Molecular Dynamics Simulations of Lysozyme in Sugar Aqueous Solutions

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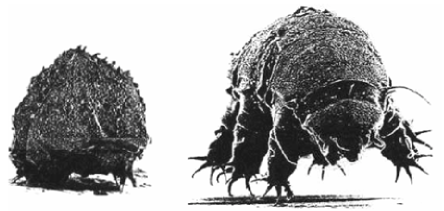
ANR program « Physique-Chimie du Vivant »
2007-2011 BIOSTAB project

Biopreservation

Anhydrobiosis

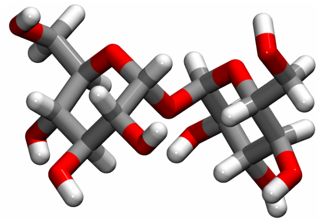
Some organisms can withstand extreme conditions of drought and/or temperature



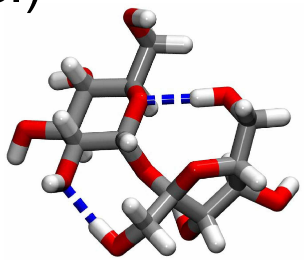
tardigrade

resurrection plant

They naturally synthesize sugars (trehalose, sucrose, etc.)



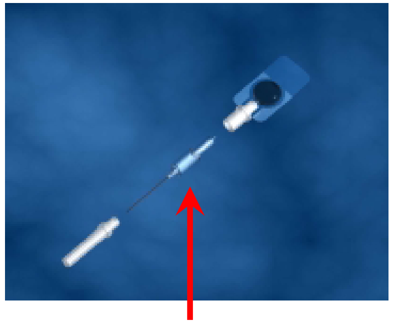
Trehalose



Sucrose

Lyophilisation (freeze-drying)

Long-term conservation of high-value products (therapeutic proteins, blood, organs, etc.)



Dried vaccine

potentially destructive process (ice formation, dehydration, pH and c° changes)

- Molecular mechanisms ?
- Why is trehalose more efficient ?

Hypotheses

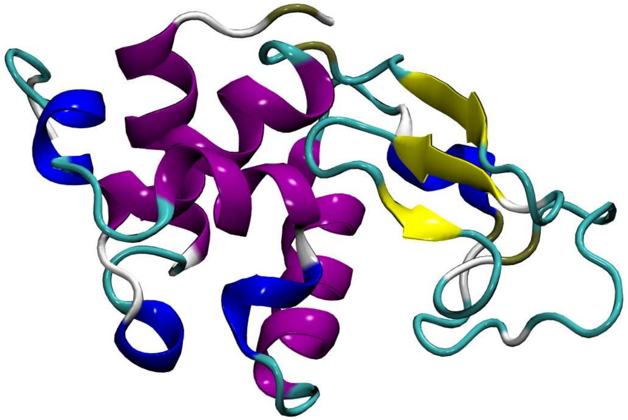
- Glass formation
- Water replacement
- Water entrapment
- T_{2H2O}/T_{α} interconversion
- Destructuring effect

