Sucrose and trehalose as agents for stabilization of proteins: insights from atomistic molecular dynamics simulations

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Outline

- The problem of protein aggregation or instable proteins

- Sucrose and trehalose: what is their use and why they are so important

- Selected proteins in this work

- Atomistic molecular dynamics (MD) simulations and their use for investigation of protein aggregation

- Simulations done in this project and their results

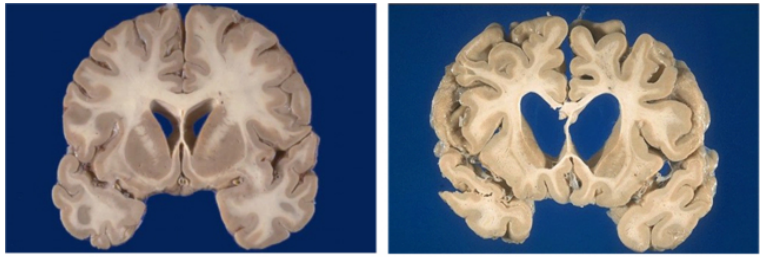
- Conclusions & Future work (experimental, computational)

The importance of prevention of protein aggregation

Neurodegenerative diseases

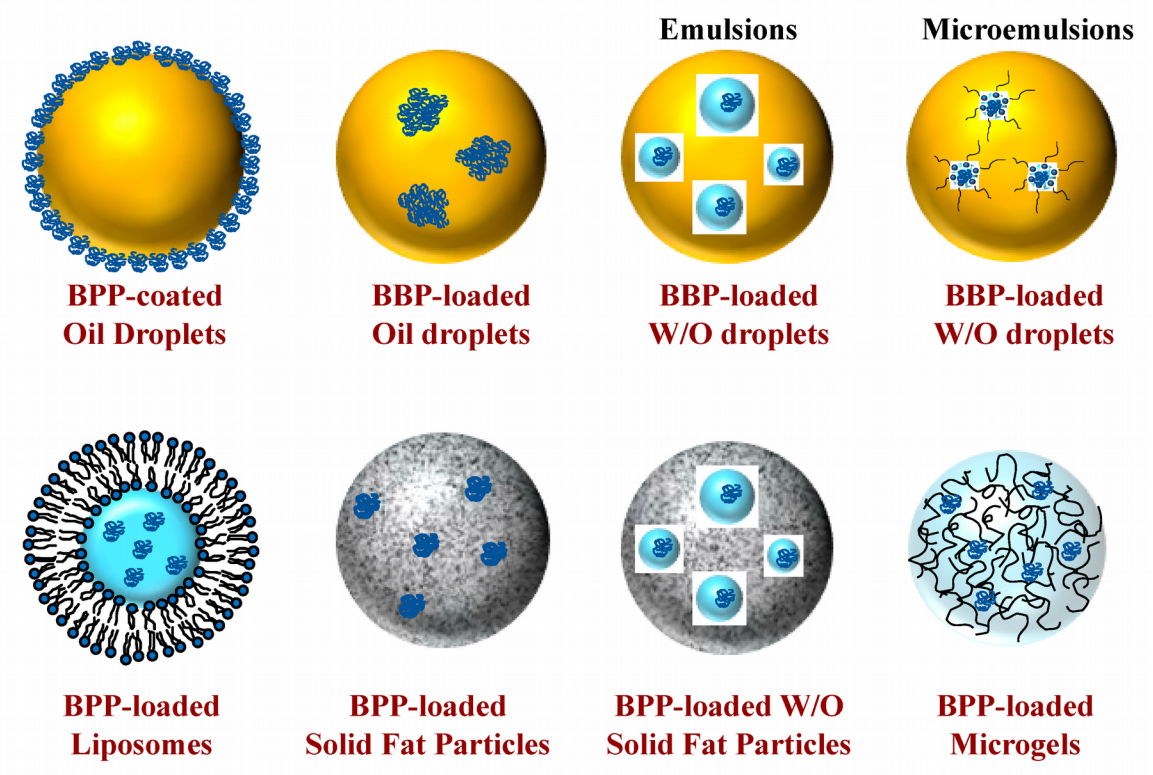
Alzheimer's disease

Huntington's disease



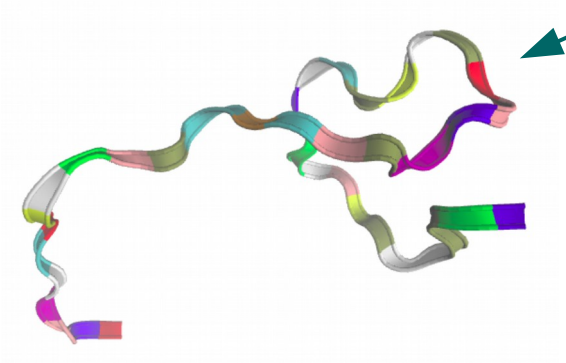
Protein aggregation is a cause of these diseases, according to amyloid hypothesis

Drug delivery & food: it is important to deliver the "right" structure

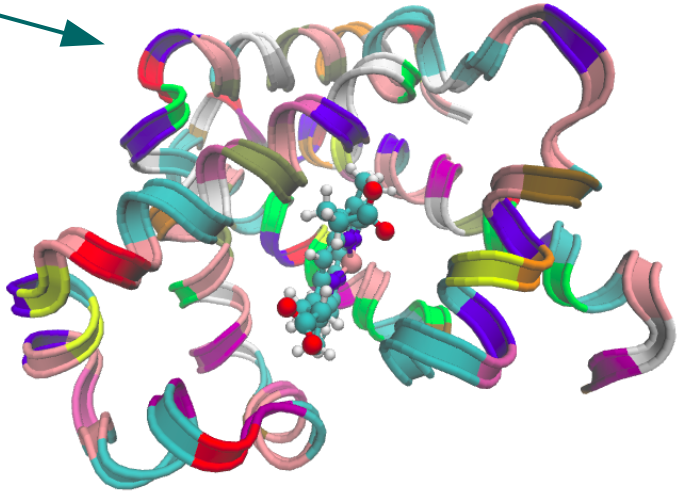


Images: Courtesy Google

Aβ(1-42) & Myoglobin



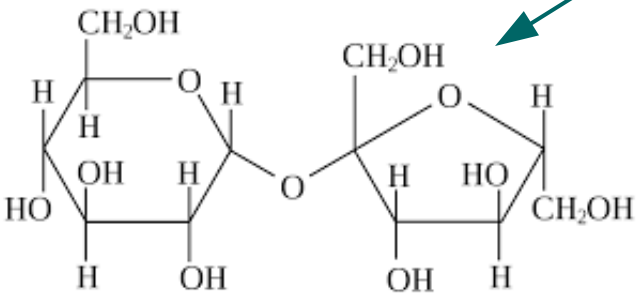
- Is a part of amyloid precursor protein
- Known to be toxic to neurons and, therefore, be a cause for neurodegenerative diseases as well as amyloidosis

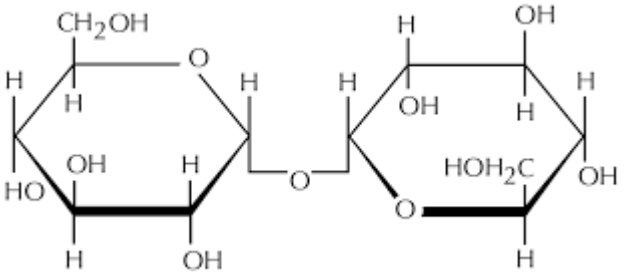


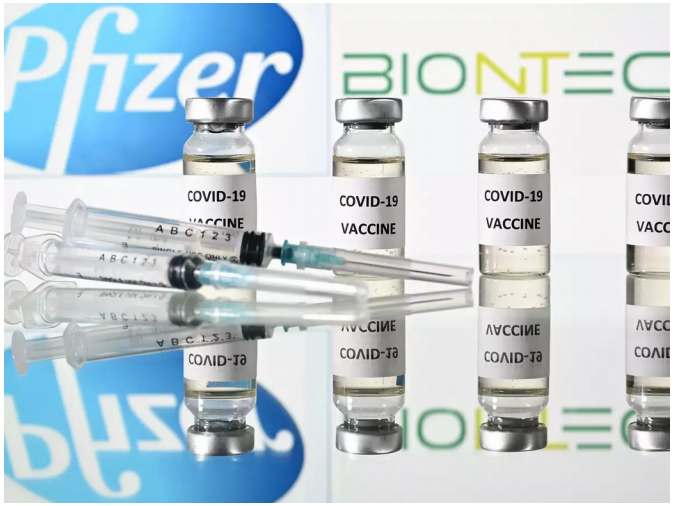
- Iron- and oxygen-binding protein
- Found in the skeletal muscle tissue in almost all of mammals
- Can also form amyloid fibrils at certain conditions

