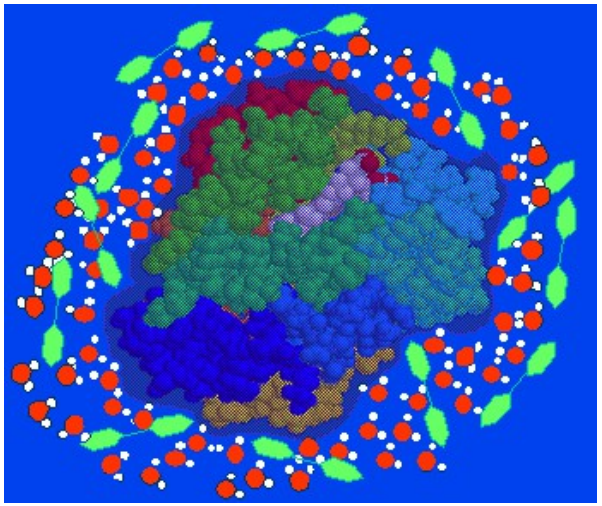
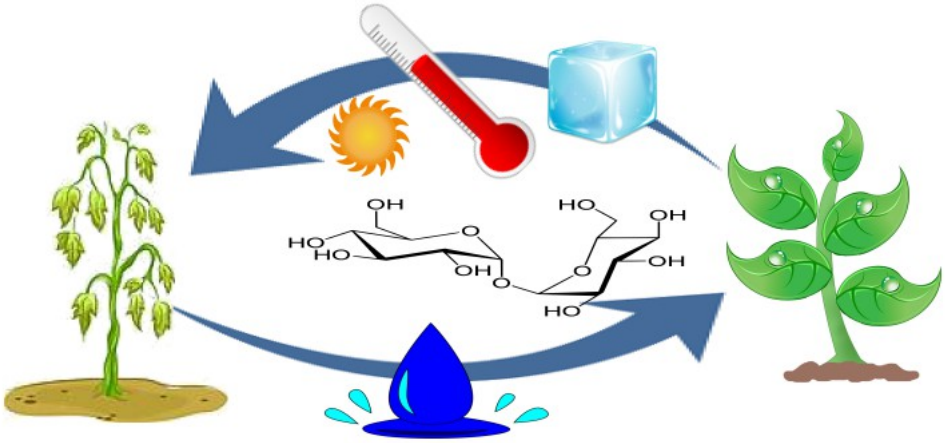
Proteins in Saccharides Matrices: the Trehalose Peculiarity and the role of water





Grazia Cottone
Dipartimento di Fisica e Chimica, Università di Palermo
School of Physics, University College Dublin

WATER AND WATER SYSTEMS
3rd Course of the ERICE School "NEUTRON SCIENCE AND INSTRUMENTATION"
Erice,July 21-31, 2016

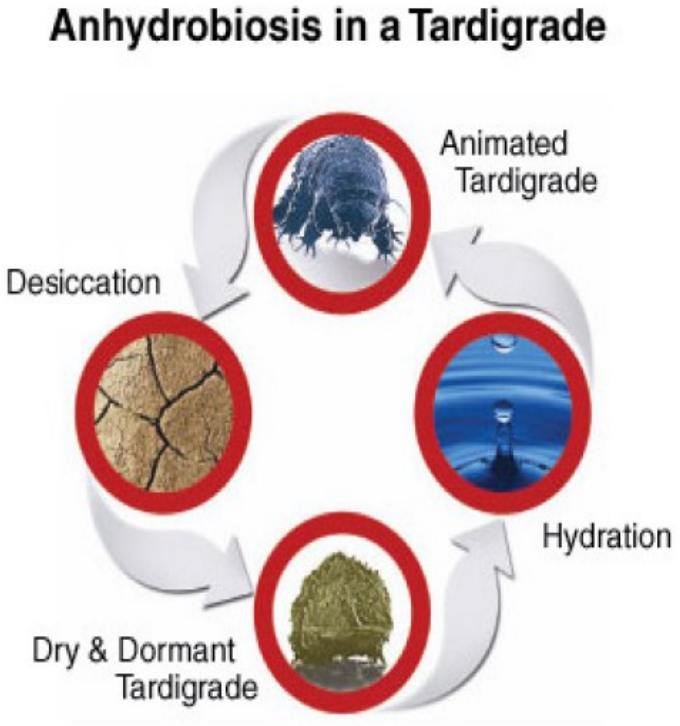
Outline

- Bioprotection by saccharides
  - The trehalose peculiarity
- Results from complementary techniques (FTIR, SAXS, DSC, Light Scattering) and MD simulations on:
  - solutions or glassy matrices of disaccharides
  - at different water content and temperatures
  - both in the presence and in the absence of proteins

- Set up a connection between the biophysical approach ("*protein-centric*"), and the pharmaceutical/applicative approach ("*stabilization-procedure centric*");
- Highligthing the role of water

Sugars are found in some organisms in Anhydrobiosis

(a) *Anastatica* in active (right) and dormant (left) form



(b) Vital cycle of a tardigrade

Bioprotection by saccharides

- A relevant topic for its applications in food industry, pharmaceutics and medecine

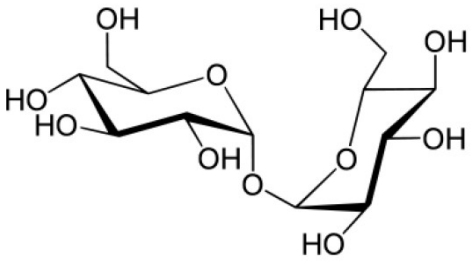
- amorphous saccharide matrices are very efficient in protecting biostructures against adverse conditions (drought, extreme temperatures)

- Need to understanding the mechanisms regulating biopreservation processes: *in vivo*, *in vitro* and *in silico*

Trehalose: the best stabilizer of biostructures

See in J.H. Crowe: *Trehalose as a "chemical chaperone": fact and fantasy*, Adv. Exp.Med. Biol, 594, 143, 2007; *Anhydrobiosis: An unsolved problem with application in human welfare*, Sub-Cellular Biochemistry, 71, 263, 2015)

Trehalose *peculiarity*

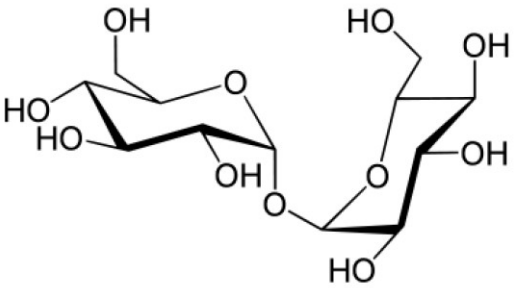


- Glassy structures in a wide *hydration range* + *hbond capability* (why not raffinose?); highest Tg Green&Angell, JPC,93,1989

- Disrupting the *tetrahedral network of water*, impairing ice formation; Branca et al, JCP 111,1999, Magazù et al 2004, Biophys J. 86, 2008
- Slowing down water dynamics (as found in protein interfacial water) Magno&Gallo, JPC Letter, 7, 2011; Corradini et al Sci. Rep. 3, 2013; increasing *water residence time* Engelsen et al, Biophys. Chem., 93, 2001, Lee e al, JCP, 122, 2005;

- Polymorphism; slow formation of dihydrate crystals keeps water in the same HB network as in solvated trehalose, capturing residual water withour disrupting the native structure of biomolecules Cesaro&Sussich, Food Chem., 106, 2008

Trehalose *peculiarity*



- Specific direct interactions with biomolecules in the dry state (*water* replacement) Carpenter & Crowe, Biochemistry, 28,1989

