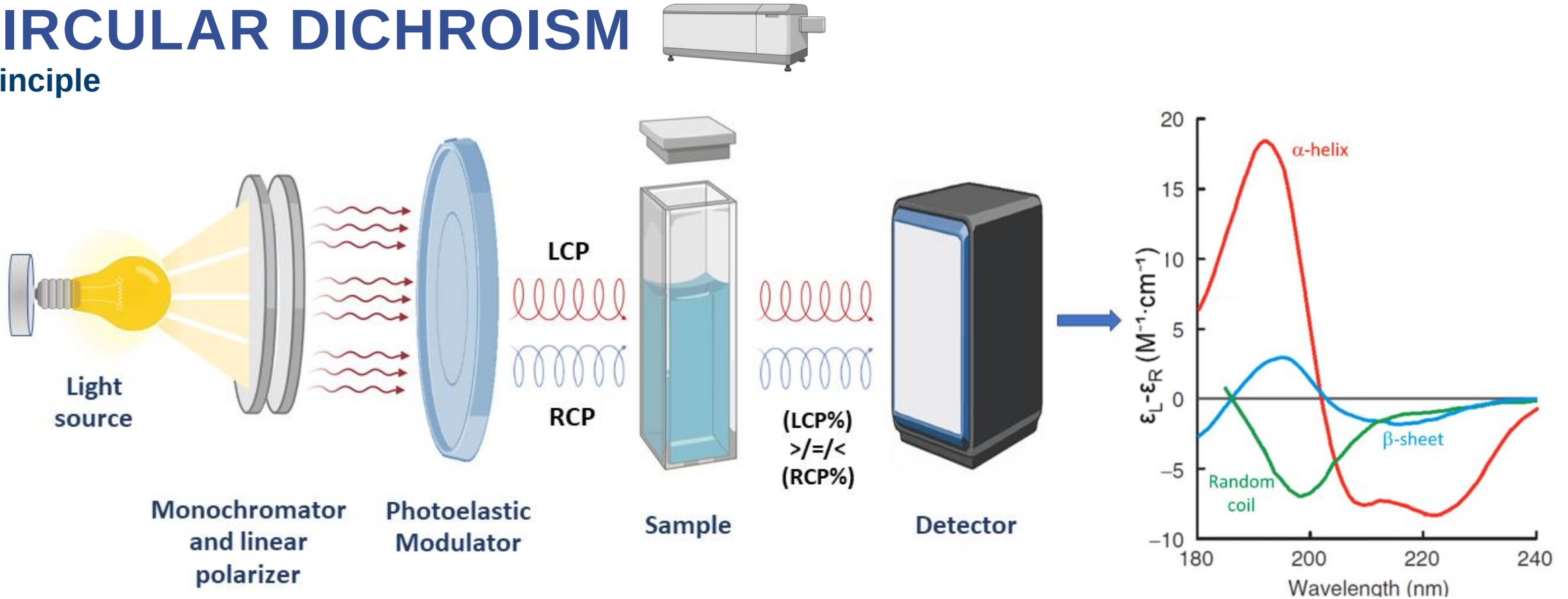
- **Apomyoglobin** (apo-Mb), myoglobin without the heme group, serves as a model system in the field of protein folding.
- It consists 8 α -helices connected by loops.
- Hydrophilicity and conformation changes with pH variation.



