

Computational Studies of Poly(N-isopropylacrylamide) [PNIPAM] in Presence of Osmolytes

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What is Poly(N-isopropylacrylamide) [PNIPAM]?

- thermosensitive polymer
- lower critical solution temperature (LCST)
 - in pure water: $32^{\circ}\text{C} \approx 305\text{K}$
 - $T > \text{LCST} \rightarrow$ phase separation
 - reversible coil-to-globule transition
- peptide bond in side chains \rightarrow proxy for proteins

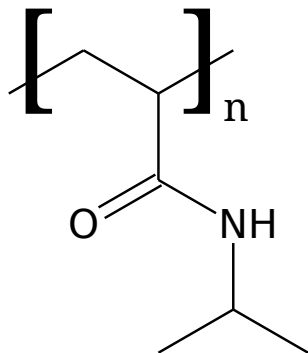


Figure: NIPAM
(monomer)

What are Osmolytes?

- molecules that affect osmosis
- protecting osmolytes (e.g. trimethylamine N-oxide [TMAO]) maintain protein structure
- denaturants (e.g. urea in high concentrations) destroy protein structure

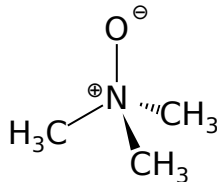


Figure: TMAO

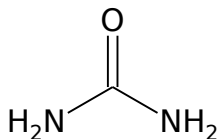
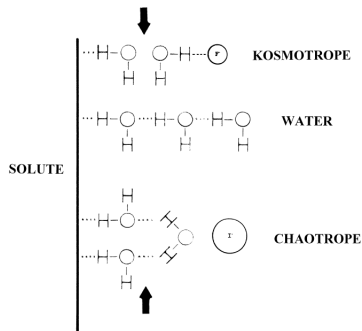


Figure: Urea

Kosmotropes and Chaotropes according to K. Collins¹

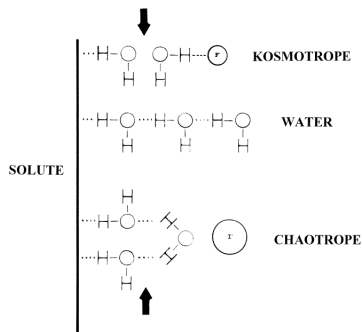
- K. Collins considers only ions
- kosmotropes: high surface charge density
 - attract water (H-bonds)
 - increase order of surrounding water
 - pull water from first hydration shell → solute minimizes solvent accessible surface area (SASA)



¹1997, Biophys. J., Vol. 72 and 2007, Biophys. Chem., Vol. 128

Kosmotropes and Chaotropes according to K. Collins¹

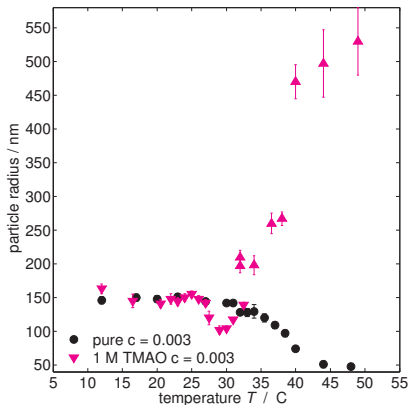
- chaotropes: low surface charge density
 - repel water (bad binding partner)
 - decrease order of surrounding water
 - push water to the first and second hydration shell → expansion of solute



¹1997, Biophys. J., Vol. 72 and 2007, Biophys. Chem., Vol. 128

Experiments

- performed by M. A. Schroer, DESY Hamburg
- PNIPAM in presence of 1 M TMAO and 4 M urea
- particle size investigated via DLS (dynamic light scattering)



Motivation

- reproduction of coil-to-globule transition
- minimal model of PNIPAM in presence of osmolytes
- explanation of the effects observed in the experiment
- hints on mechanism of interaction between osmolytes and PNIPAM
 - one generic mechanism?
 - direct or indirect mechanism?

Approach

- all-atom MD in GROMACS
- simulated systems: 24-NIPAM
 - in pure TIP3P water
 - in 1 M TMAO solution
 - in 4 M urea solution
 - at $T = 295, 310$ K: above and below LCST, using Berendsen thermostat
- force field preparation (PNIPAM, TMAO and urea)
 - structure via PRODRGserver
 - force fields via ACPYPE using GAFF
 - partial charges of PNIPAM changed according to Du et al.²
- reaction coordinate: radius of gyration R^{gyr} of C-backbone

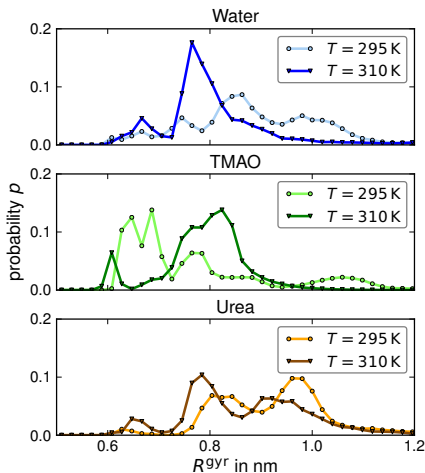
²2010, J. Phys. Chem. B, 114

Investigation of Free Energy Landscapes

- explore phase space, unfold the polymer
 - tool: Metadynamics (with PLUMED)
- yield free energy landscapes
 - tool: Umbrella Sampling (with PLUMED) and WHAM
 - coil-to-globule transition reproduced?
 - qualitative effects of the osmolytes?