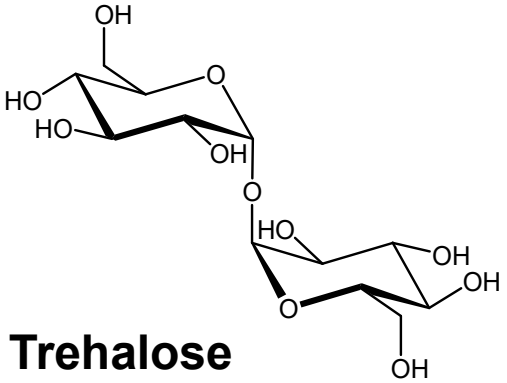
- Water entrapment at the interface by glass formation, preserving the native solvation (*water* entrapment) Belton & Gll, Biopolymers, 34,1994; Timasheff Biochemistry, 41, 2002

- High viscosity matrices: reducing large scale motions leading to protein denaturation Sampedro&Uribe, Mol.Cell.Biochem.,256, 2004

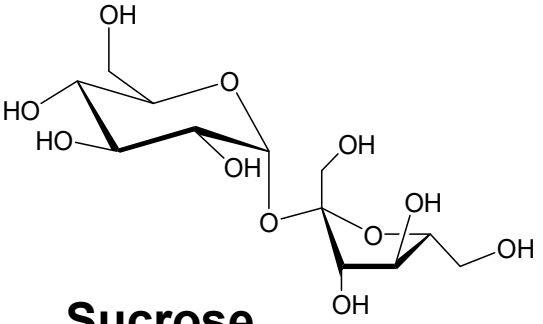
- Stabilizing native tertiary and folded secondary structure, in solutions: via thermal stability of the HB network Hedoux et al JPCB, 110, JPCB, 110, 2006; J Phys.: Condens. Matter,19, 2007; JPCB, 113, 2009, Seo et al, JPCB, 114, 2010, Lerbret et al, JCP, 24, 2009

" ..there is no clear structural explanation for the relative efficiency of trehalose over other sugars that also act as dessication protectants except that it is not related to the number or the position of hydroxyl groups available for hydrogen bonding".

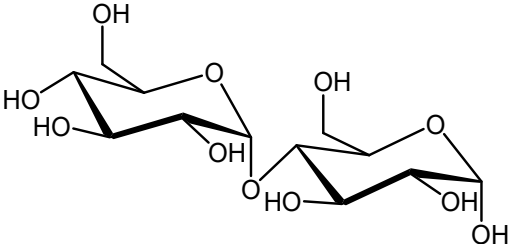
Green & Angell, JPC, 93, 1989



Trehalose



Sucrose



Maltose

Our work

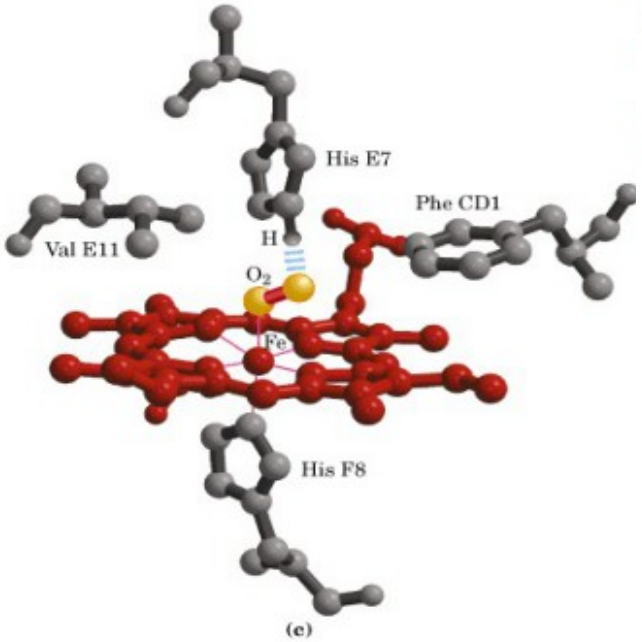
- *A set of complementary techniques*
  - Atomistic: **IR**, **MD**, neutron scattering, Mössbauer spectroscopy, Flash Photolysis...
  - Supramolecular: SAXS
  - Thermodynamics: DSC
- *Two different states (at least...)*
  - 50% w/w rubbery, 90% w/w amorphous
- *Different sugars*
  - **Trehalose**, **sucrose**, **maltose**, mono and oligosaccharides
- *Different proteins*
  - **Myoglobin**, but also lysozyme, Hb, BSA: charge and size effects
- *Ternary (or even quaternary) vs. binary systems*

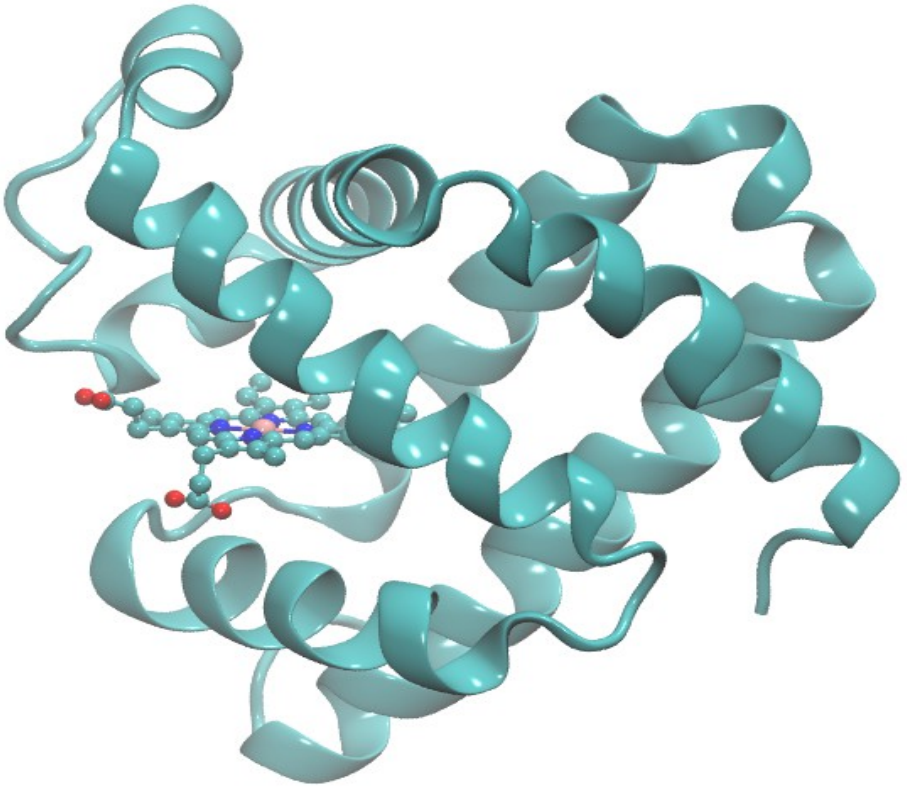
Myoglobin: The hydrogen atom of biology and a paradigm of complexity

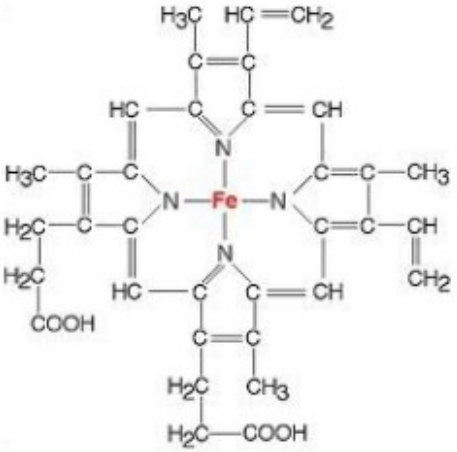
PNAS | July 22, 2003 | vol. 100 | no. 15 | 8615–8617