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

CIRCULAR DICHROISM

Principle



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

Beer–Lambert law, $\Delta A = (\epsilon_L - \epsilon_R)cl$

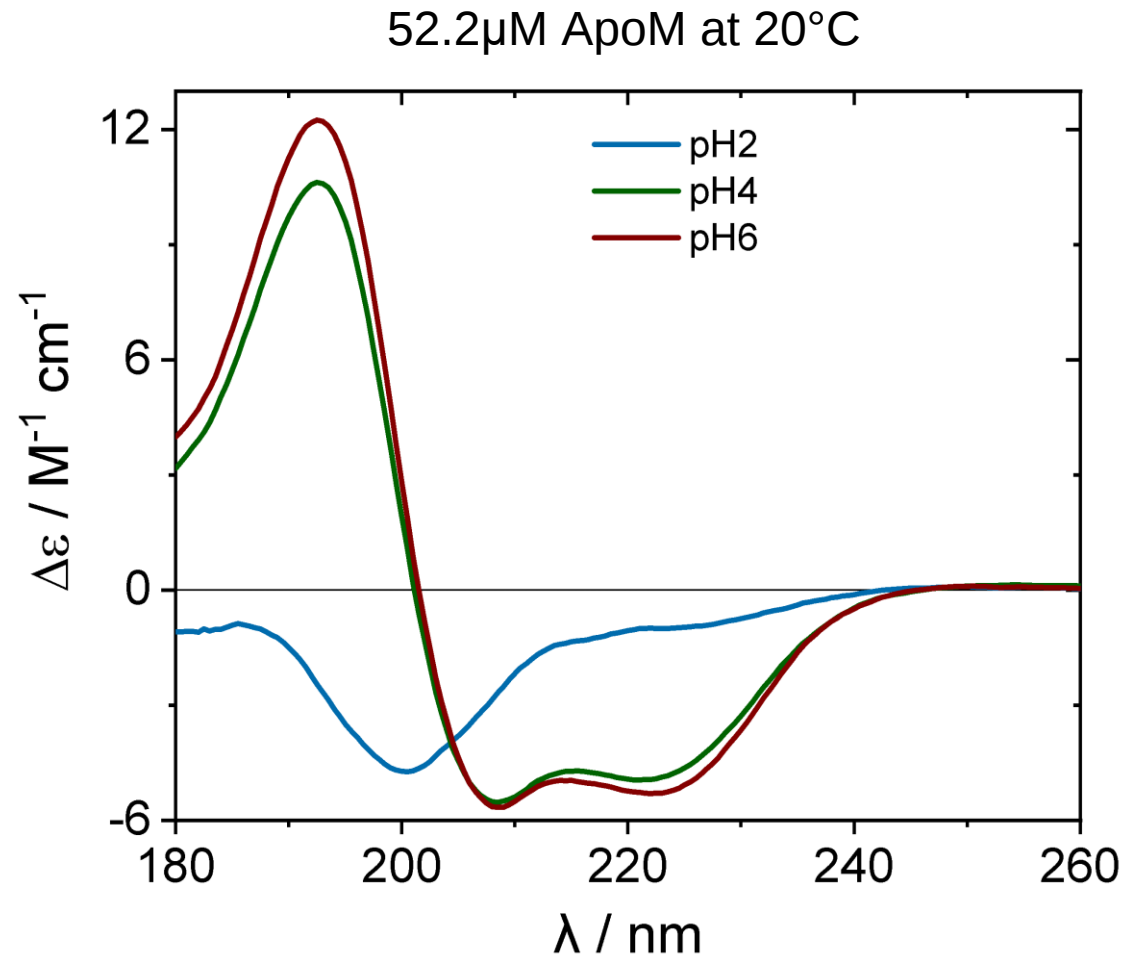
ϵ = molar extinction coefficient

c = molar concentration

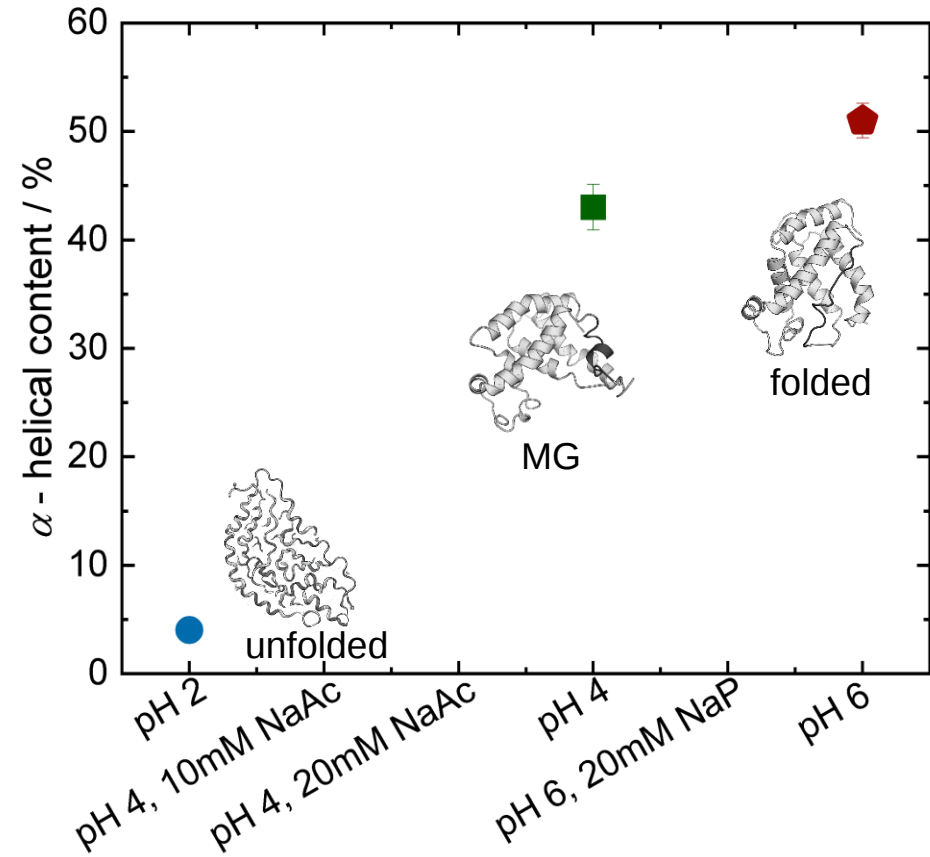
l = path length (cm)