None fully satisfactory

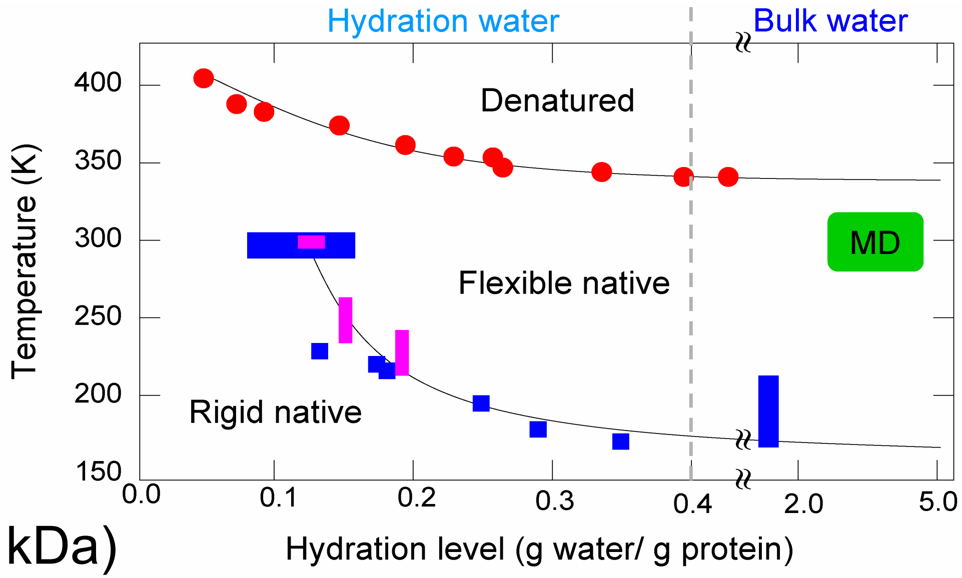
Simulation details

Model protein

Lysozyme (PDB : 193L)

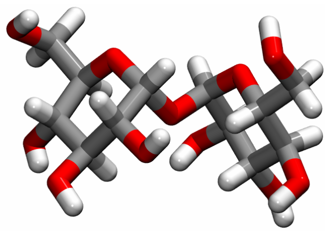


Small globular protein (129 residues, 14.3 kDa)

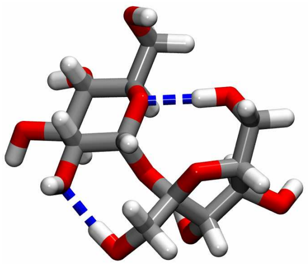


Gregory, in « The Properties of Water in Foods », 1997

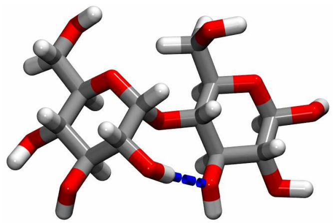
Disaccharides



Trehalose



Sucrose



Maltose

- Same chemical formula ($C_{12}H_{22}O_{11}$)
- Same number of OH groups (8)
- Different topologies

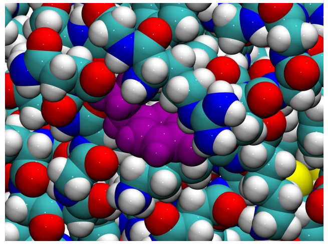
MD program	Force fields	Concentrations	T (K)	Δt	t_{sim}
CHARMM	W SPC/E D CSFF L CHARMM22	0, 37, 50, 60 wt % protein-free basis (~ 14-16 katoms)	300	2 fs	10-17 ns

Fractional solvent accessibilities

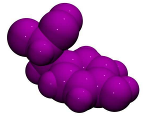
$$f_{sa} = \frac{SASA_{res,protein}}{SASA_{res,free}}$$