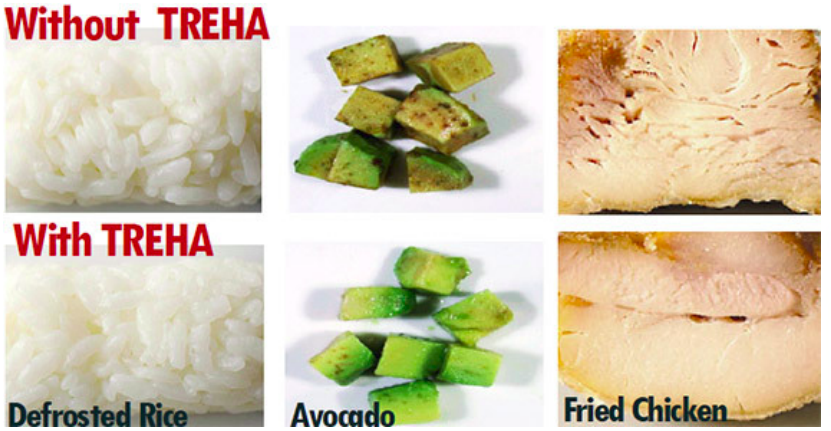
Two different proteins, different structures and sizes. Interesting to compare the behavior in the aqueous solution together with sugars

Sucrose & Trehalose









Sugars are already widely used as preservatives in various products!

Images: Courtesy Google

Earlier studies with scattering techniques

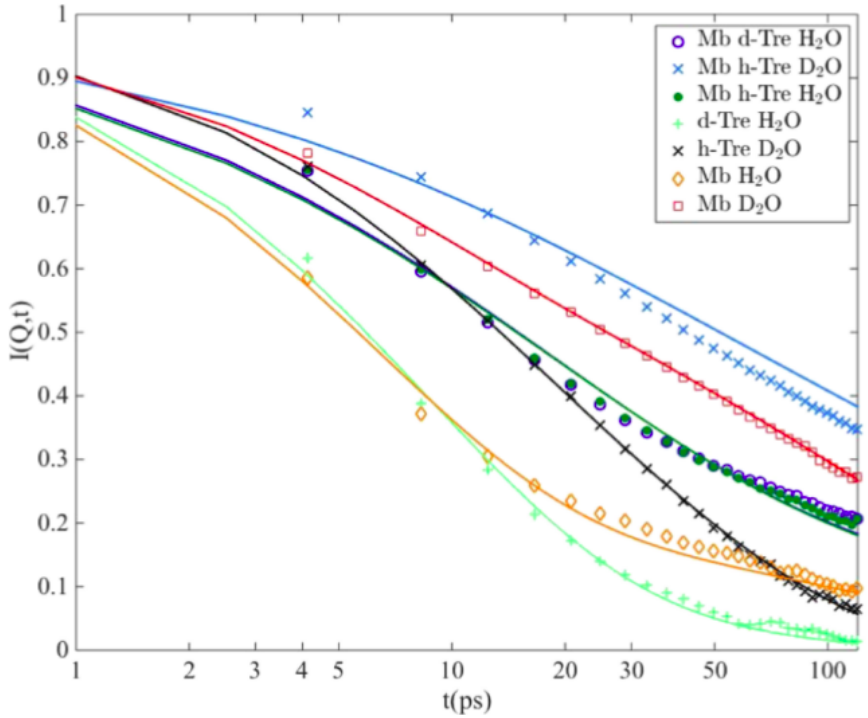


Figure 3. Intermediate scattering functions for all samples at a representative value of Q = 1.06 Å^{-1} and 300 K. Symbols represent experimental data and solid lines represent fits obtained by solving the set of nonlinear equations in eq 2 based on the use of stretched exponentials.

Trehalose stabilized myoglobin in aqueous solution.
(C.Olsson et al., J. Phys. Chem. B 2019, 123, 3679−3687)

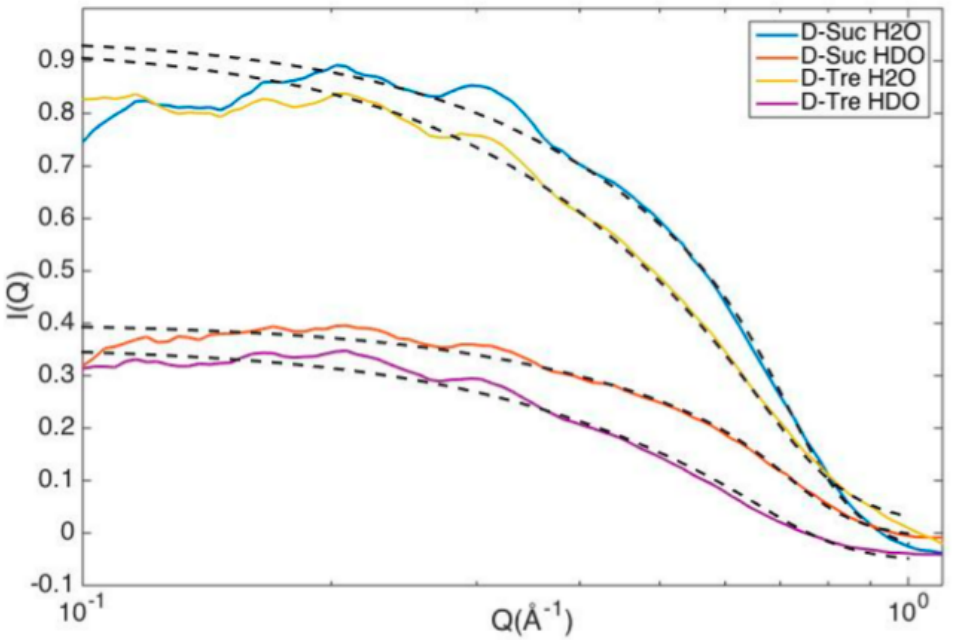


Figure 4. Small-angle data fitted for samples containing deuterated disaccharides in either H_2O or HDO. Dotted lines show the fits to the data.

Structural studies revealed small differences in structures of aqueous solutions of sucrose and trehalose .
(C.Olsson et al., J. Phys. Chem. B 2020, 124, 3074−3082)

Atomistic MD simulations

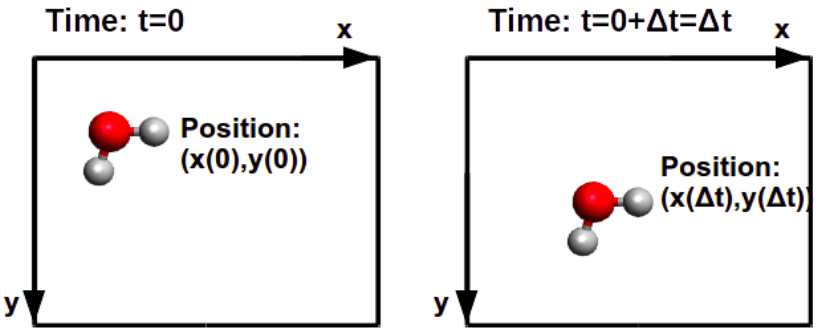
Newtonian equations of motion

$$\dot{r}_i = v_i \quad \text{(velocity)}$$

$$\dot{v}_i = \frac{F_i(r)}{m_i} \quad \text{(acceleration)}$$

$$F_i(r) = -\nabla_i U(r)$$

(force) (energy)

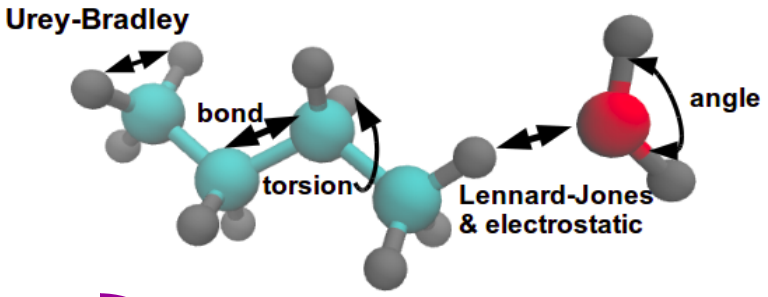


Position update in time

Here: r_i – coordinates of a particle i, m_i – mass of a particle i

MD simulations can give valuable information about structures and dynamics of systems. They can help to understand reasons behind certain behaviors of compounds.