CIRCULAR DICHROISM

Results



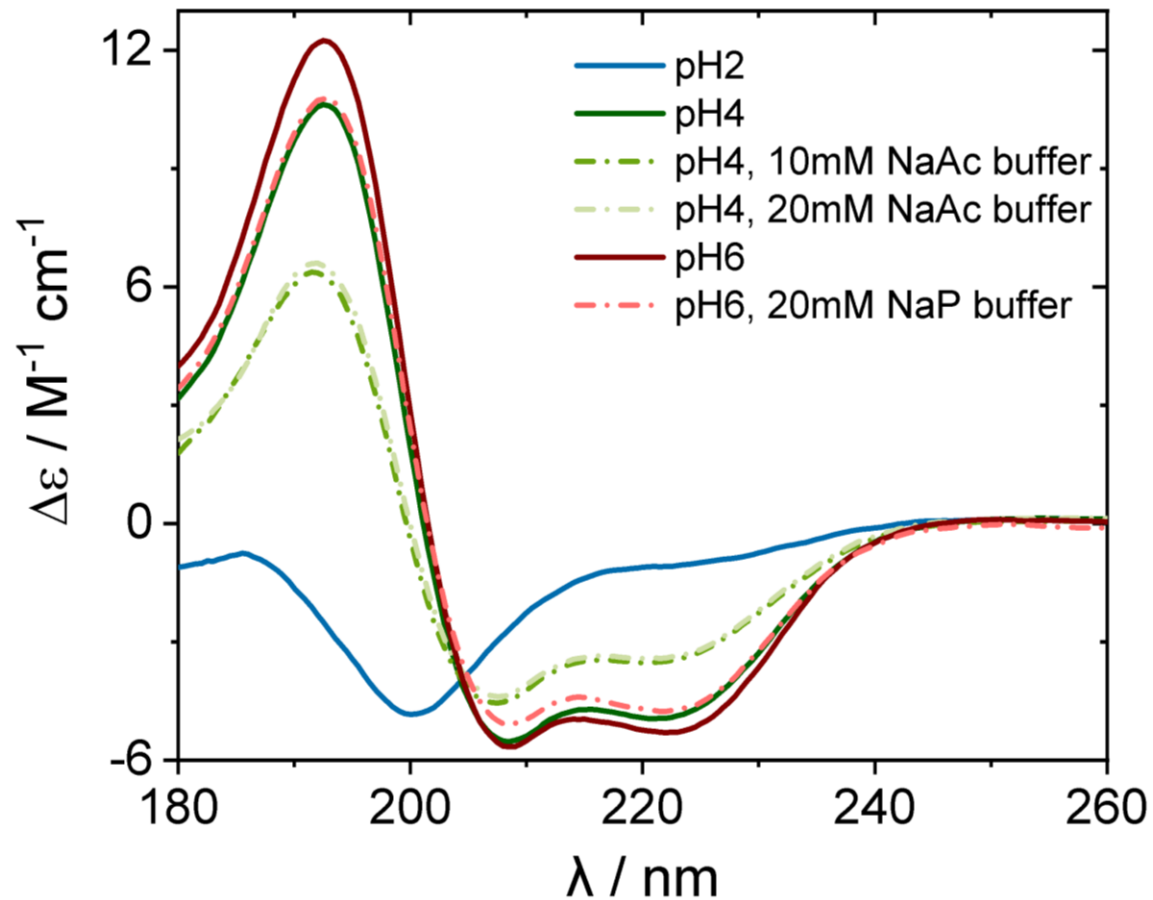
α -helical content: pH6 > pH4 > pH2



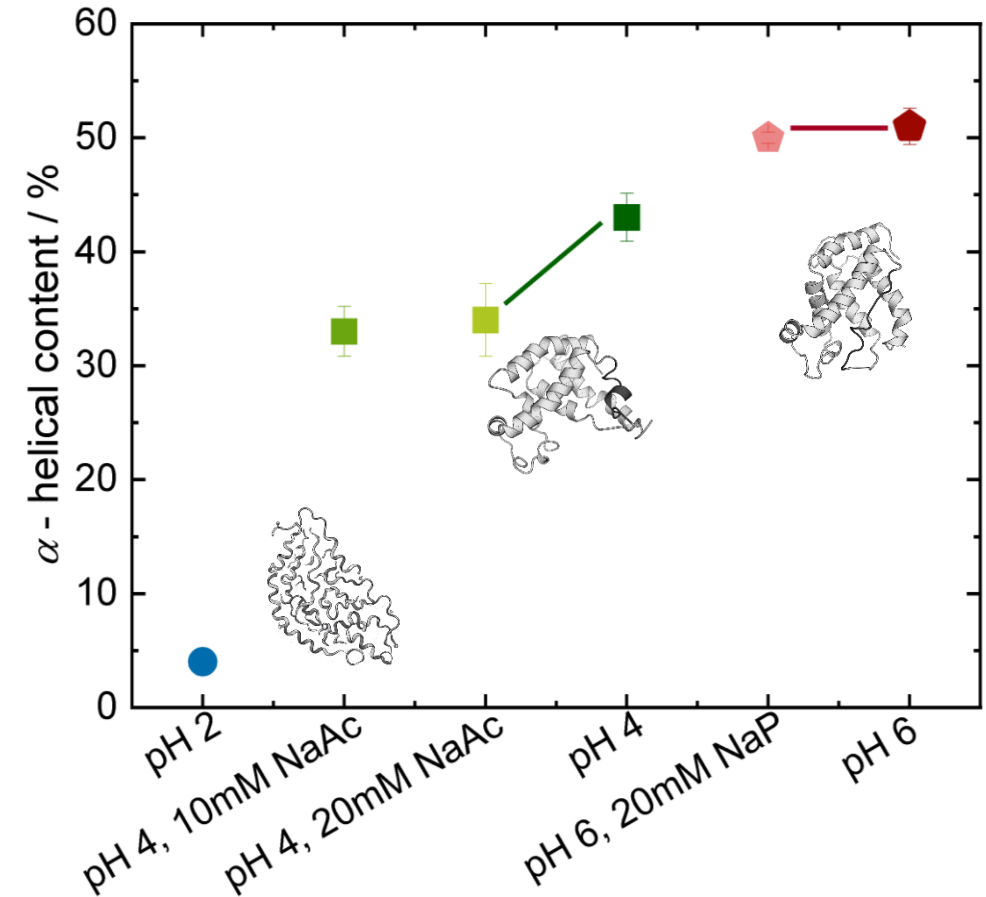
CIRCULAR DICHROISM

Results

52.2 μ M ApoM at 20°C



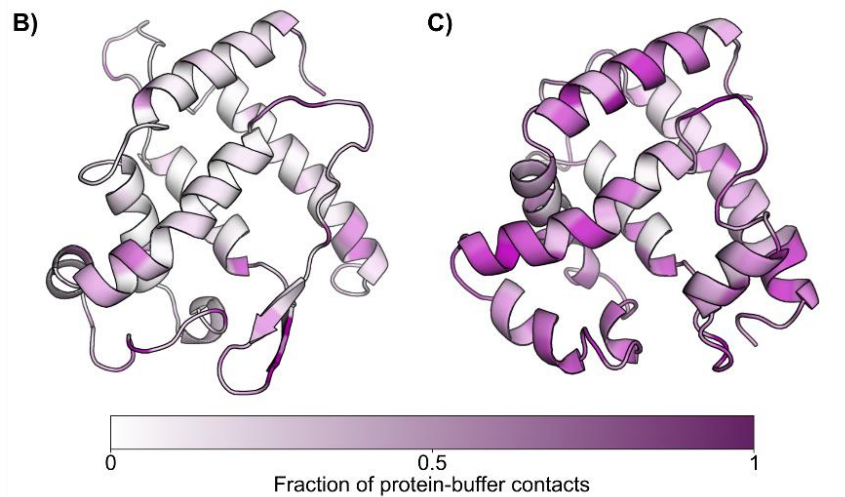
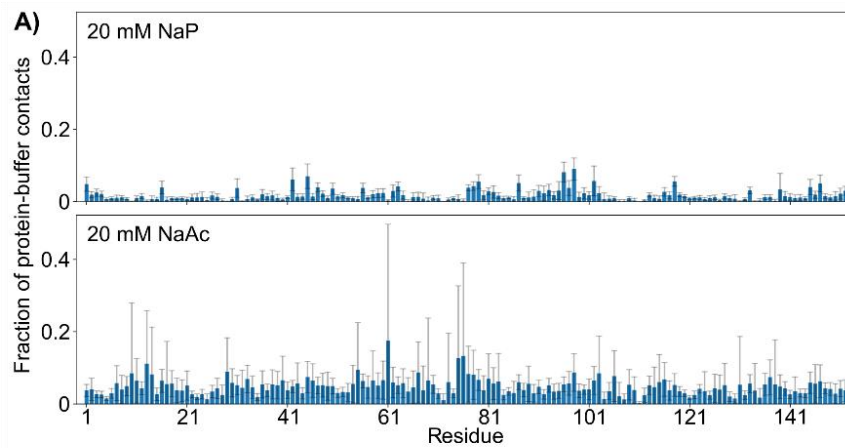
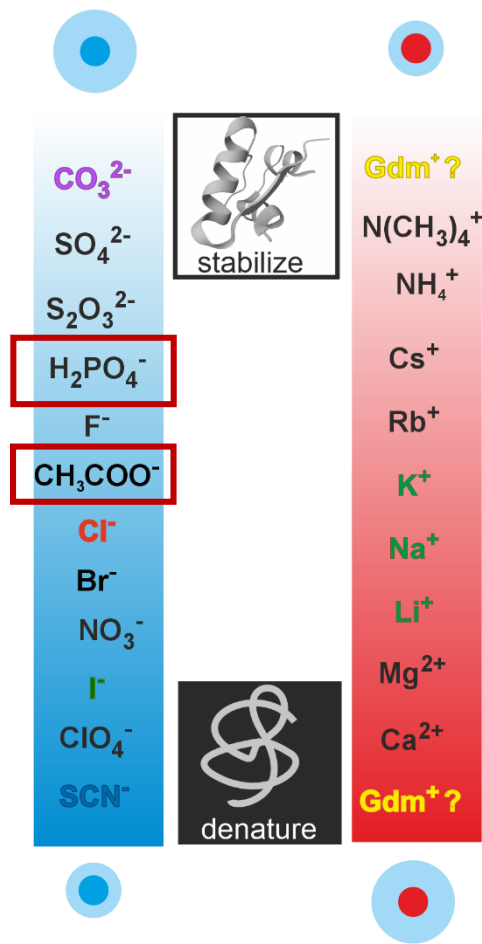
α -helical content: **pH6** > **pH4** > **pH2**