Ex. : Tyr20

1 Protein envt



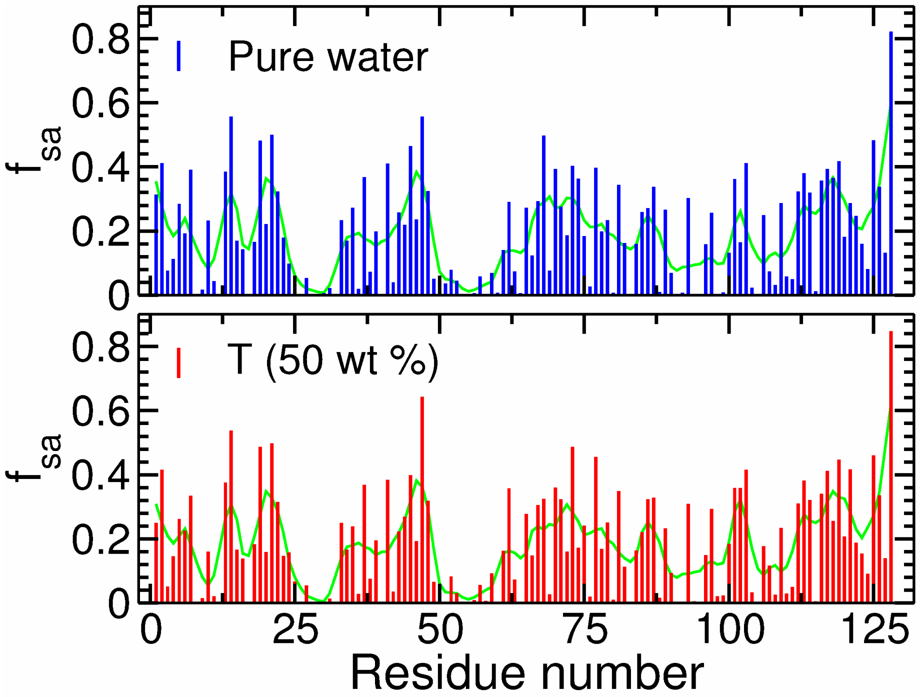
2 Free envt

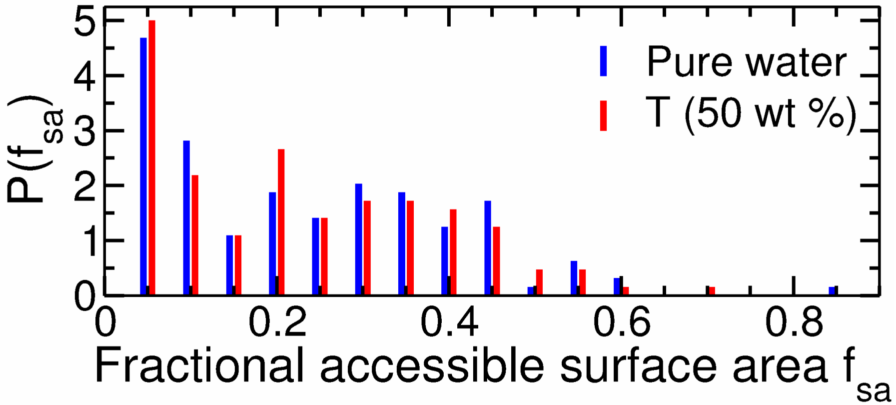


1 SASA = 70 Å^2

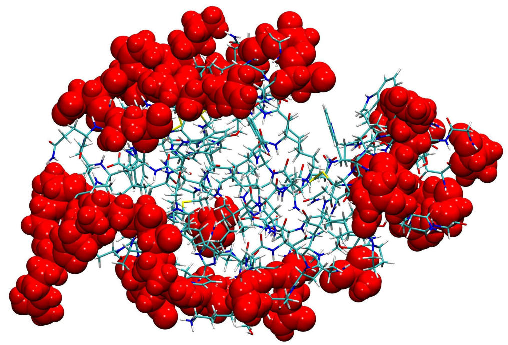
2 SASA = 347 Å^2

f_{sa} = 0.20



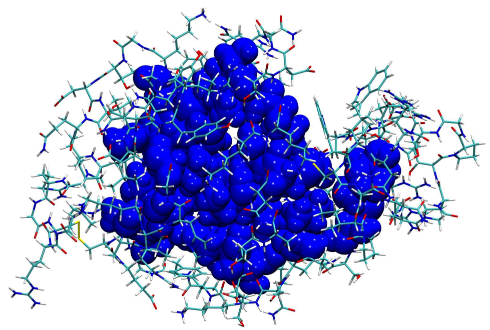


30 most exposed residues :