H. Frauenfelder*†, B. H. McMahon‡, and P. W. Fenimore*

*Center for Nonlinear Studies, MS B258, and ‡Theoretical Biology and Biophysics Group (T-10), MS K710, Los Alamos National Laboratory, Los Alamos, NM 87545



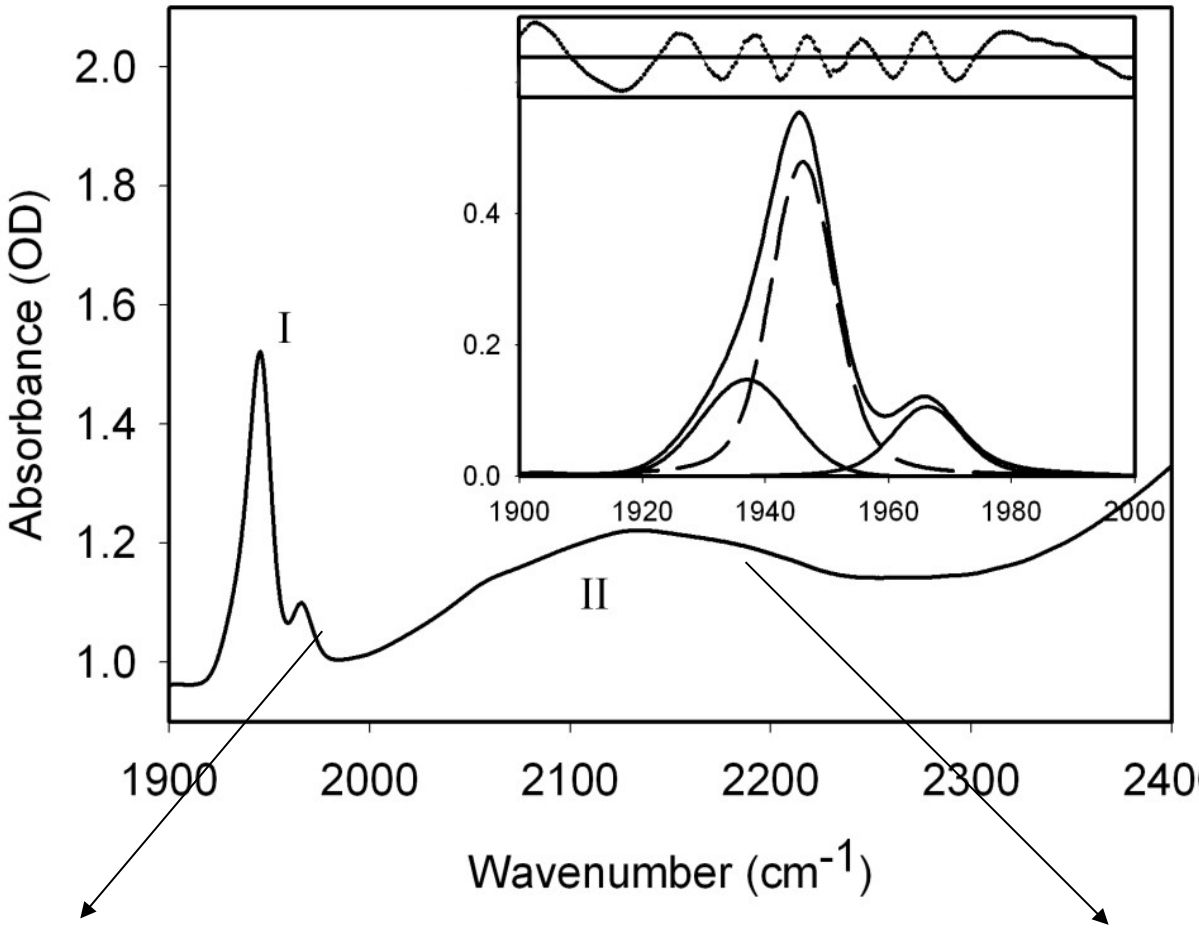


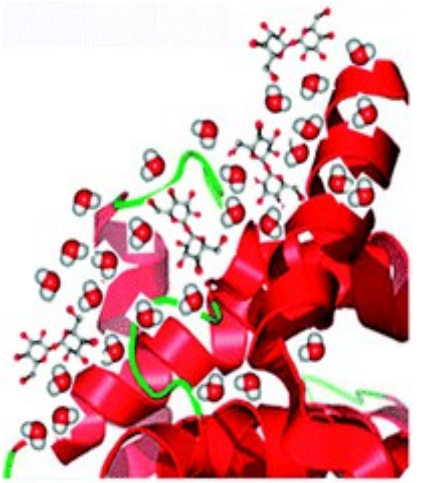


A small monomeric protein, oxygen storage in the muscles. 153 amino acids, ~ 2600 atoms, 8 helices. Active center: heme group with a central Fe atom; O_2, CO, NO

A prototype for complex systems, yielding insight into chemistry and physics of soft matter and chemical reactions

FTIR: protein and matrix bands





CO Stretching Band (COB)

three sub-bands (taxonomic or A substates)

Water Association Band (WAB):
bending mode of water + intermolecular water–water (but also water-non water) modes

Giuffrida et al, JPCB, 107, 2003; Cordone et al, BBA-Proteins, 1749,2005; J.Phys.: Condens. Matter, 19,2007; Chem.Phys, 345, 2008;Giuffrida et al, J. Non-Cryst.Solids, 357, 2011;Eur. Phys.J.E, 36,2013; Cordone et al, Curr Org Chem. 19,2015

FTIR: WAB, a marker for HB in trehalose amorphous matrices

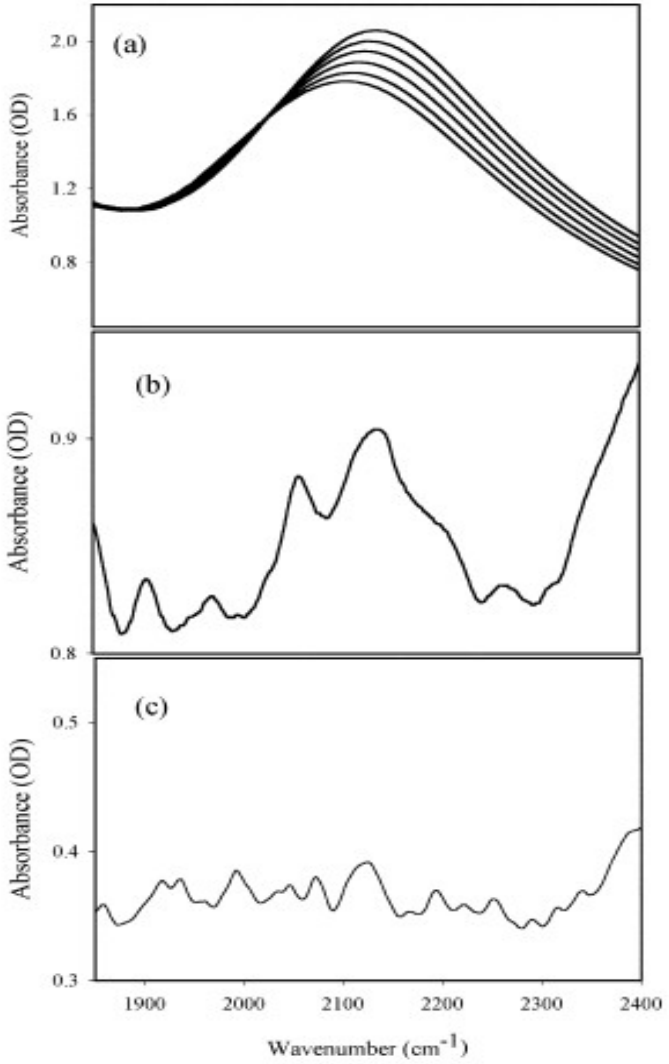


Figure 2. (a) Water association band in pure water, in the temperature range 280−330 K; (b) water association band in dihydrate trehalose powders, at room temperature; (c) absorption profile of anhydrous sucrose powders in the range 2000−2400 cm^{-1}, at room temperature.