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

PHOSPHATE VS ACETATE BUFFER

HOFMEISTER SERIES



20 mM NaP

20 mM NaAc

(Simulation – IBG-4)



Steffen
Docter



Stephan
Schott-
Verdugo



Holger
Gohlke

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

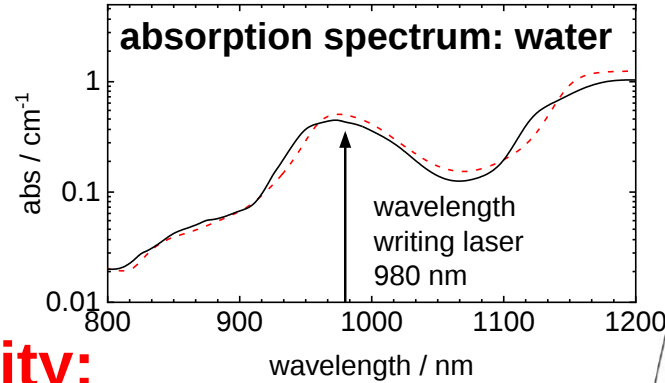
B. A. Rudani et al. JCP **160**, 214502 (2024).

Member of the Helmholtz Association

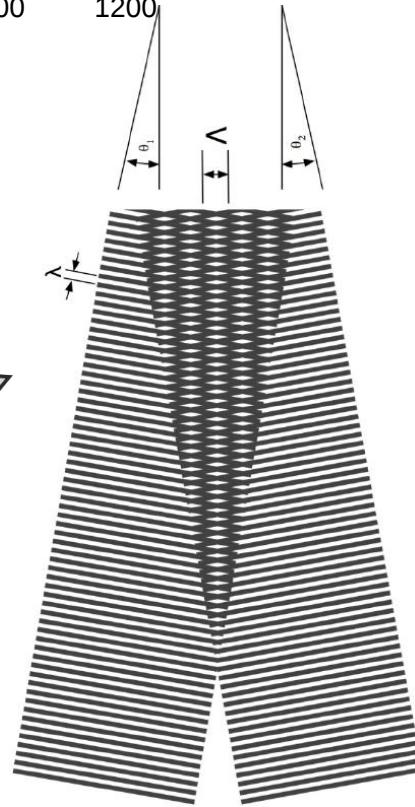
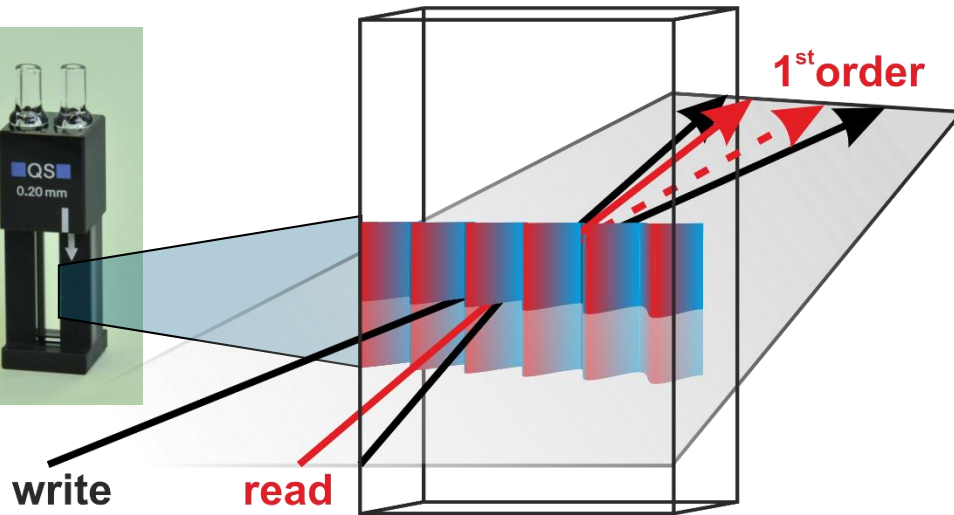
B. A. Rudani et al., Langmuir **41**, 28322 (2025).

TDFRS: HOW DO WE MEASURE?

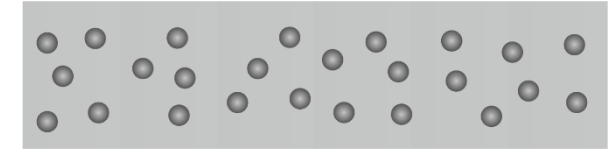
Refractive index grating



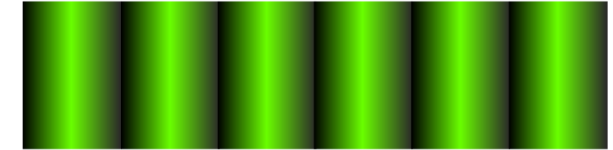
**Measured quantity:
intensity of diffracted reading
beam**



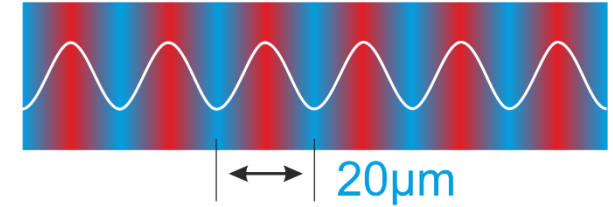
homogen



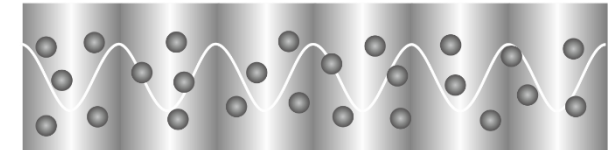
laser
grating



temperature
grating
(20-100 μ K)

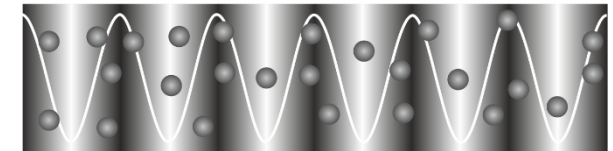


refractive index
grating
(temperature)