The force fields CHARMM36 (protein) and GAFF (sugars)



$$U_{bonds} = \sum_{bonds} k_r (r - r_0)^2$$

$$U_{Urey-Bradley} = \sum_{Urey-Bradley} k_b (b - b_0)^2$$

$$U_{torsions} = \sum_{torsions} k_\phi (1 + \cos(n\phi - \delta))$$

$$U_{angles} = \sum_{angles} k_\theta (\theta - \theta_0)^2$$

Bonded interactions

$$U_{Lennard-Jones} = \sum_{i,j \neq i} 4\epsilon_{ij} \left[\left(\frac{\sigma_{ij}}{r_{ij}} \right)^{12} - \left(\frac{\sigma_{ij}}{r_{ij}} \right)^{6} \right]$$

$$U_{el.st.} = \sum_{i,j \neq i} \frac{q_i q_j}{4\pi\epsilon_0 r_{ij}}$$

Non-bonded interactions

$$U_{force-field} = U_{bonds} + U_{angles} + U_{torsions} + U_{Urey-Bradley} + U_{Lennard-Jones} + U_{el.st.}$$

GAFF has a similar description, but no Urey-Bradley term.

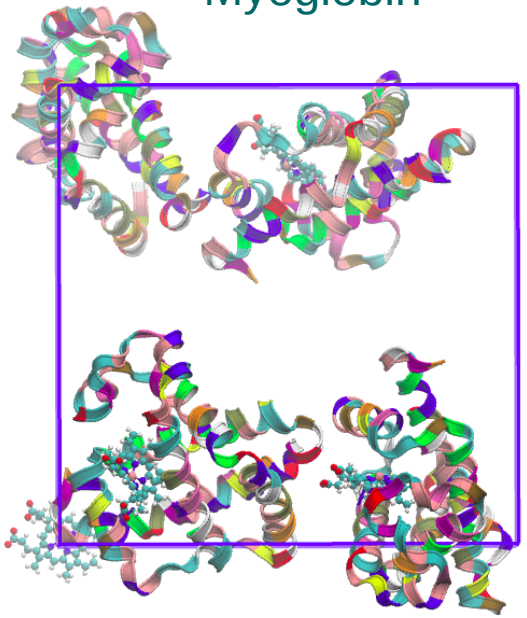
Simulations' set-ups

System	# of protein molecules	# of sugars	# of Na ions	# of Cl ions	# of water (TIP3p)
Myoglobin (no sugar)	4	204	8	0	7824
Myoglobin (sucrose)	4	204	8	0	7824
Myoglobin (trehalose)	4	204	8	0	7824
Aβ(1-42) (no sugar)	15	204	45	0	7824
Aβ(1-42) (sucrose)	15	204	45	0	7824
Aβ(1-42) (trehalose)	15	204	45	0	7824
Myoglobin (no sugar)	4	204	21	13	7824
Myoglobin (sucrose)	4	204	21	13	7824
Myoglobin (trehalose)	4	204	21	13	7824
Aβ(1-42) (no sugar)	15	204	58	13	7824
Aβ(1-42) (sucrose)	15	204	58	13	7824
Aβ(1-42) (trehalose)	15	204	58	13	7824

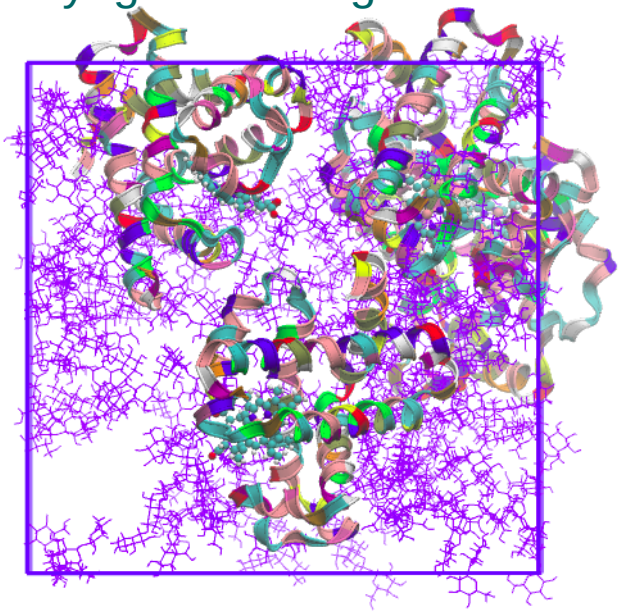
All MD simulations were equilibrated for 100 ns and ran for 1 μs in NPT ensemle with an isotropic pressure coupling scheme. The temperature was 298 K and the pressure was 1 atm.