A gaussian band in liquid water

Approximately the same shape above 30% w/w water content

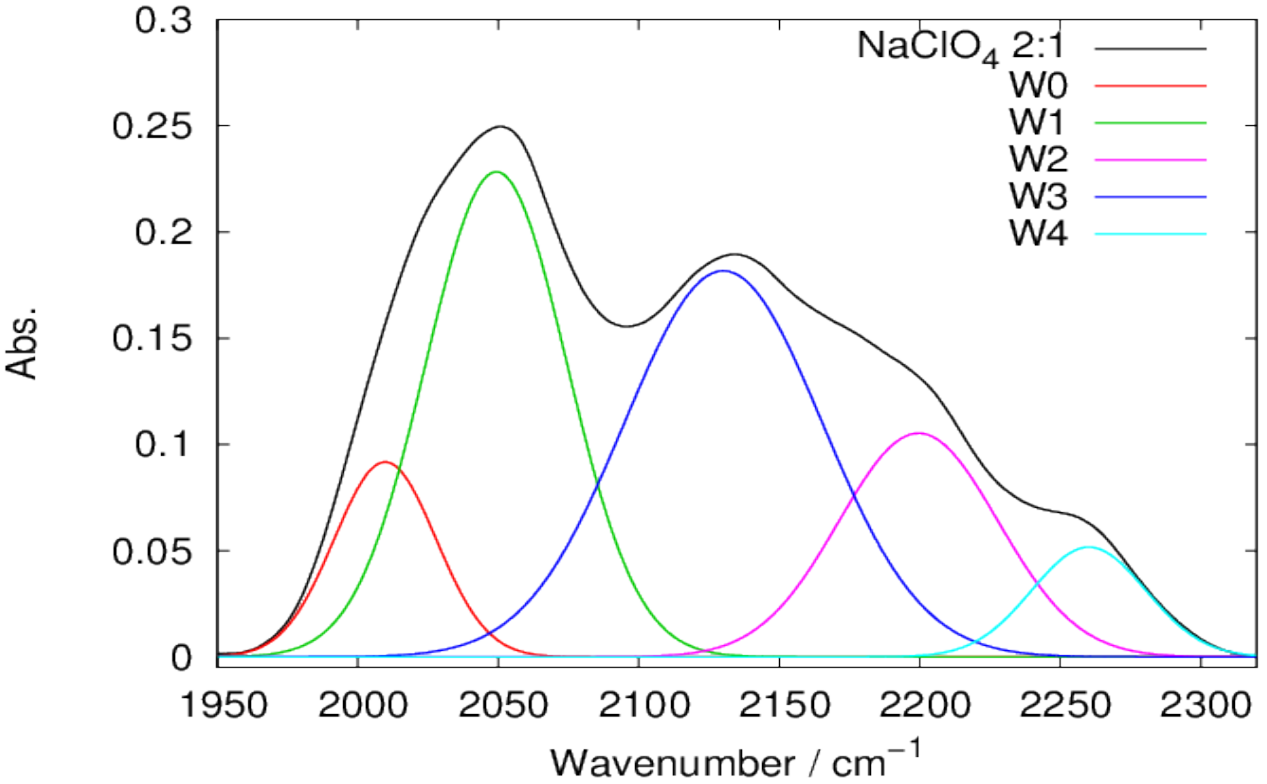
Structured in sugar samples at low water content

Intermolecular vibrational modes involving also non water OH groups

FTIR: WAB in trehalose + Hofmeister salts

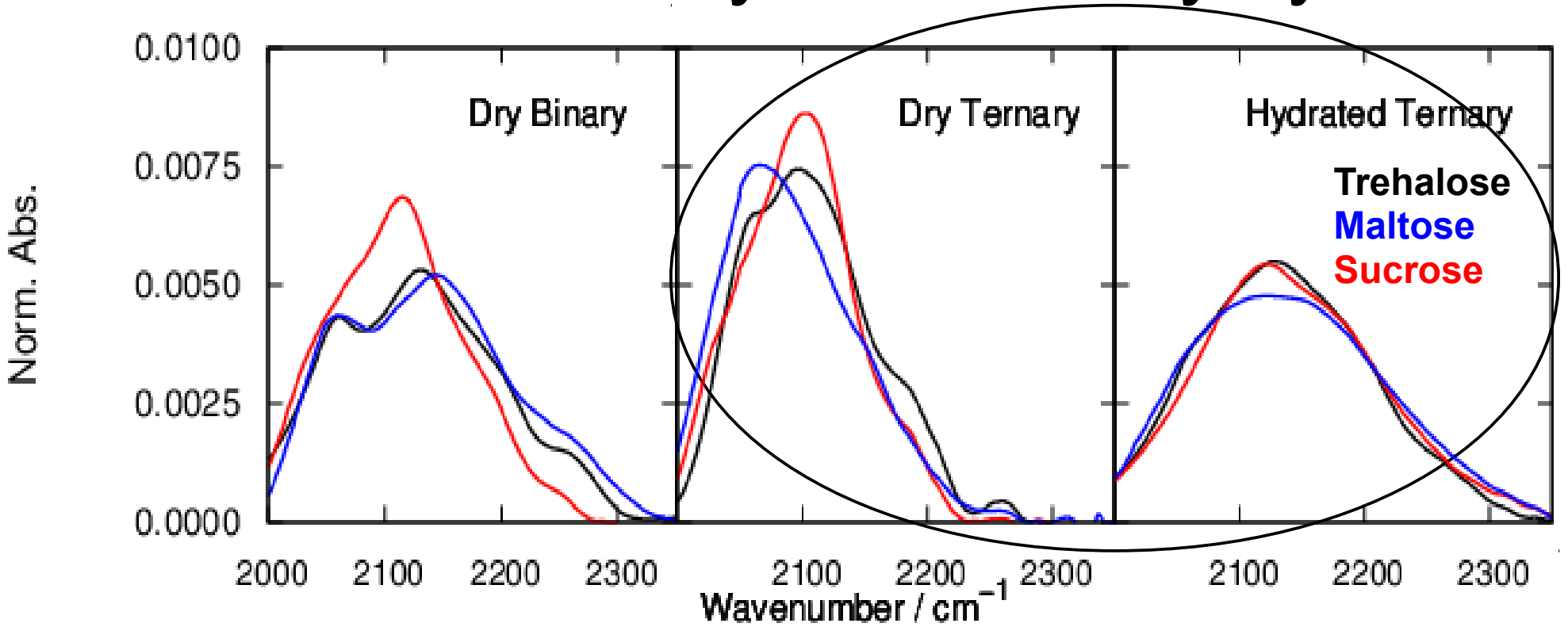
W0	2010-2030 cm^{-1}
W1	2050-2070 cm^{-1}
W2	2100-2140 cm^{-1}
W3	2180-2210 cm^{-1}
W4	2240-2270 cm^{-1}