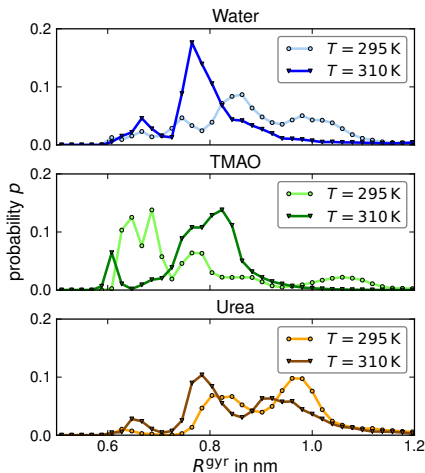
Results: Probability Distributions

- pure water, $T = 295$ K
 - broad maximum at $R^{gyr} \simeq 0.86$ nm
 - coil states allowed
- pure water, $T = 310$ K
 - sharp maximum at $R^{gyr} \simeq 0.78$ nm
 - no coil states
- coil-to-globule transition reproduced



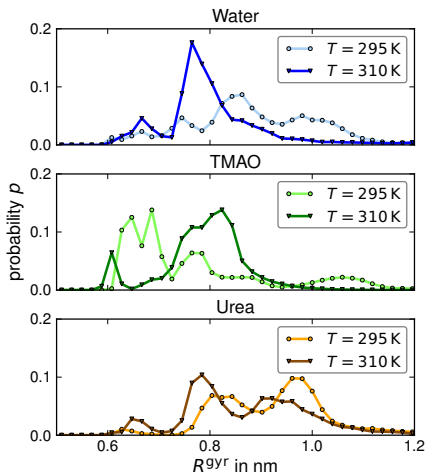
Results: Probability Distributions

- TMAO solution, $T = 295$ K
 - steep maximum at $R^{gyr} \simeq 0.70$ nm
 - very small states allowed
 - almost no coil states
- TMAO solution, $T = 310$ K
 - similar to pure water
- TMAO acts mainly at lower temperatures
- earlier adaption of globule states
- LCST decreases



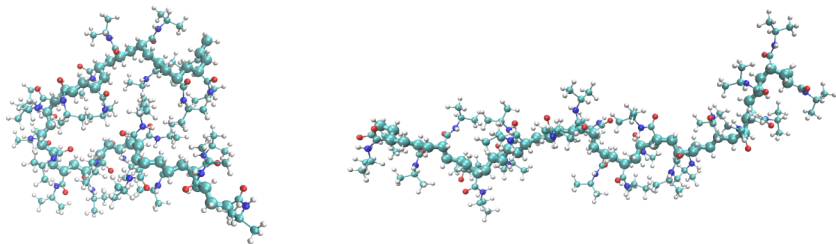
Results: Probability Distributions

- urea solution, $T = 295$ K
 - maximum at $R^{gyr} \approx 0.99$ nm
 - coil states favored
 - almost no states at $R^{gyr} < 0.8$ nm
- urea solution, $T = 310$ K
 - smaller maximum at $R^{gyr} \approx 7.9$ nm
 - coil states still probable
 - very small states still improbable
- urea pushes the system to coil conformations
- LCST increases



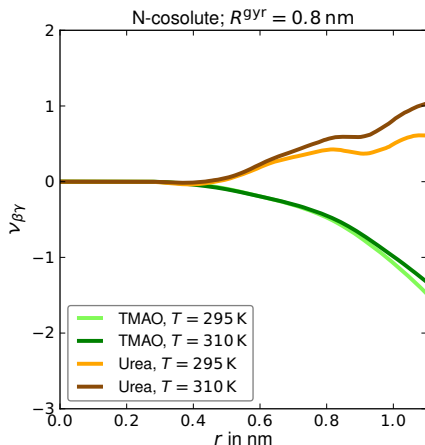
Investigation of Coil and Globule States

- tool: 20 ns NVT simulation with position restraints at $R^{gyr} = 0.8, 1.4$ nm
- preferential binding behaviour of osmolytes?
- quantity and quality of hydrogen bonds to PNIPAM?
- effects on solvent accessible surface area (SASA)?



Results: Preferential Binding

- $F_{\text{trans}} = -RT\nu_{\beta\gamma}$
- C-backbone and peptide bonds investigated
- \rightarrow TMAO is excluded from all groups
- \rightarrow urea binds to all groups



Results: Hydrogen Bond and SASA Analysis

- analyze hydrophobic, hydrophilic and total SASA
- analyze number of H-bonds between PNIPAM and water/osmolytes
- Luzar-Chandler analysis yields
 - average activation free energy ΔF^* (energy needed to break H-bond)
 - average hydrogen bond life times τ

Results: Hydrogen Bond and SASA Analysis

- TMAO draws water from PNIPAM:
 - weaker and less H-bonds around PNIPAM
 - no H-bonds between PNIPAM and TMAO
 - SASA unchanged
- urea displaces water around PNIPAM:
 - stronger H-bonds with PNIPAM than water (activation free energy $\sim 20 - 30\%$ higher)
 - less H-bonds between water and PNIPAM
 - hydrophilic SASA increases for globule conformations

Conclusion

- TMAO
 - placed in the second hydration shell
 - draws water from PNIPAM
 - makes water a “worse solvent” for PNIPAM
 - → suggestion: acts entirely as Collins Kosmotrope
- urea
 - placed in first hydration shell
 - enthalpic gains due to stronger H-bonds
 - PNIPAM gains hydrophilic SASA
 - → suggestion: big urea molecules push polymer side chains apart in a mechanical manner

Conclusion

- no generic mechanism observed
- TMAO: indirect mechanism
- urea: direct mechanism
- small effects in minimal model
- for thorough analysis: higher degree of polymerization

Thank you for your attention!