Results: How the systems look like

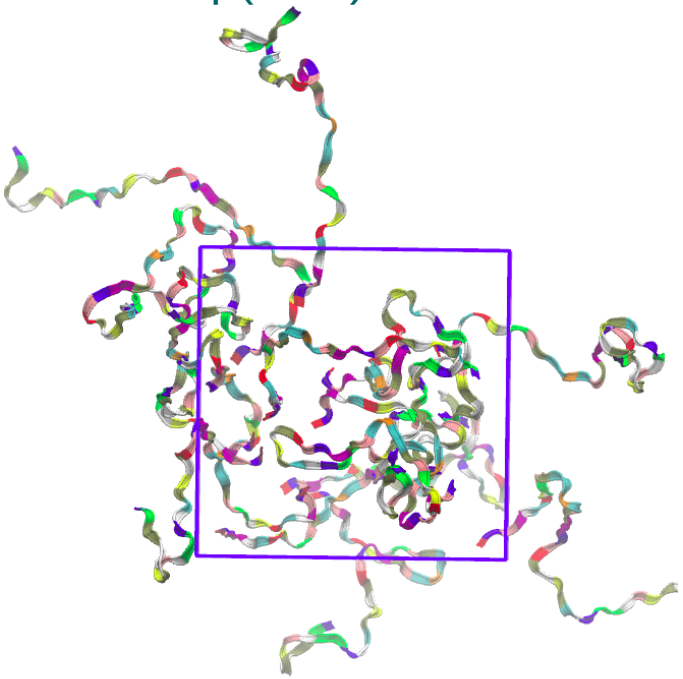
Myoglobin



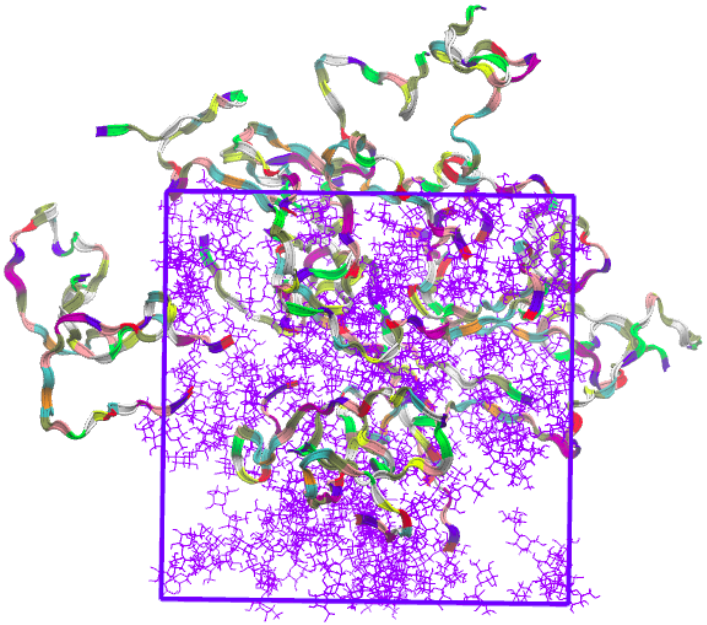
Myoglobin & sugar



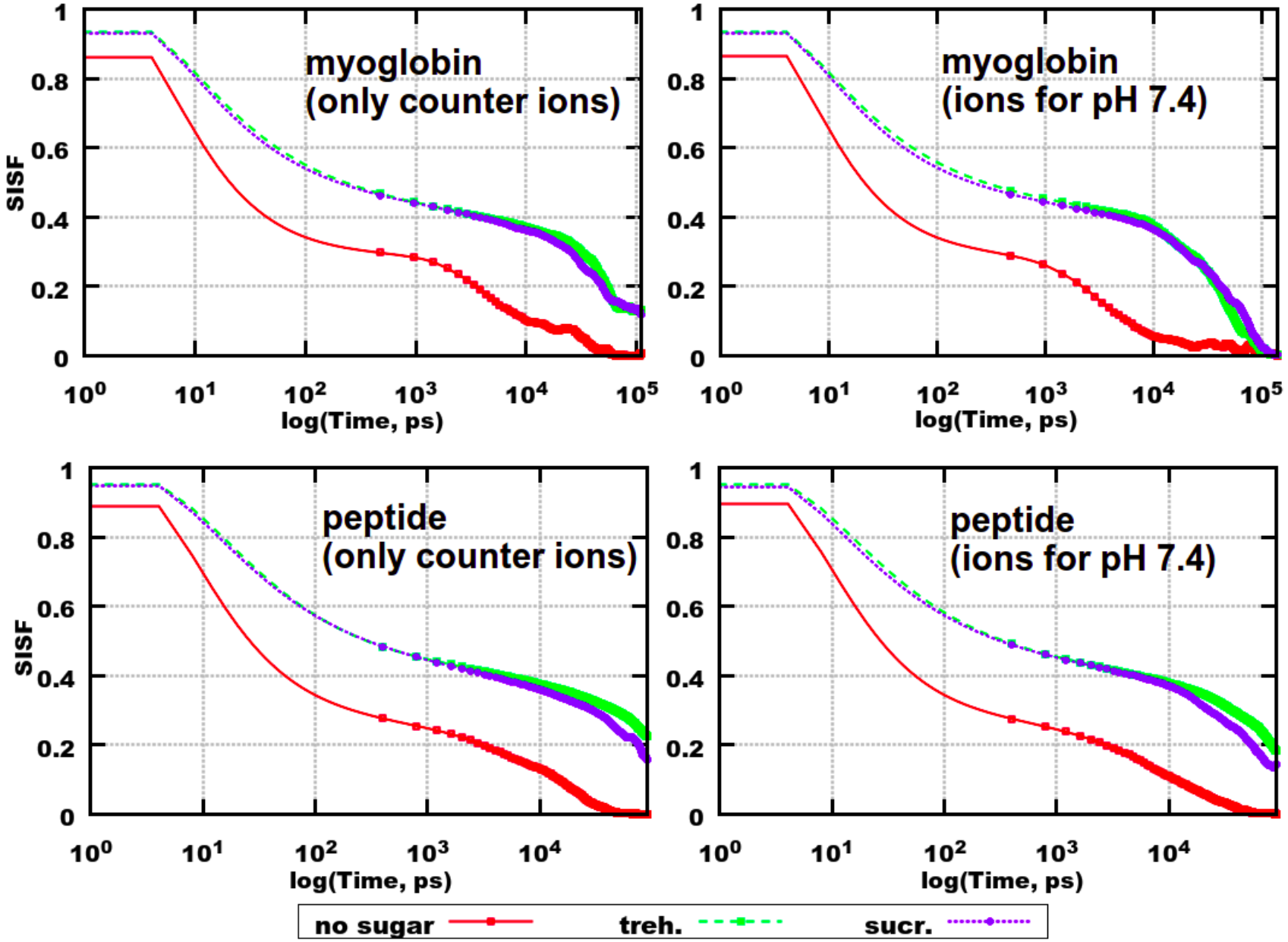
Aβ(1-42)