W0	Strongly destructured
W1	Destructured
W2	Weakly structured *Bulk like*
W3	Structured
W4	Strongly structured *Ice like*



assignment of the sub-bands to different *water classes*

FTIR: WAB in binary and ternary systems

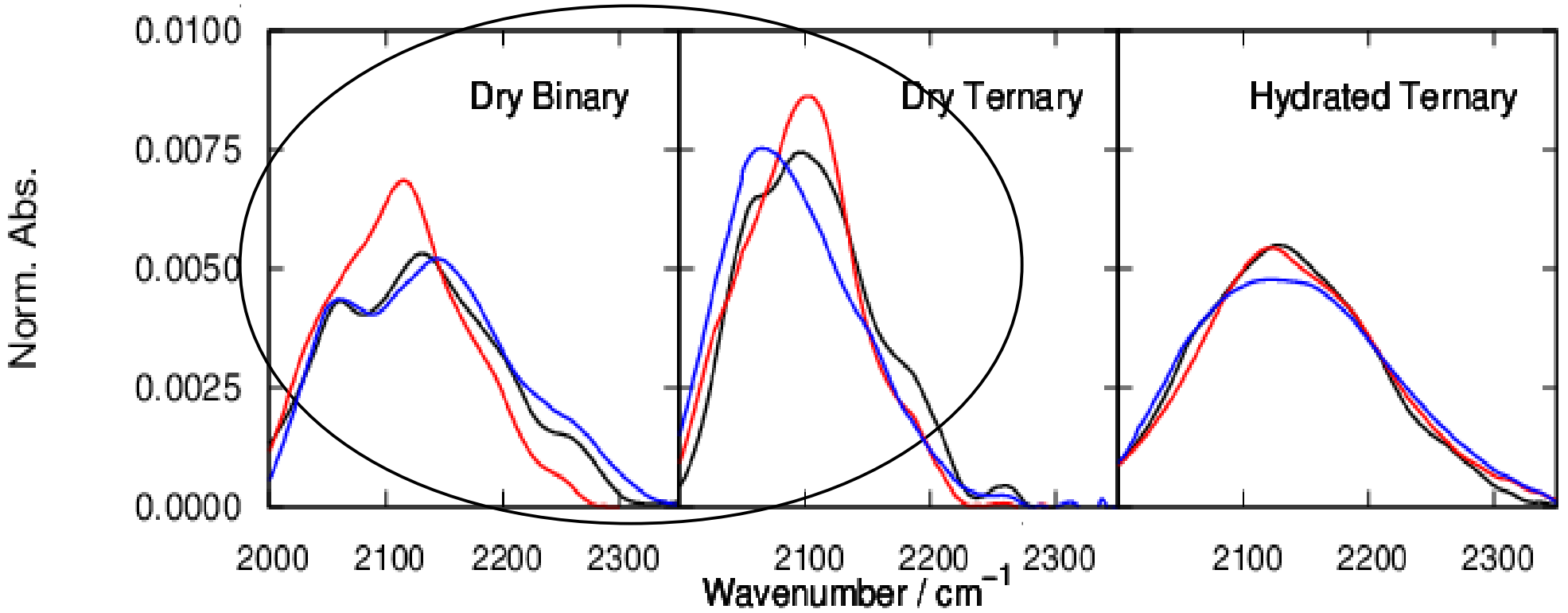


- **high hydration ternary**:
  NO saccharide-specific features
  Shape as in pure water

Sample	Water concentration (M)	~Water/protein (g_{H_2O}/g_{MbCO})	~Water/sugar (g_{H_2O}/g_{Sugar})
Very dry	0.06	10 (0.01)	0.3 (0.02)
Dry	0.36	70 (0.07)	1.8 (0.09)
Humidified	5.4	1080 (1.080)	27 (1.4)

- **low hydration ternary**:
  Saccharide-specific features
  Different pattern of population of HB local structures

FTIR: WAB in homologous disaccharides

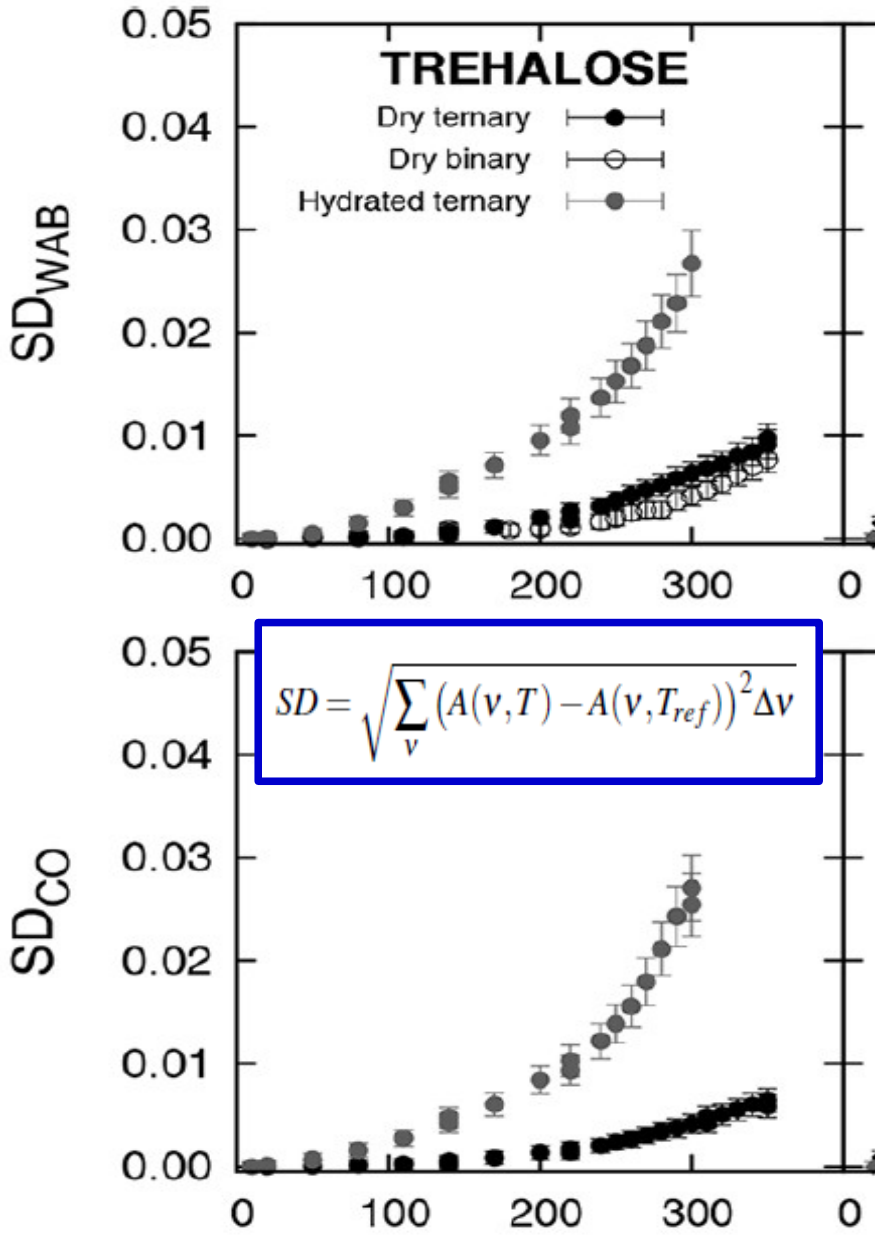


Binary vs ternary systems:

trehalose and **maltose**: an increase in the low-frequency components, weaker HB (water-protein)