Surface residues

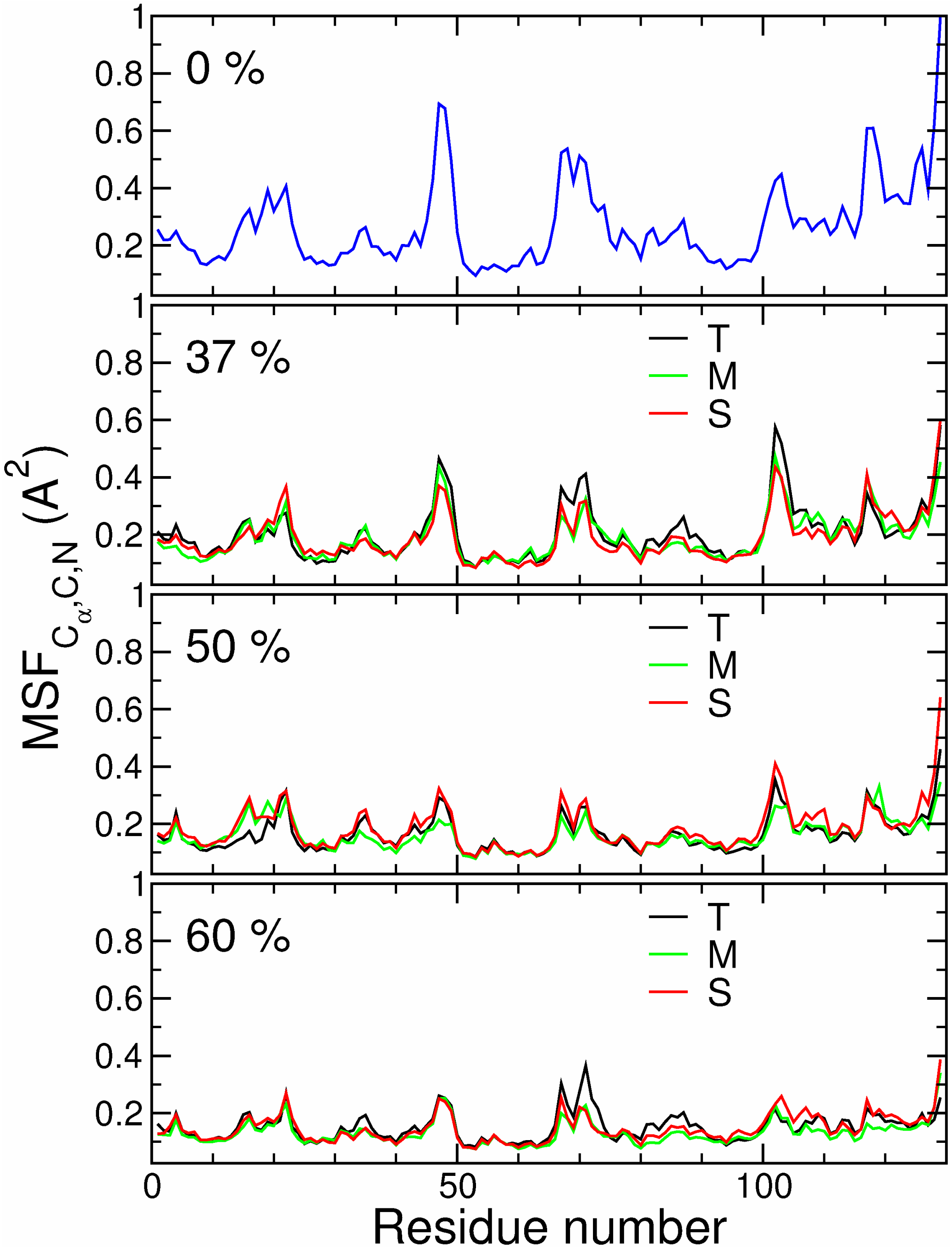
30 least exposed residues :



Core residues

No effect of sugars on the native conformation of lysozyme

Mean-square fluctuations <u^2>



- High heterogeneity of local environments
  - similar to residues f_{sa}
- Addition of sugars
  - significant reduction of <u^2>
    - high T°-shift of the denaturation temperature T_m
  - No change of the global shape
    - no preferential interaction with specific parts
  - Similar effect on lysozyme