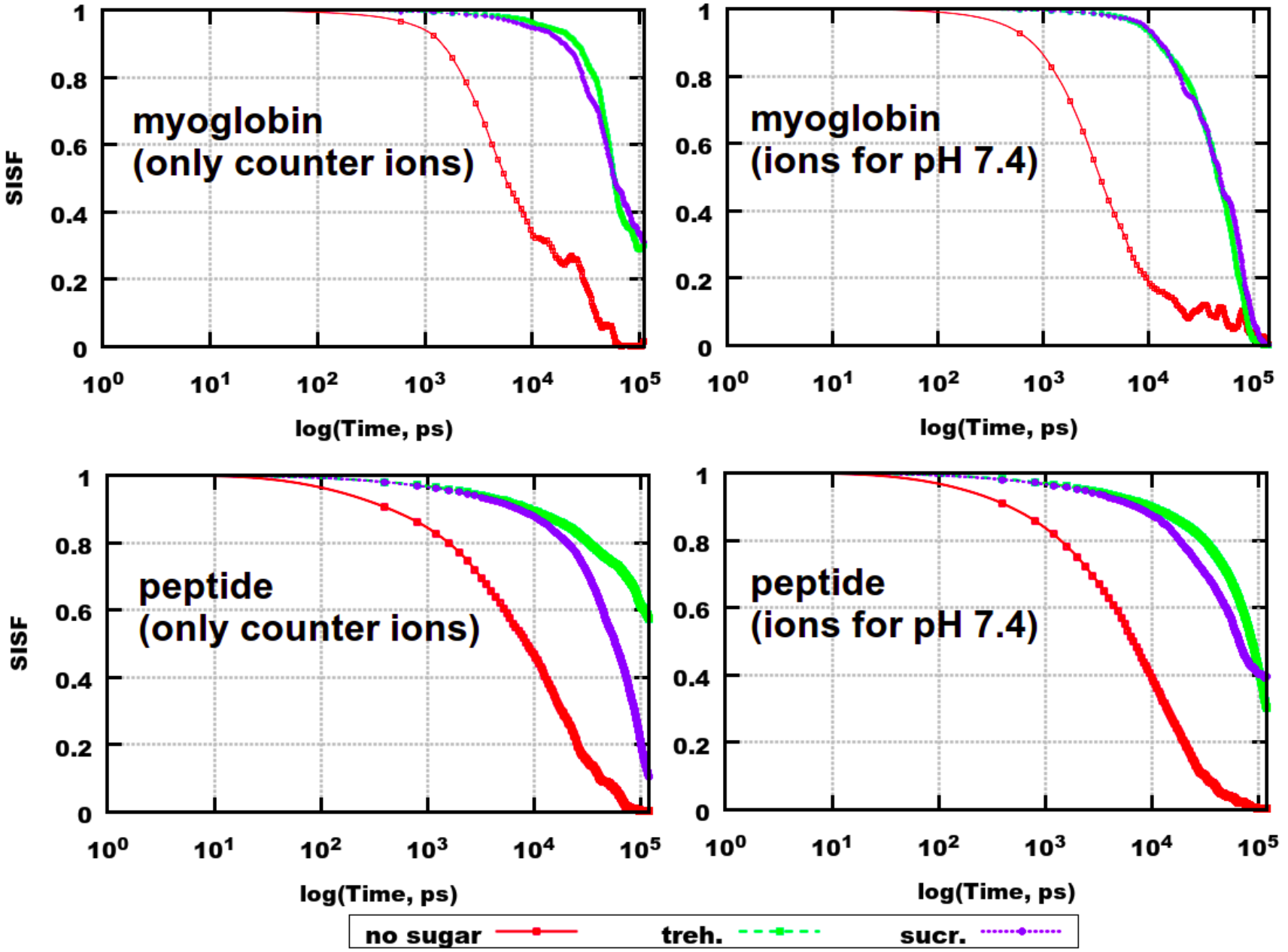
Aβ(1-42) & sugar



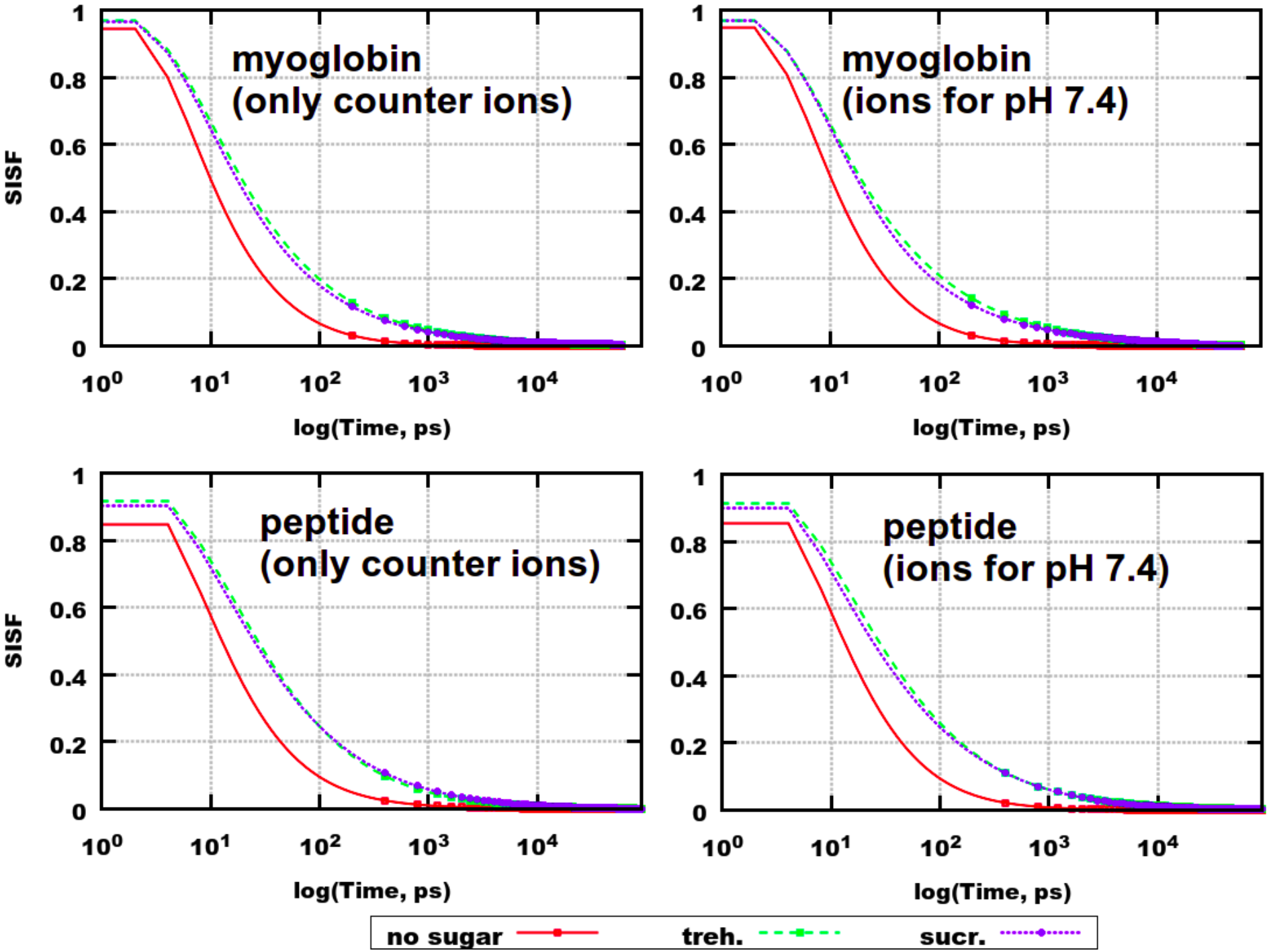
Results: Self-intermediate scattering functions (systems), q=1.52 Å^{-1}
The same q value was used for all other slides.



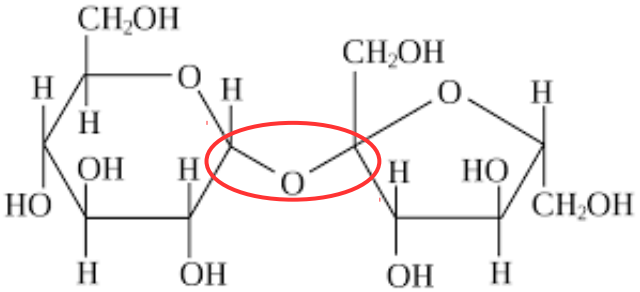
Results: Self-intermediate scattering functions (protein)

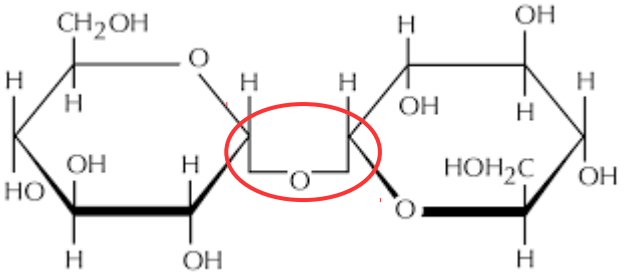


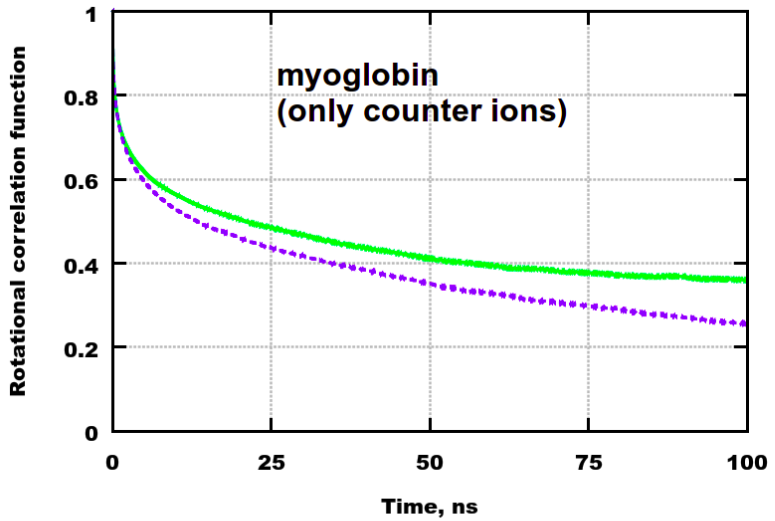
Results: Self-intermediate scattering functions (water)

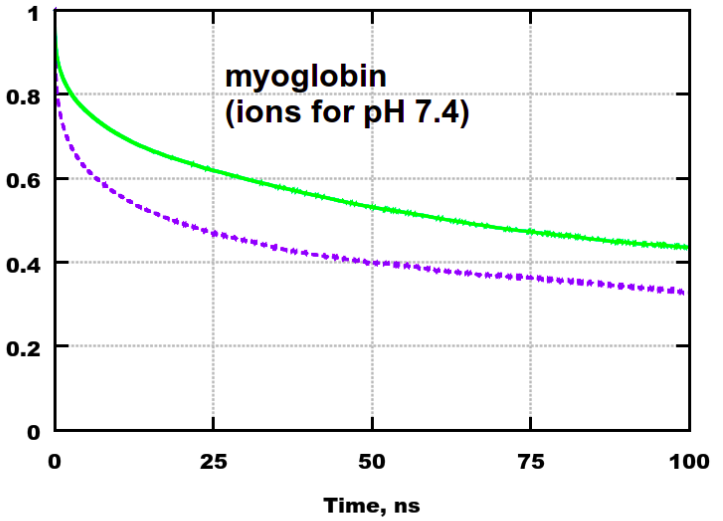


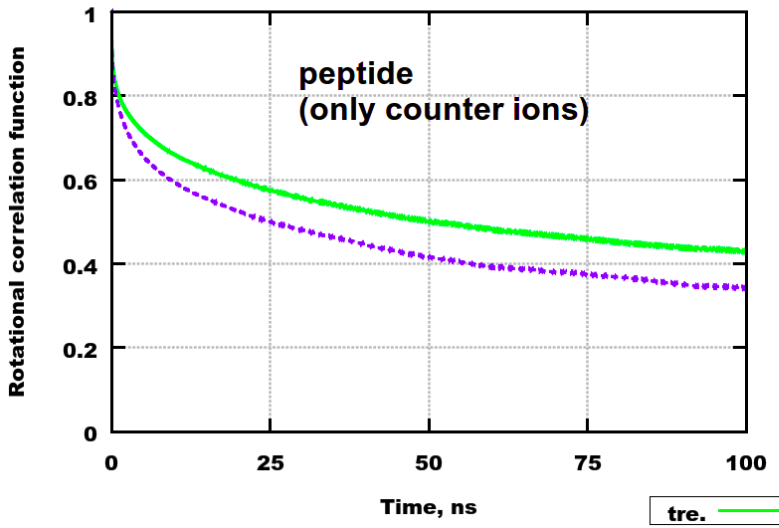
Results: Rotational correlation functions (2nd order Legendre polynomial)