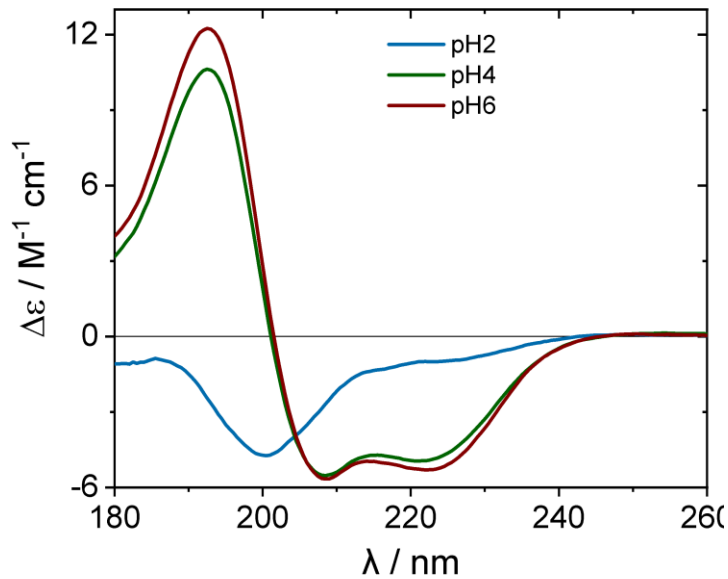
Thermodiffusion

refractive index
grating
(concentration)



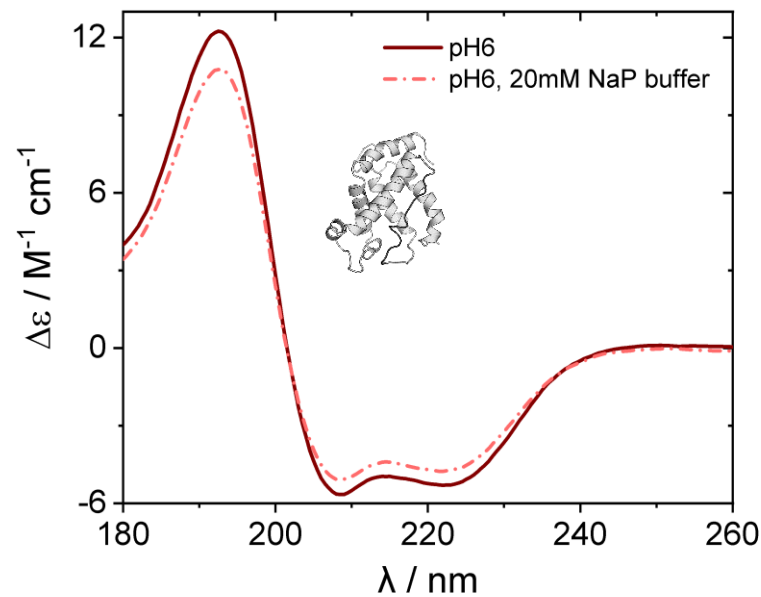
DISCUSSION OF TDFRS RESULTS IN STEPS

No buffer



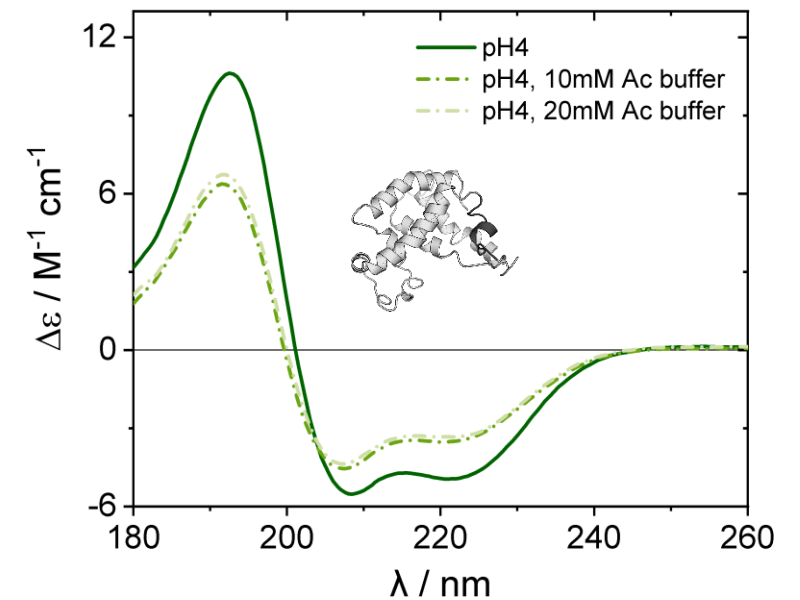
(1)

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



(2)

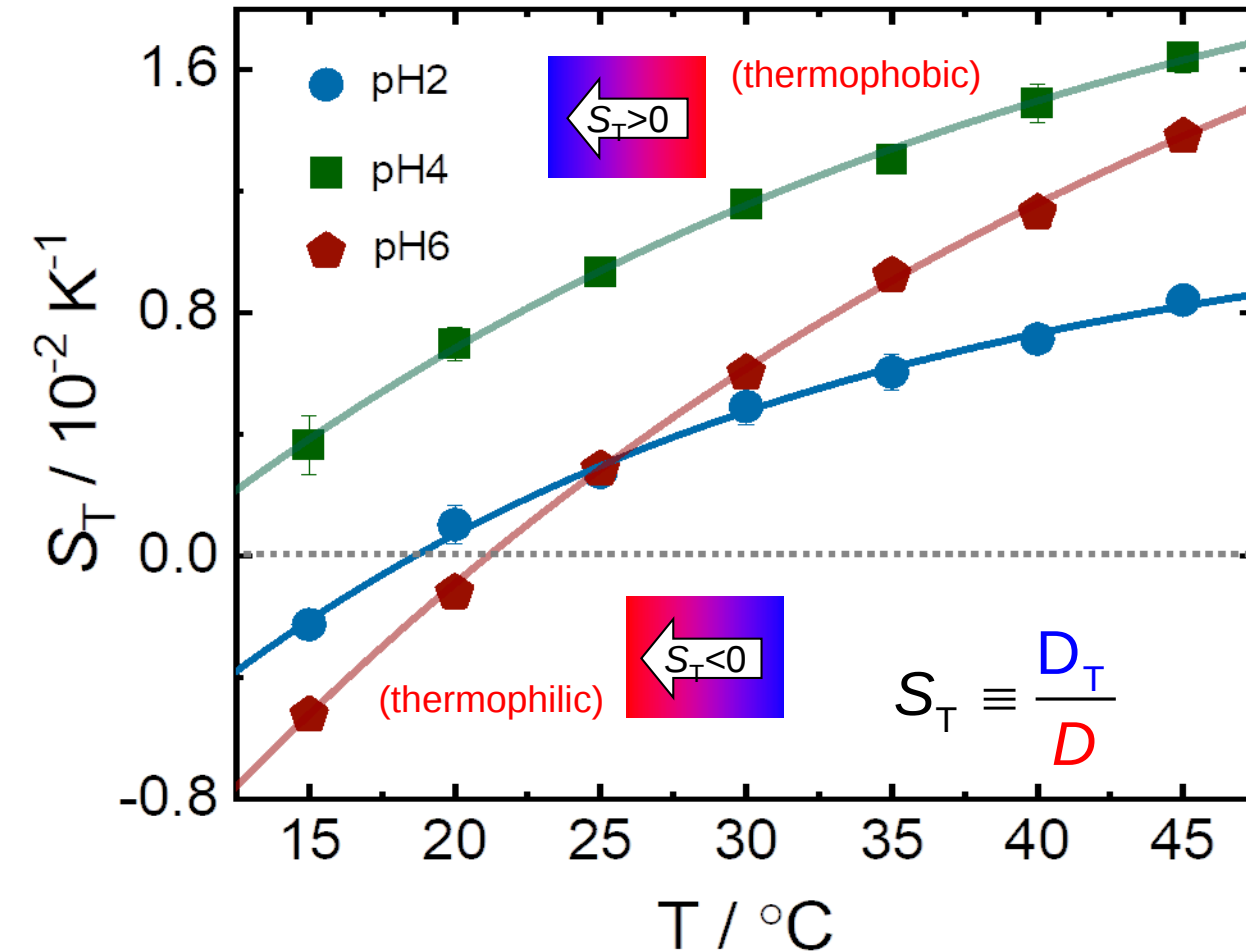
Ac buffer influences
CD spectrum at **pH 4**