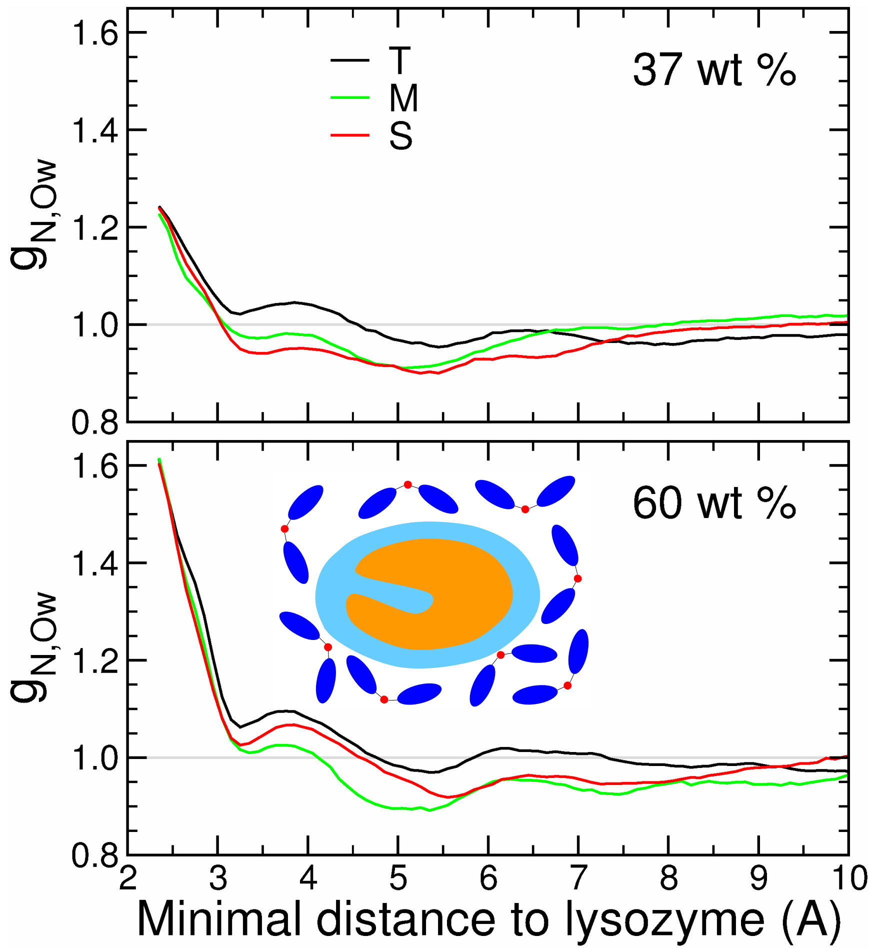
Local concentration of water around lysozyme

$$g_{N,Ow}(r) = \frac{\dfrac{n_{Ow}(r)}{n_{Ow}(r)+n_{Os}(r)}}{\dfrac{N_{Ow}}{N_{Ow}+N_{Os}}}$$

n/N : local/total numbers of atoms

r : minimal distance to any heavy atom of lysozyme

- $g_{N,Ow} > 1$ → excess of water
- $g_{N,Ow} < 1$ → lack of water

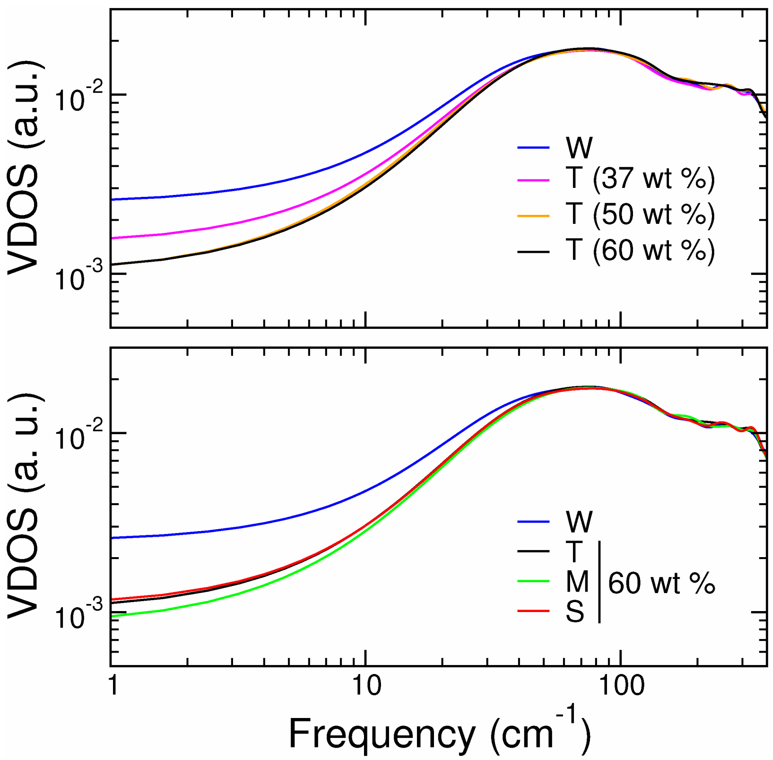


- preferential exclusion of sugars
- agreement with the *preferential hydration* hypothesis [Timasheff, *Biochem.* 41 (2002),89]