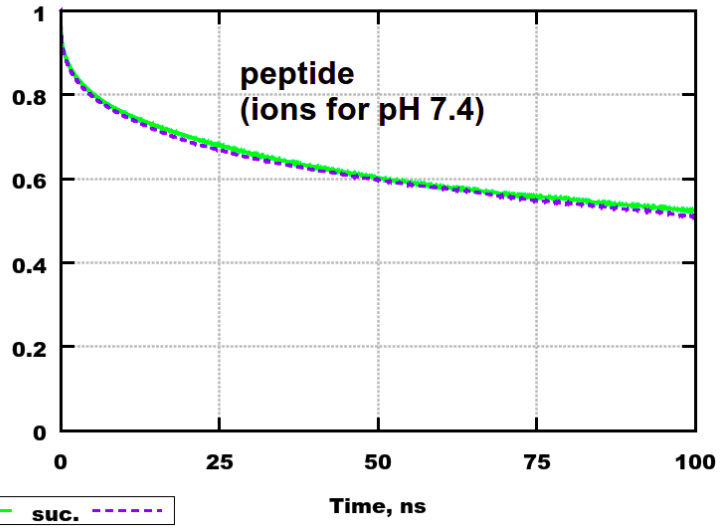






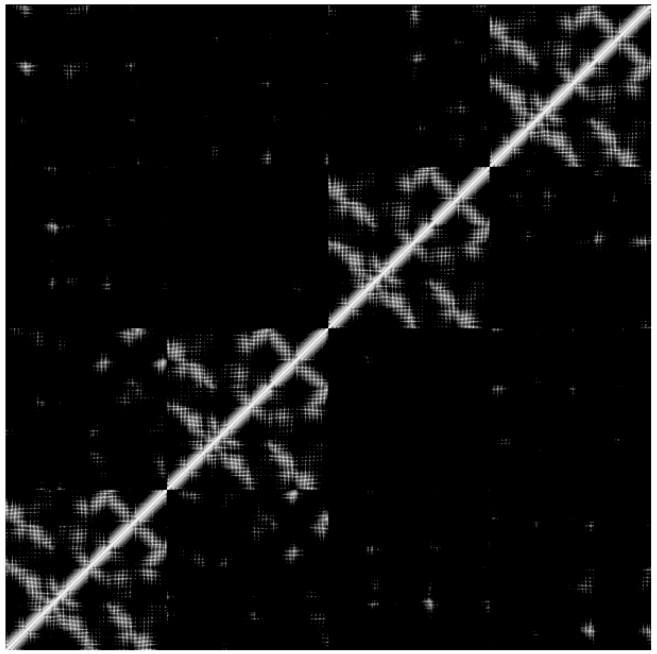




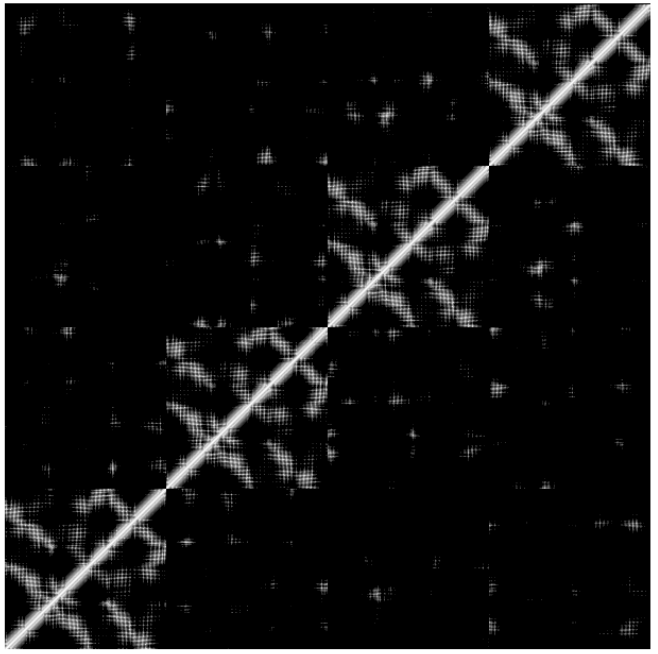


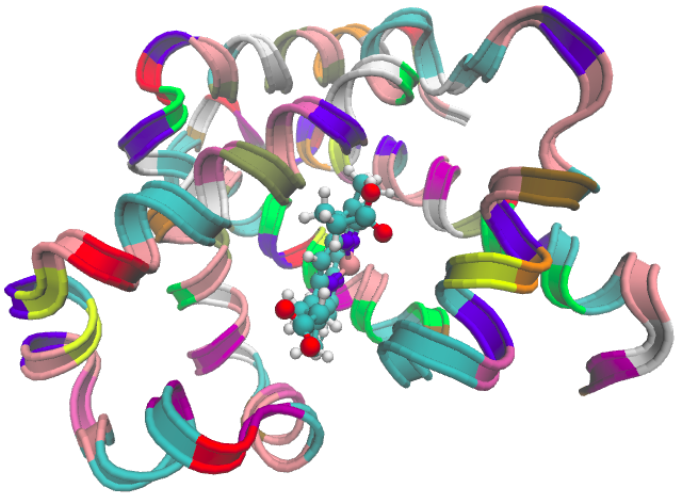
Results: Contact maps for myoglobin (last 50 ps)

SUCROSE

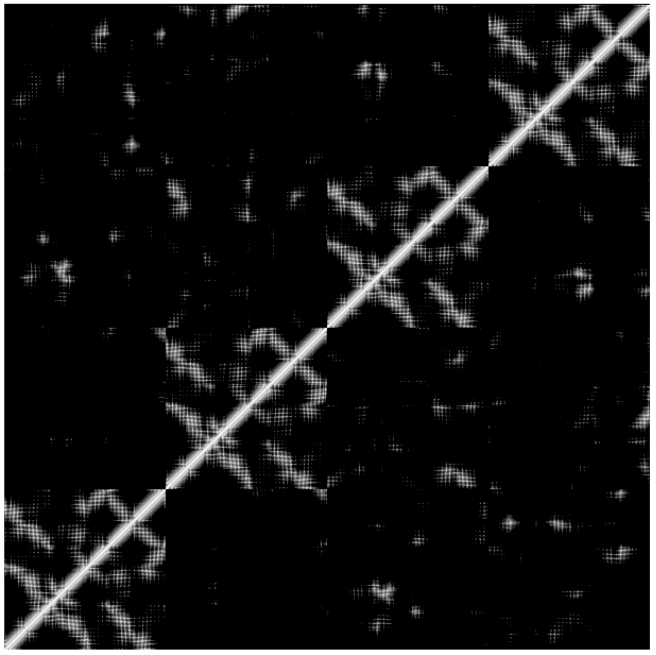


TREHALOSE





NO SUGAR



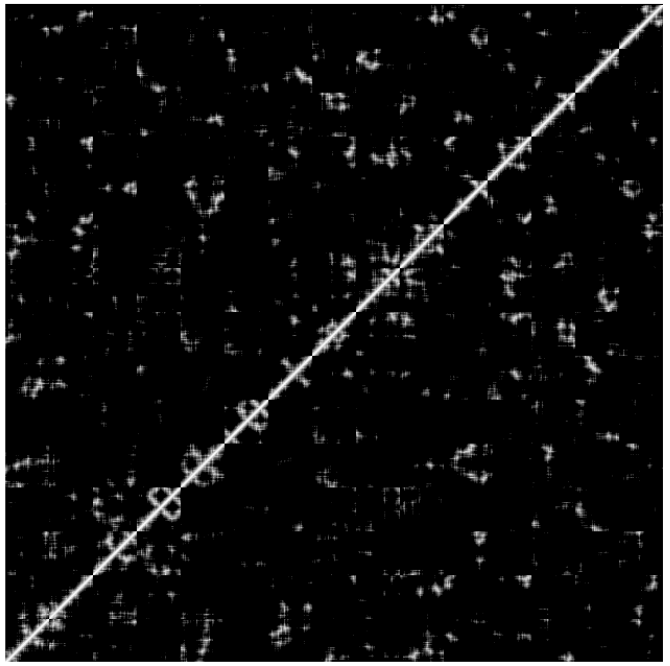
Contact maps show the number of contacts between various amino acid residues in proteins.

Everything on diagonal shows contacts for a single protein, while spots away from diagonal elements show contacts between separate proteins.

4 molecules of myoglobin can be clearly seen from those maps.