(3)

1. SORET COEFFICIENT (S_T) – NO BUFFER

412 μM ApoM



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

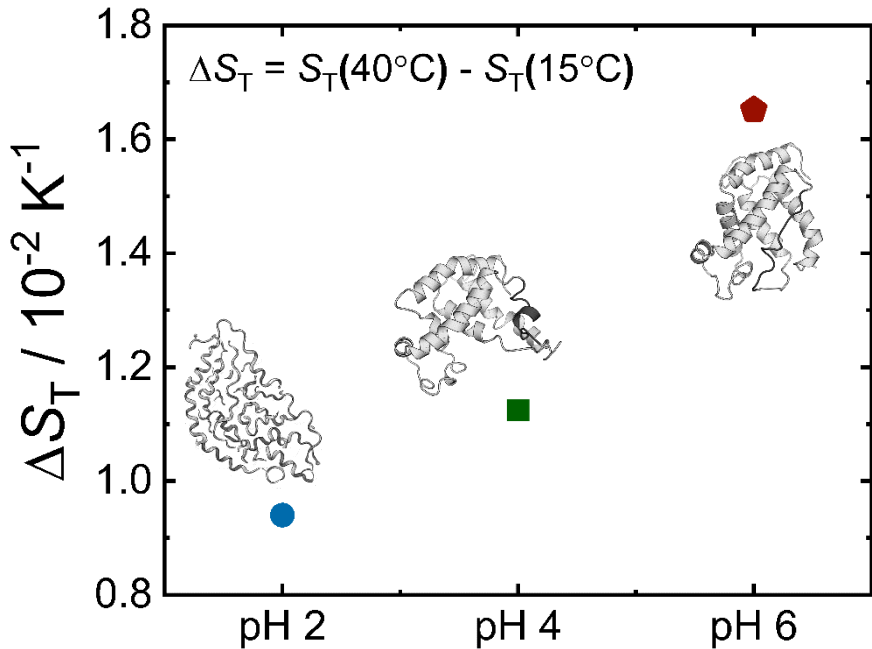
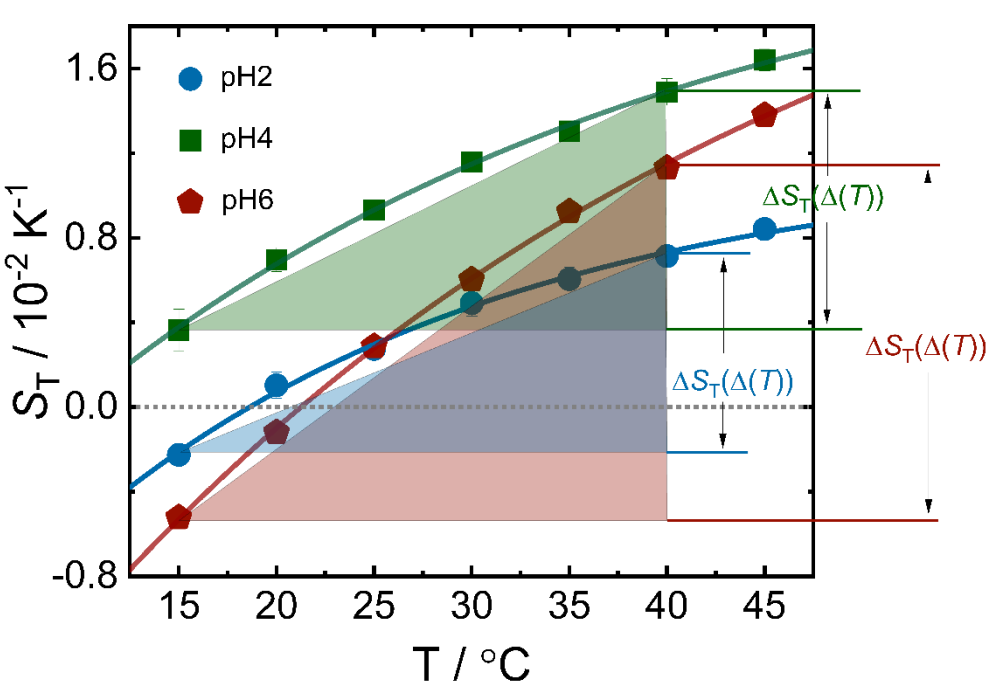
$$S_T(T) = S_T^\infty + A \cdot \exp(-T/T_0)$$

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

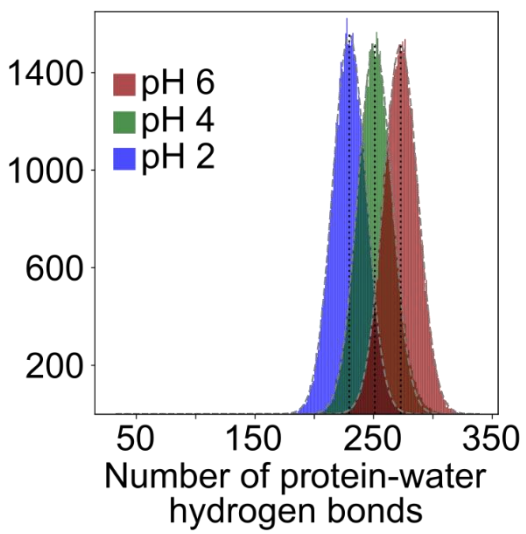
- What can we learn from it?