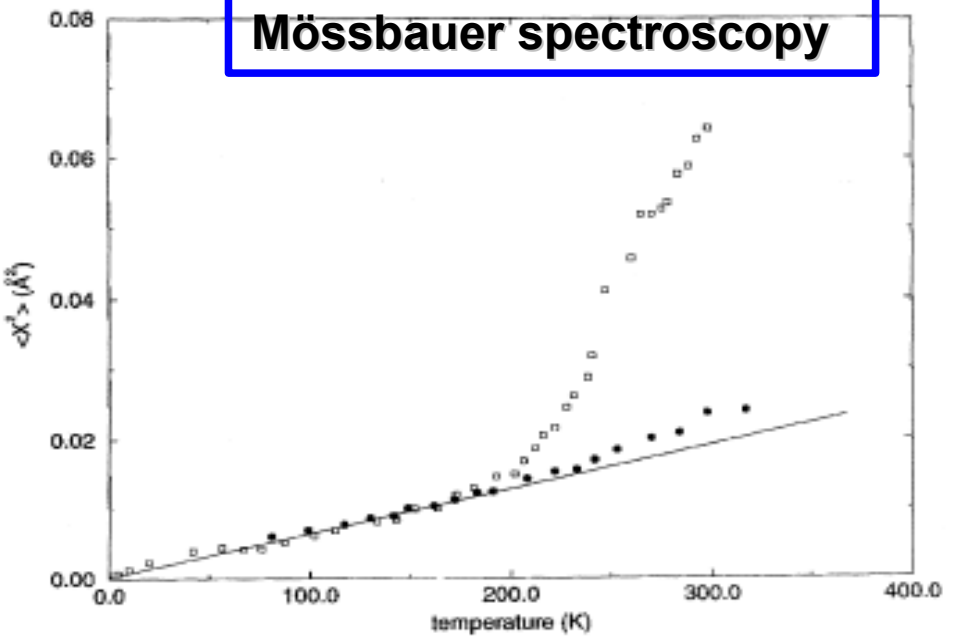
sucrose: smaller changes, the distribution of water molecules is not strongly altered, protein has a weak influence