Results: Contact maps for Aβ(1-42) (last 50 ps)

SUCROSE



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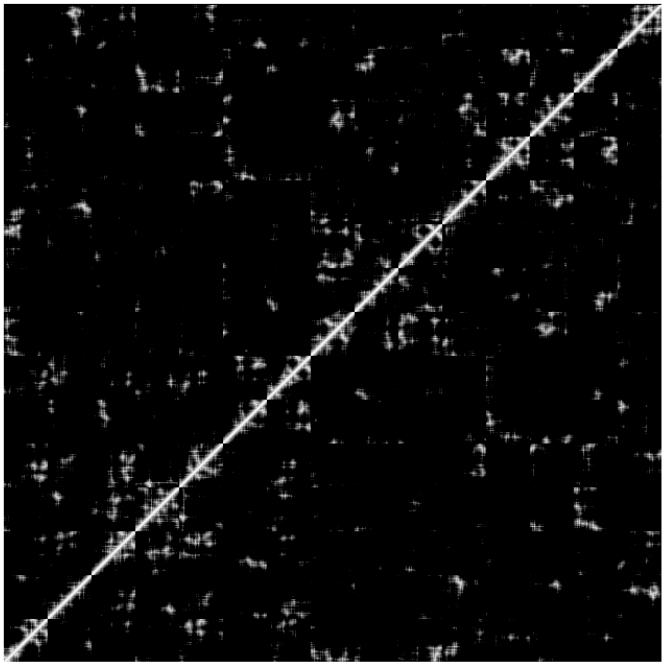

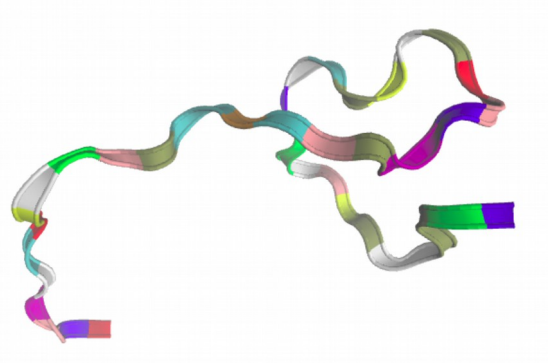

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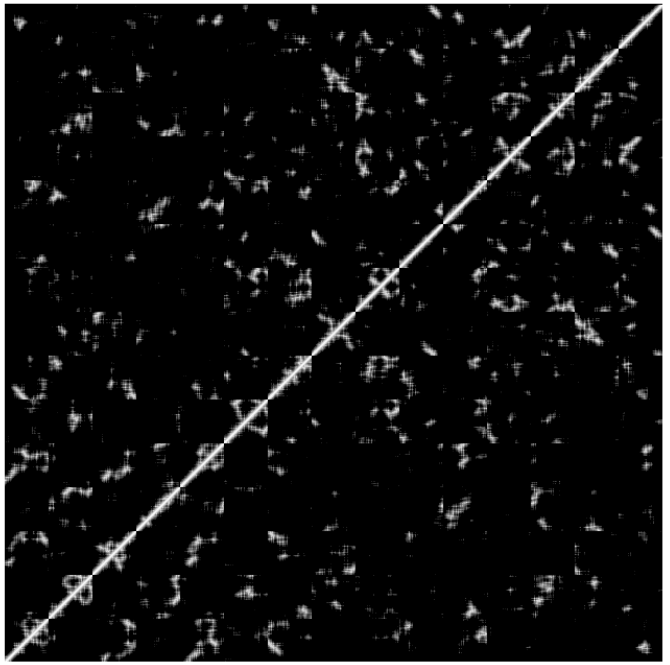
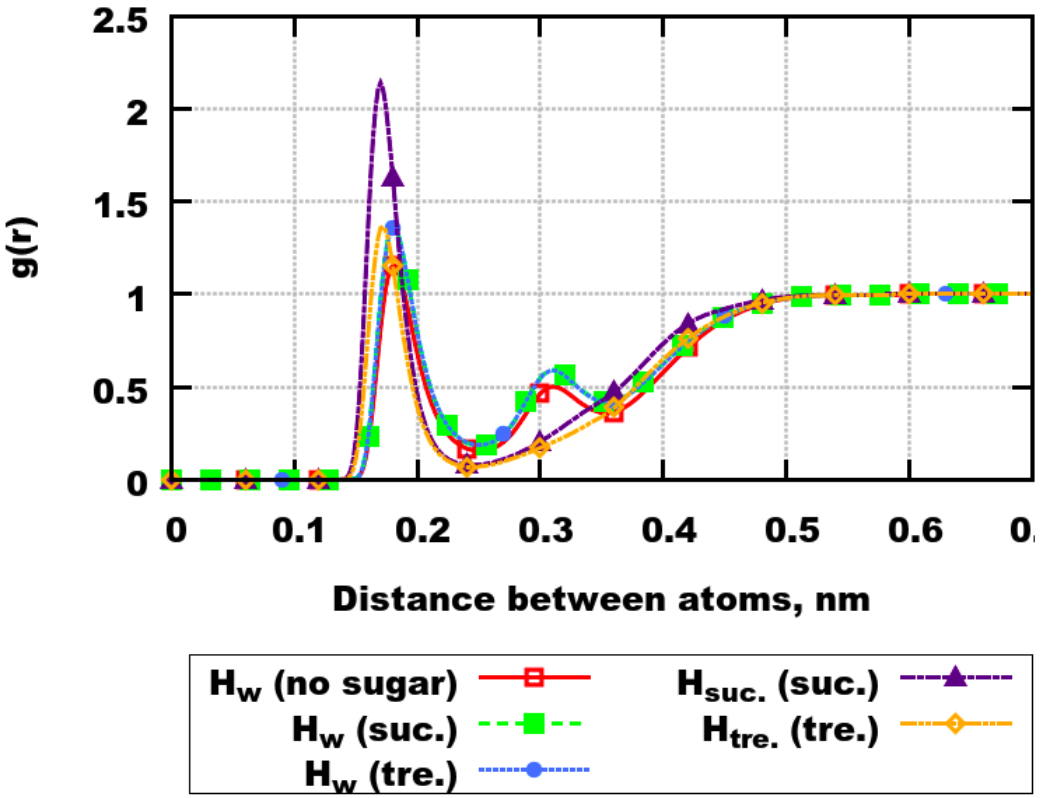
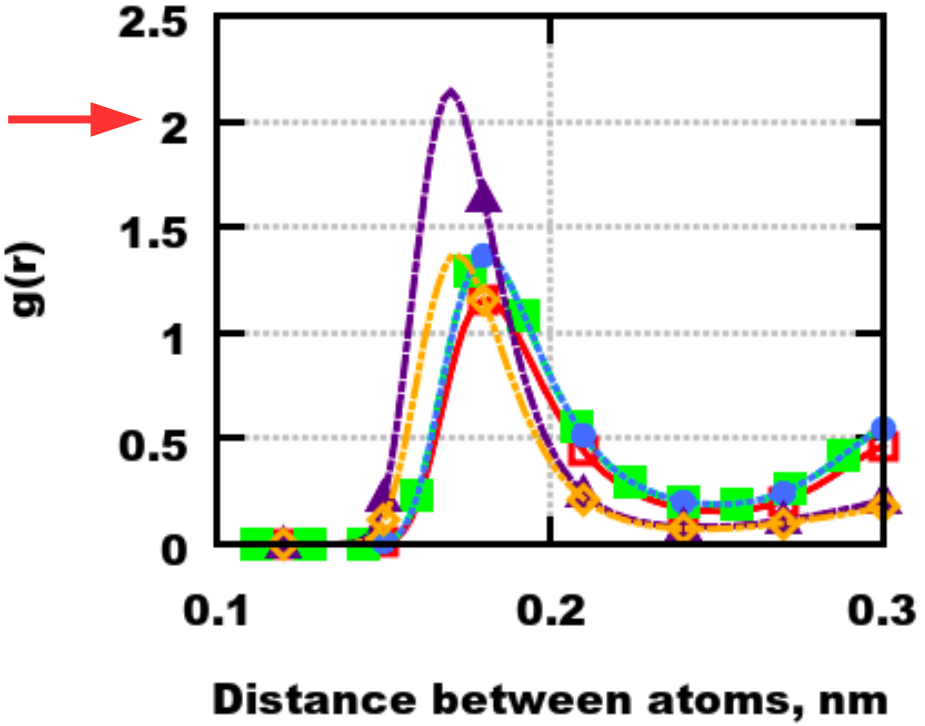
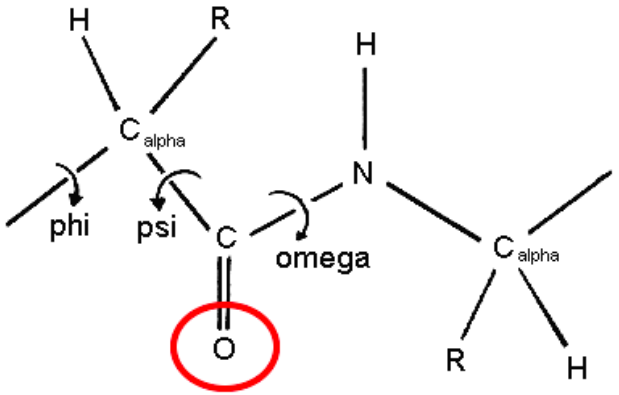


Here systems contained 15 peptides each.