Temperature sensitivity of S_T at different pH

No buffer



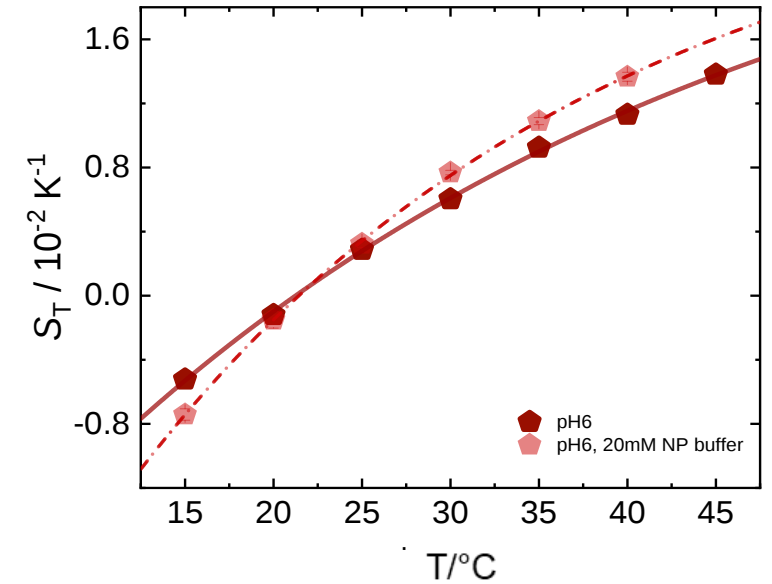
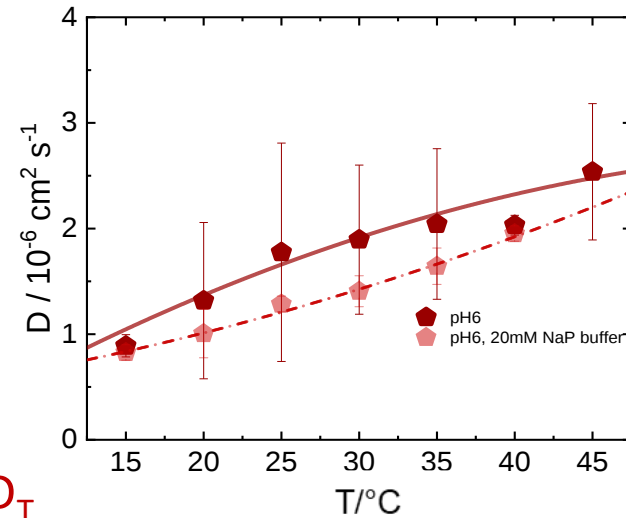
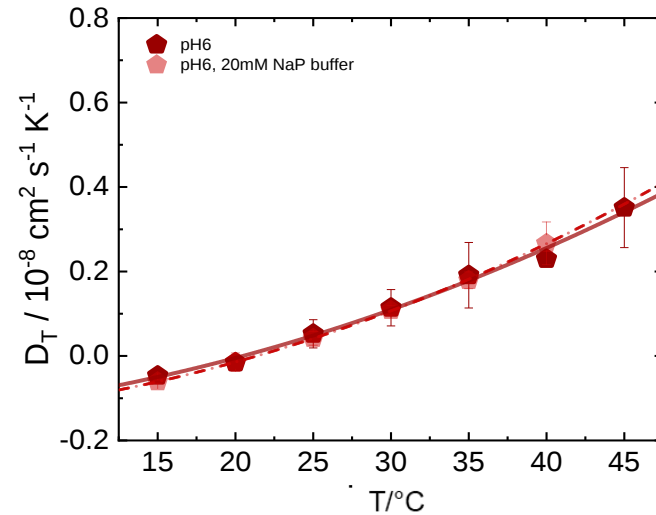
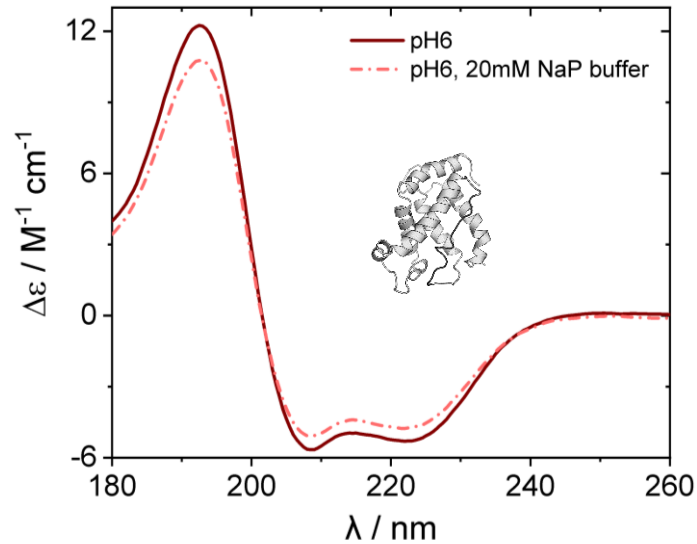
Simulation – IBG-4