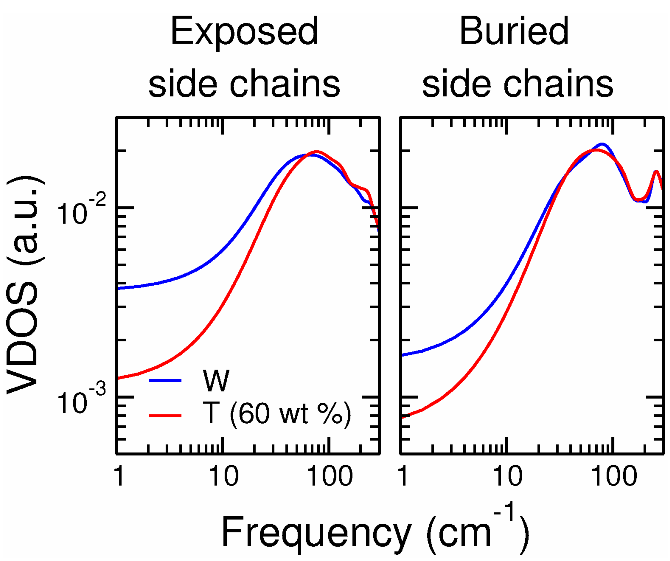
- T : slightly more excluded → larger hydration number n_H (T: 7.95, M : 6.50, S: 6.33)

[Kawai et al., Cryobiol. 29 (1992), 599]

Vibrational density of states (1)
Lysozyme

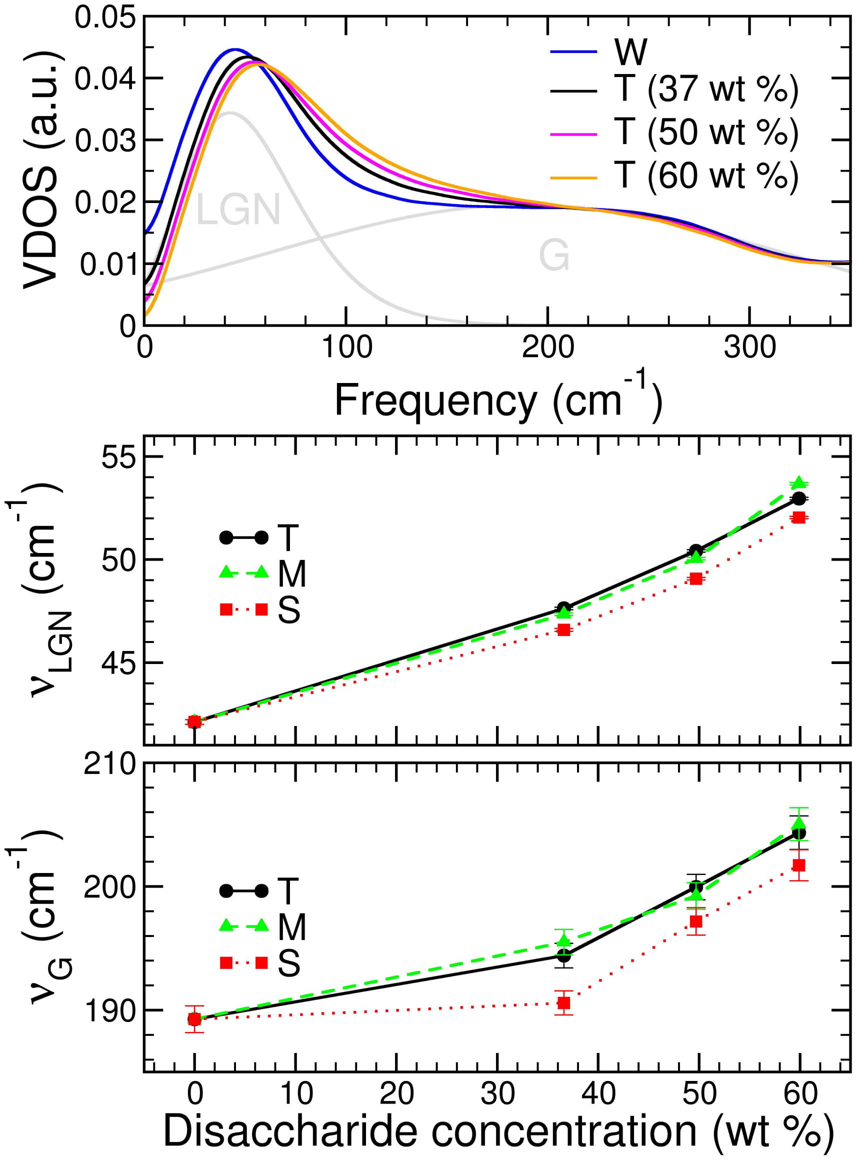


- Decrease of the VDOS with sugar concentration
  - → more rigid lysozyme
  - → stabilization at high temperatures
  - → Small differences between sugars, within simulation accuracy