FTIR: changing temperature, 20-350K

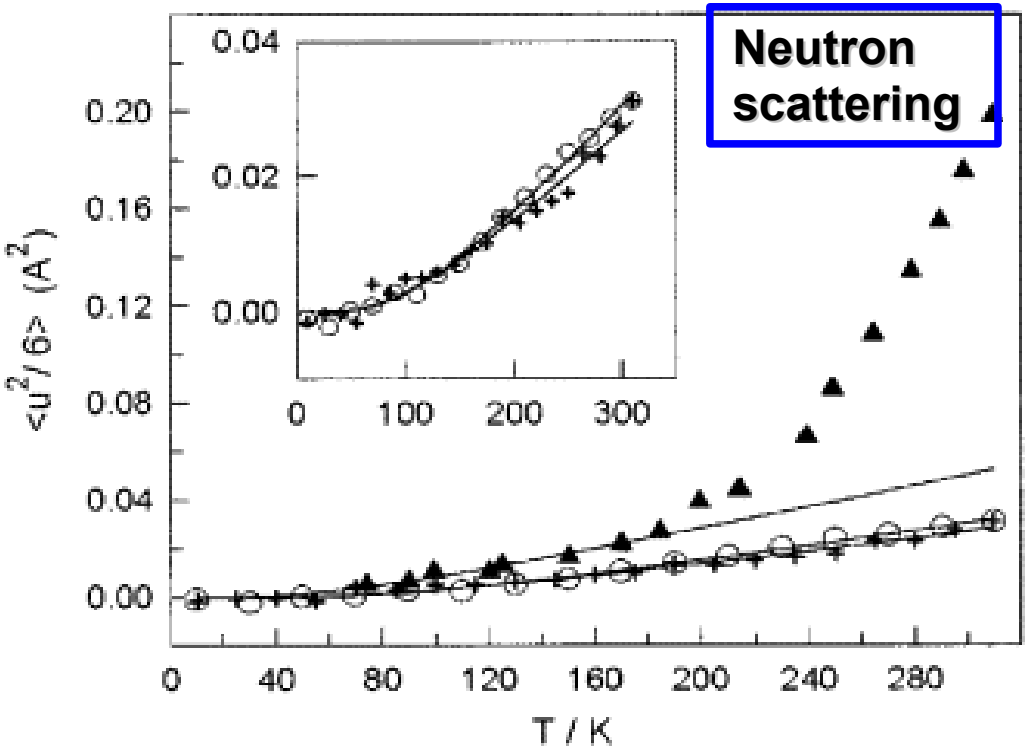


$$SD = \sqrt{\sum_{\nu} \left(A(\nu,T) - A(\nu,T_{ref})\right)^2 \Delta\nu}$$

Mössbauer spectroscopy

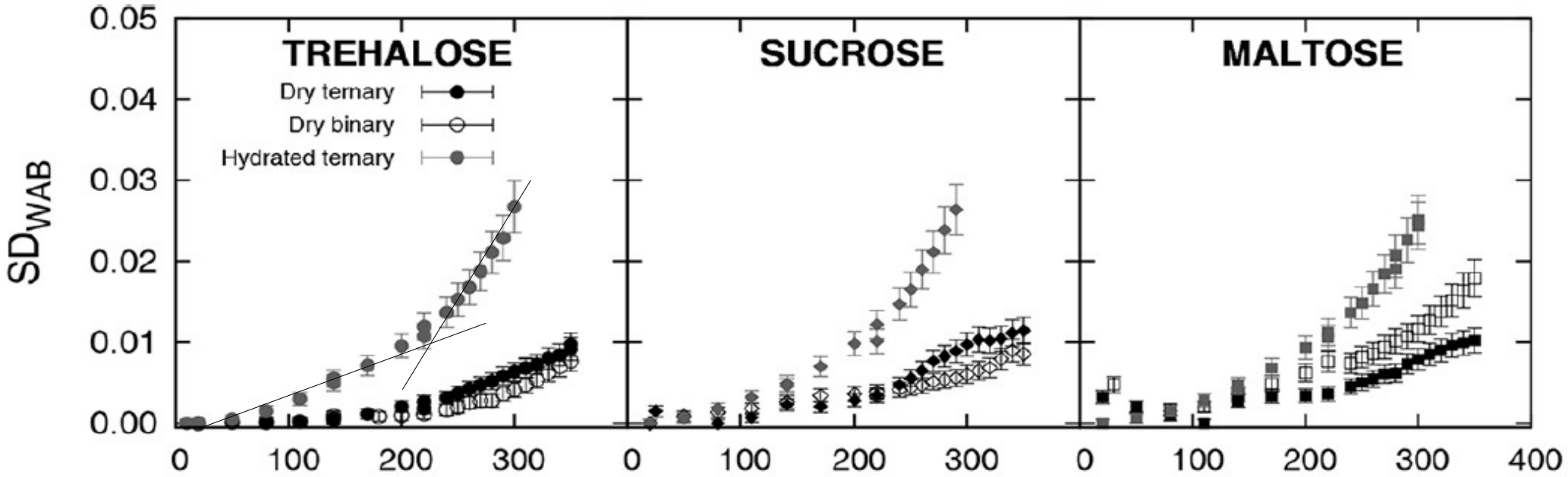


Neutron scattering

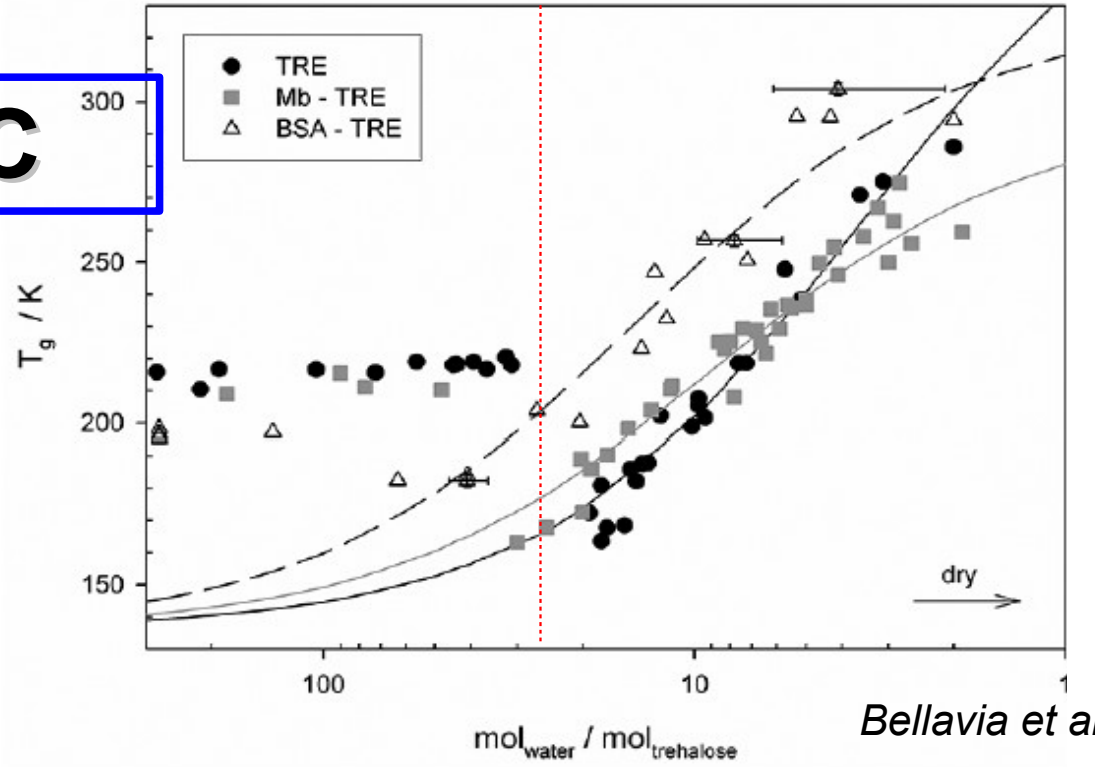


Giuffrida et al, Biophys. J.91 , 2006
Cordone et al, Eur. Biophys.27, 1998
Cordone et al, Biophys j. 76, 1999

FTIR: changing temperature, 20-350K



DSC



Bellavia et al., JPCB, 113, 2009; 115, 2011

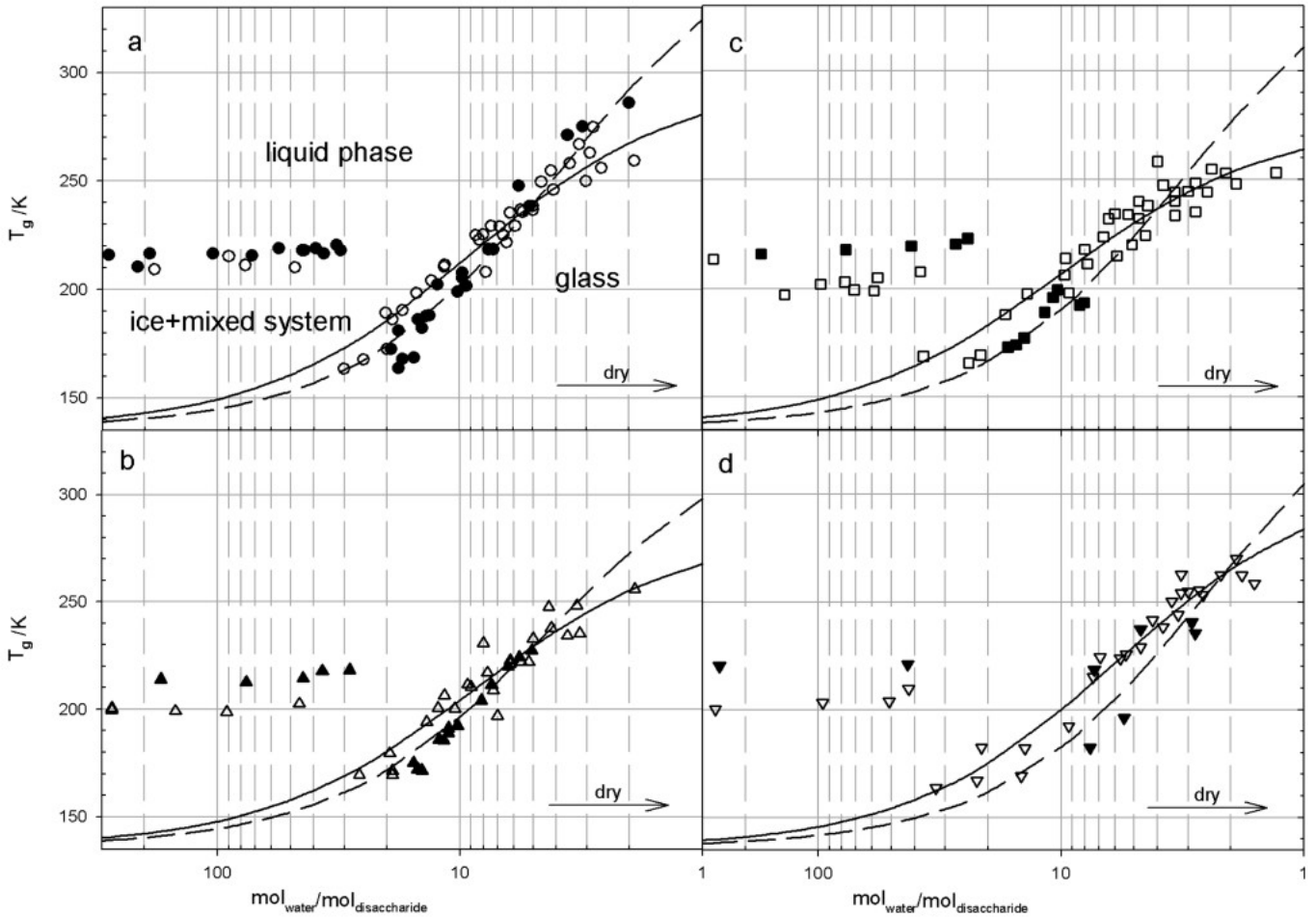


Figure 5. Glass transition temperature as a function of hydration in Mb−disaccharide−water systems (empty symbols and continuous lines) in comparison with the correspondent binary disaccharide−water systems (solid symbols and dashed lines). Lines are fittings in terms of the Gordon−Taylor formula (eq 1 in the text). Panel a: trehalose (circles).[48] Panel b: sucrose (triangles up). Panel c: maltose (squares). Panel d: lactose (triangles down).

TABLE 1: Parameters Obtained from Fittings of T_g vs Water/Disaccharide Molar Ratio in Terms of the Gordon−Taylor Expression (eq 1 in the text)

disaccharide	$T_g(f \to 0)$/K (binary[a])	$T_g(f \to 0)$/K (ternary)	k (binary)	k (ternary)
trehalose	373	297 ± 13	4.9 ± 0.4	2.1 ± 0.5
sucrose	335	283 ± 16	4.6 ± 0.3	2.2 ± 0.6
maltose	368	277 ± 12	6.1 ± 0.8	1.9 ± 0.5
lactose	374	309 ± 17	7.8 ± 2.6	3.3 ± 0.8

$$T_g(f) = \frac{T_g(f \to 0) + T_g(f \to \infty)\, k \frac{MW_w}{MW_s} f}{1 + k \frac{MW_w}{MW_s} f} \qquad (1)$$

where MW_w and MW_s are the water and disaccharide molecular weights, respectively; f is the water/disaccharide molar ratio; $T_g(f \to 0)$ and $T_g(f \to \infty)$ are the glass transition temperatures of the system in the absence of water and of disaccharide, respectively; and k is the nonlinearity parameter.[53]