The folded protein is more hydrophilic than the unfolded

2. Comparison of S_T at pH6 with NaP buffer

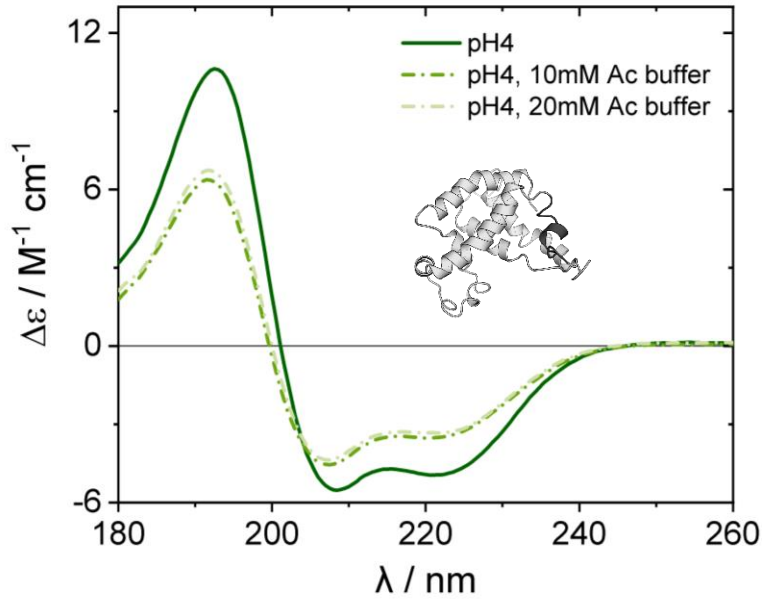
CD spectrum



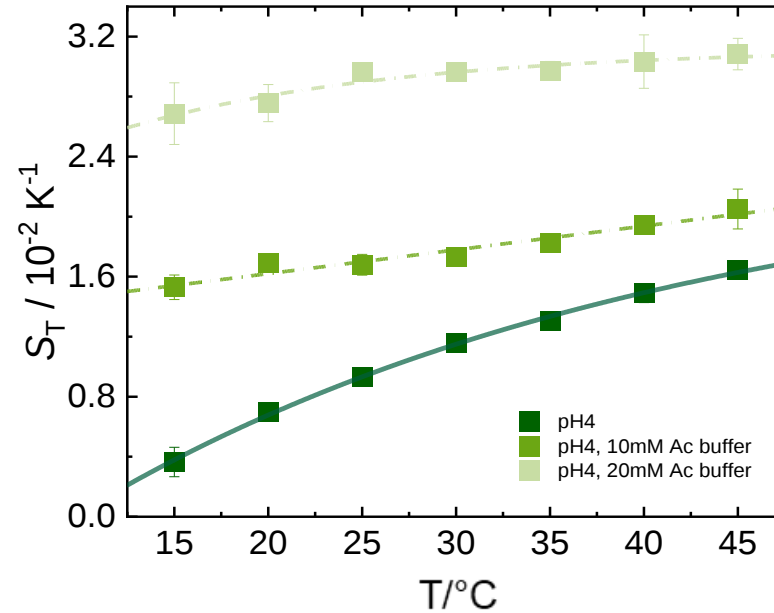
- pH6: Use of buffer does not change D_T
- Buffer leads to stronger aggregation, reflected in a lower diffusion coefficient

3. Comparison of S_T at pH4 with NaAc buffer

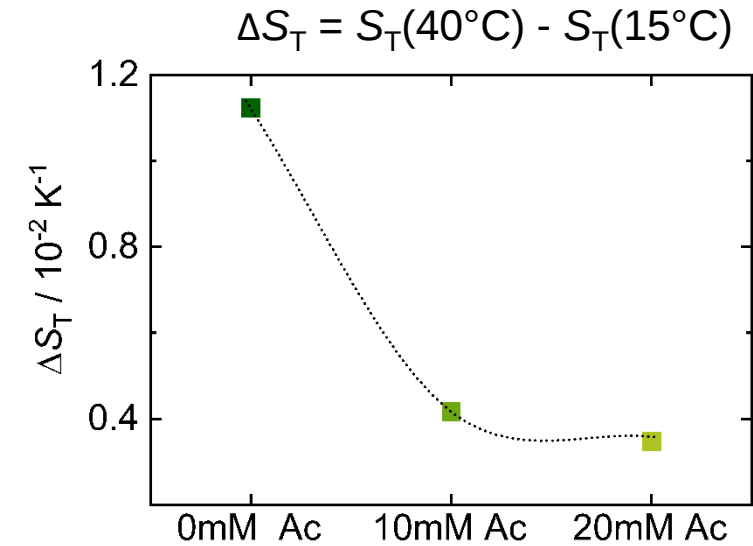
CD spectrum



Soret coefficient



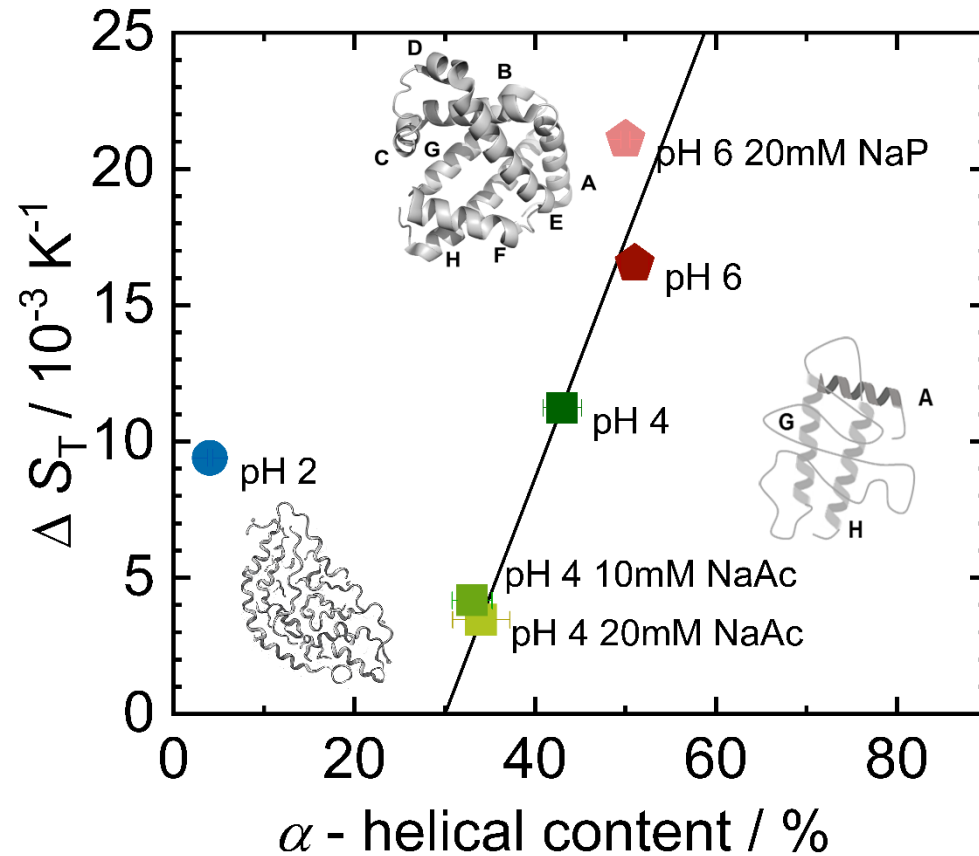
Temperature sensitivity ΔS_T



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

(Simulation – IBG-4)



Simone
Wiegand
(Supervisor)



Andreas Stadler
(protein, JCNS-1)



Johan Buitenhuis
(CD measure-
ments)



Steffen
Dochter

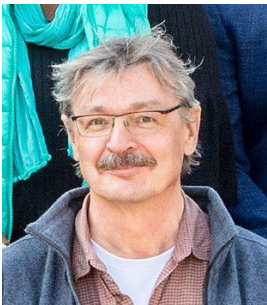


Stephan
Schott-
Verdugo



Holger
Gohlke

Group of IBI-4



Hartmut
Kriegs
(technical
support)



Wim Briels
(theory of thermo-
diffusion of salts)



Peter Lang
(head IBI-4)

Member of the Helmholtz Association

BioSoft
Biophysics and Soft Matter

JÜLICH
Forschungszentrum

THANK YOU