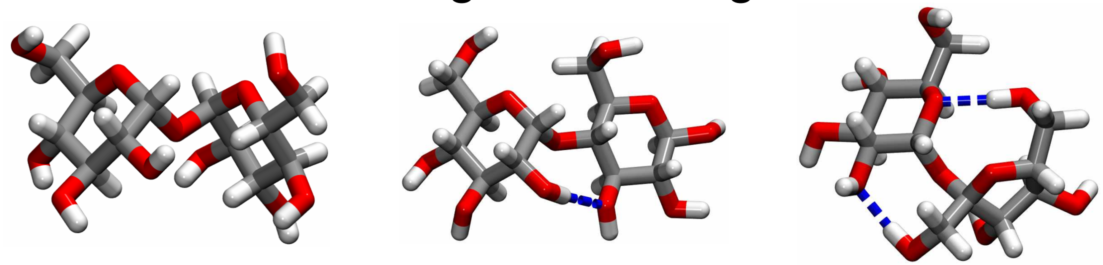
- Reduced VDOS for buried residues
  - → lower mobility, in line with $\langle u^2 \rangle$
- Lower effect of sugars on buried residues
  - → indirect influence

Vibrational density of states (2)
Water



- Broadening and high freq. shift of the 1st peak
  - → Increased **heterogeneity** and **rigidity** of water local environments

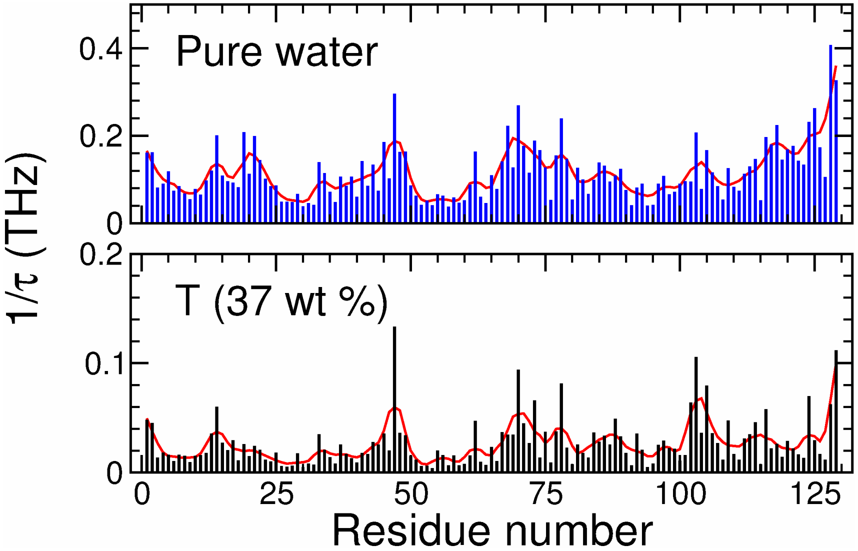
- Fit with a log-normal (LGN) and a gaussian (G) functions :
  - → strengthening of the water HB network
  - → T~M > S
    - Lower nb of sugar-water HBs
    - Larger nb of sugar intra HBs