Results: RDFs between carbonyl oxygens in Aβ(1-42) and hydrogens in water and hydroxyl group of sugars







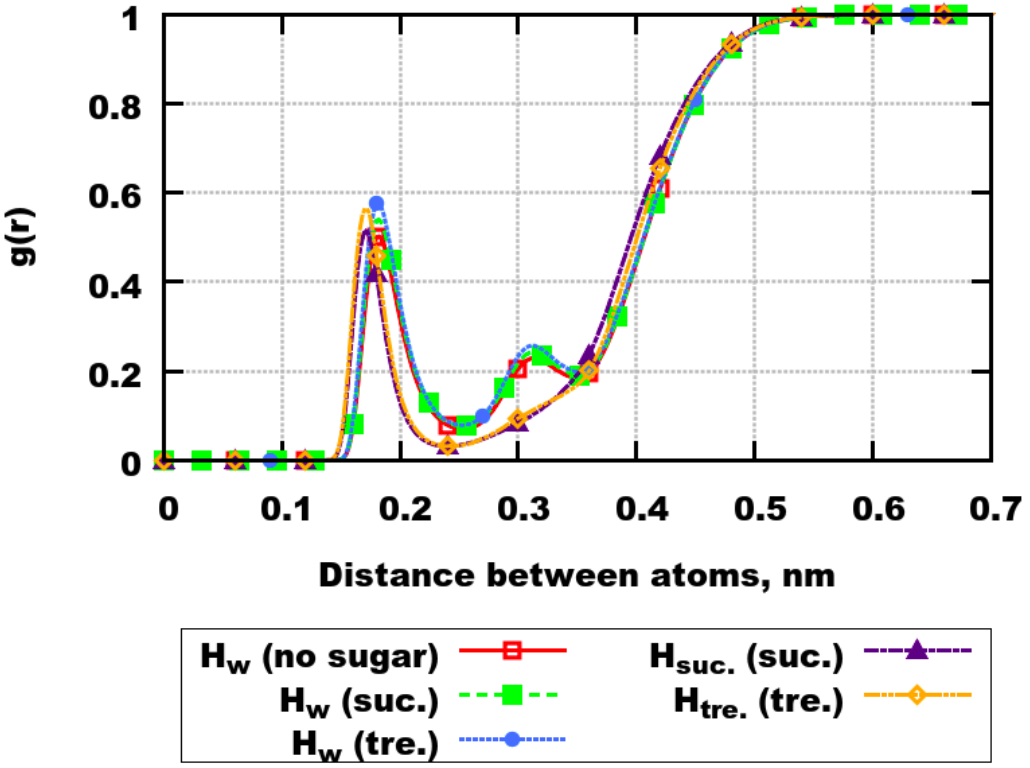
Here:

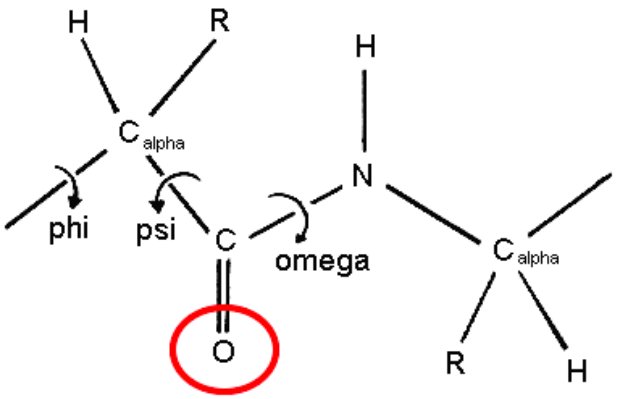
H_w – hydrogens from water molecules

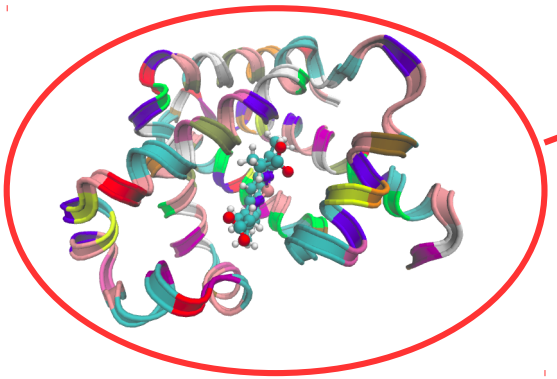
$H_{suc.}$ - hydrogens from hydroxyl group in sucrose

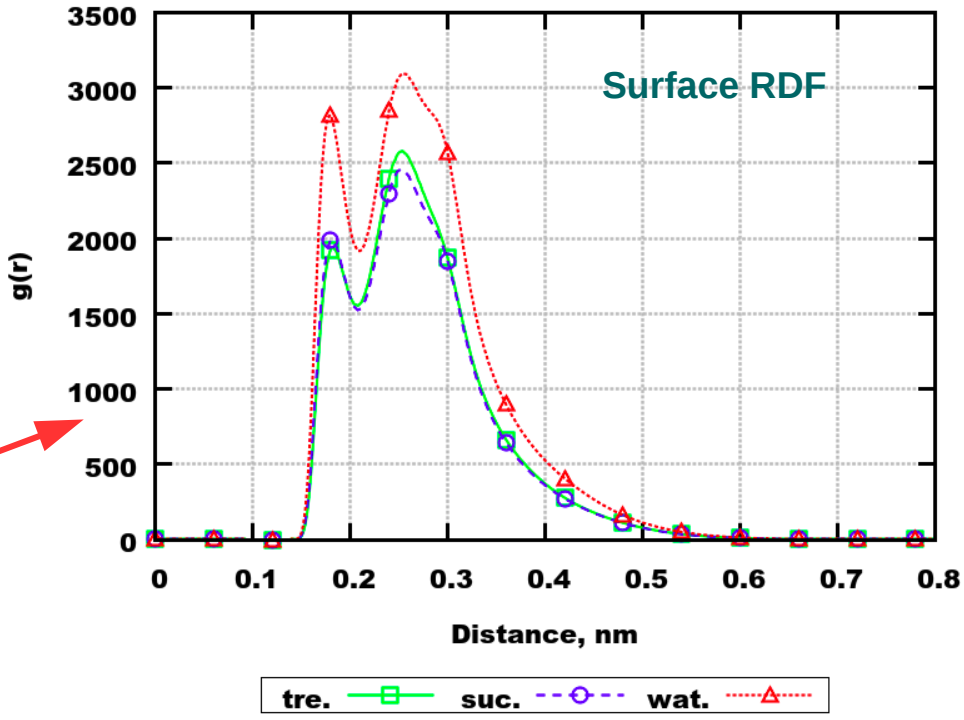
$H_{tre.}$ - hydrogens from hydroxyl group in trehalose

Part 2: RDFs between carbonyl oxygens in myoglobin and hydrogens in water and hydroxyl group of sugars









Conclusions & Future work

- From the resulting simulations trehalose seems to be the best stabilizing agent for aqueous solutions of proteins in systems containing small amounts of water

- Such properties can be due to small structural differences of two sugars

- Both sugars are good for inhibiting the protein aggregation in both cases of myoglobin and Aβ(1-42). However, in case of the peptide in order to reach the good separation higher concentrations of sugar might be needed.

*** As future studies we are planing to do SANS and spin-echo experiments in order to compare with existing simulations.

*** Also free energy calculations are needed in order to make conclusion from the thermodynamical point of view.

Acknowledgements

- My supervisor, professor Jan Swenson

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Thank you for listening!
Questions?