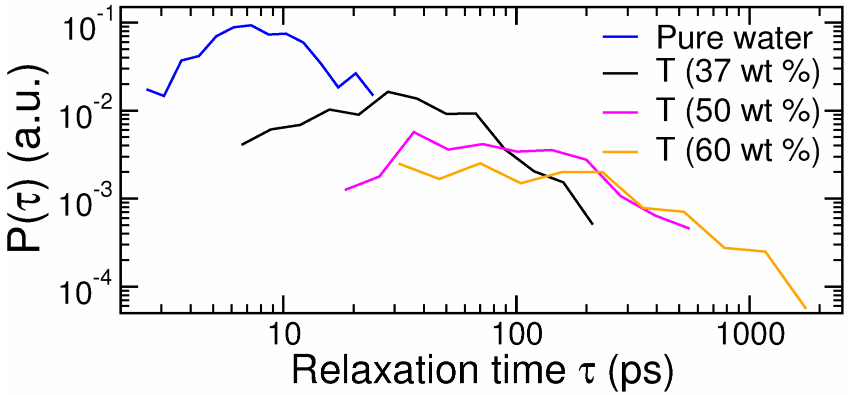


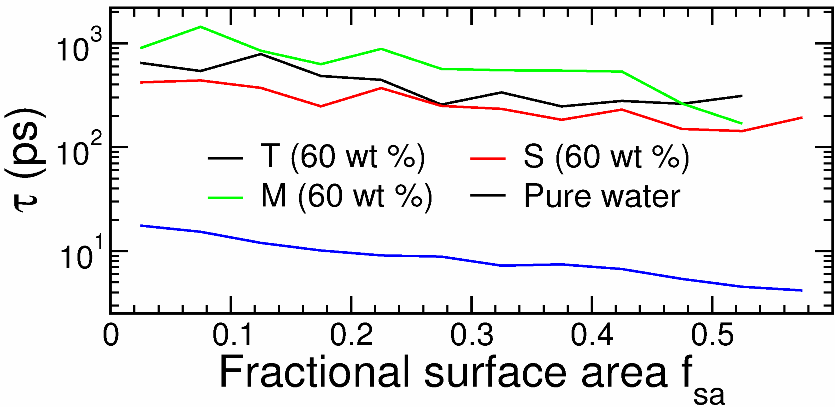
T M S

- Differences between sugars too small to be observed on the protein VDOS

Relaxation properties







τ = 1/*e* decay time of the incoherent intermediate scattering function S_{inc}(Q=2.29 Å^{-1},t)

☑ Distributions similar to f_{sa} and $\langle u^2 \rangle$

- $\tau_{core} > \tau_{surface}$ (as expected)

☑ Significant slowing down of lysozyme's :

- Global translation/rotation
- Internal dynamics ($\langle u^2 \rangle$)

☑ On average :

- $\log(\tau) \sim f_{sa}$
- τ (M) > τ(T) > τ(S)
  - sugar cluster size $\langle n_S \rangle$: M > T > S

Conclusions

- Influence of sugars on lysozyme
  - ☑ none on its native conformation → stabilizing solutes
  - ☑ significant reduction of $<u^2>$ → shift of its heat denaturation temperature T_m
  - ☑ preferential exclusion of sugars → non–specific interaction
  - ☑ strengthening of the water HB network → protein-solvent dynamical coupling via its water hydration shell
  - ☑ more important for exposed than for buried residues

- Comparison between sugars
  - ☑ rather similar effects on native lysozyme

Similar effects on denatured lysozyme ? → MD simulations of denatured lysozyme needed

Selected papers

- *Analysis of sugar bioprotective mechanisms on the thermal denaturation of lysozyme from Raman scattering and differential scanning calorimetry investigations*, A. Hédoux, J.-F. Willart, R. Ionov, F. Affouard, Y. Guinet, L. Paccou, A. Lerbret, M. Descamps, *J. Phys. Chem. B* **2006**, *110*, 22886-22893.

- *How do trehalose, maltose, and sucrose influence some structural and dynamical properties of lysozyme ? Insight from molecular dynamics simulations*, A. Lerbret, P. Bordat, F. Affouard, A. Hédoux, Y. Guinet, M. Descamps, *J. Phys. Chem. B* **2007**, *111*, 9410-9420.

- *Molecular dynamics simulations of lysozyme water/sugar solutions*, A. Lerbret, F. Affouard, P. Bordat, A. Hédoux, Y. Guinet, M. Descamps, *Chem. Phys.* **2008**, *345*, 267-274.

- *Low-frequency vibrational properties of lysozyme in sugar aqueous solutions: a Raman scattering and molecular dynamics simulation study*, A. Lerbret, F. Affouard, P. Bordat, A. Hédoux, Y. Guinet, M. Descamps, *J. Chem. Phys.* **2009**, *131*, 245103.