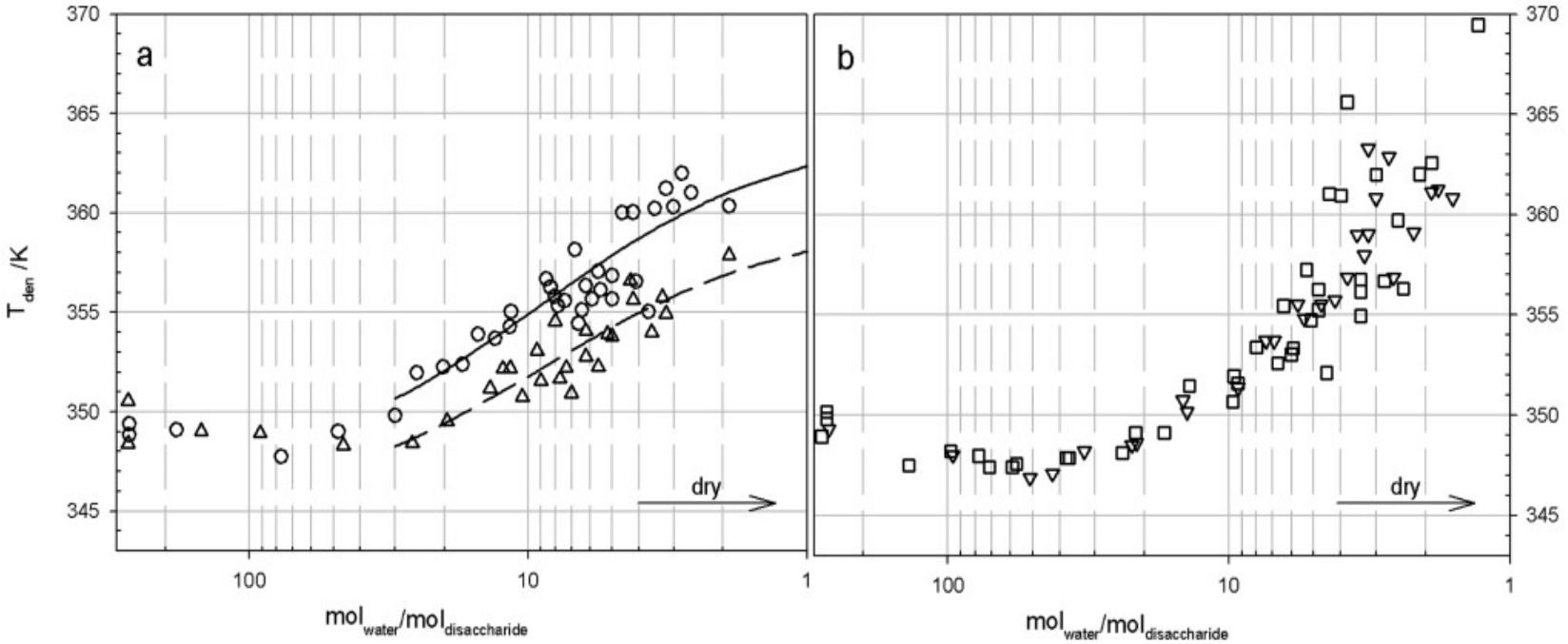


Figure 3. Denaturation temperature of myoglobin as a function of hydration. Panel a: in trehalose (circle)[48] and in sucrose (triangle up) systems. Panel b: in maltose (square) and in lactose (triangle down) systems. Lines are fittings in terms of eq 3.

$$T_{den}(f) = T_{den}(f \to \infty) + \frac{\partial T_{den}}{\partial T_g} \frac{T_g(f \to 0) - T_g(f \to \infty)}{1 + k\left(\frac{MW_w}{MW_s} f\right)} \quad (3)$$

Weak hydration dependence at high water content (> 30 water/sugar)

Saccharide specific Tden increase at low water content, steeper for trehalose than sucrose

In reducing saccharides Tden increase is monotonic up to 5 water/sugar

11046 J. Phys. Chem. B 2005, 109, 11046−11057

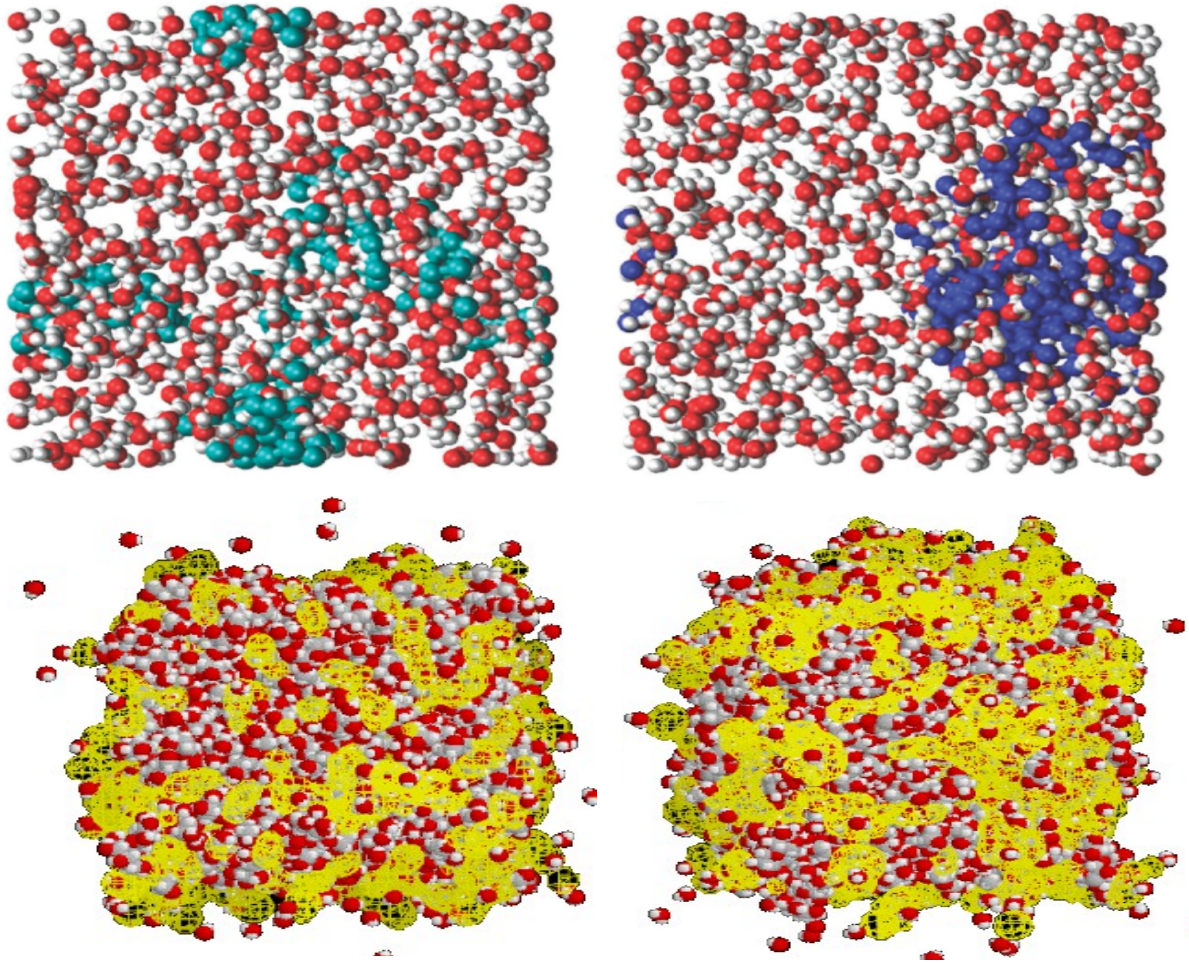
How Homogeneous Are the Trehalose, Maltose, and Sucrose Water Solutions? An Insight from Molecular Dynamics Simulations

A. Lerbret,* P. Bordat, F. Affouard, M. Descamps, and F. Migliardo

THE JOURNAL OF PHYSICAL CHEMISTRY Letters
LETTER
pubs.acs.org/JPCL

Understanding the Mechanisms of Bioprotection: A Comparative Study of Aqueous Solutions of Trehalose and Maltose upon Supercooling

A. Magno† and P. Gallo*



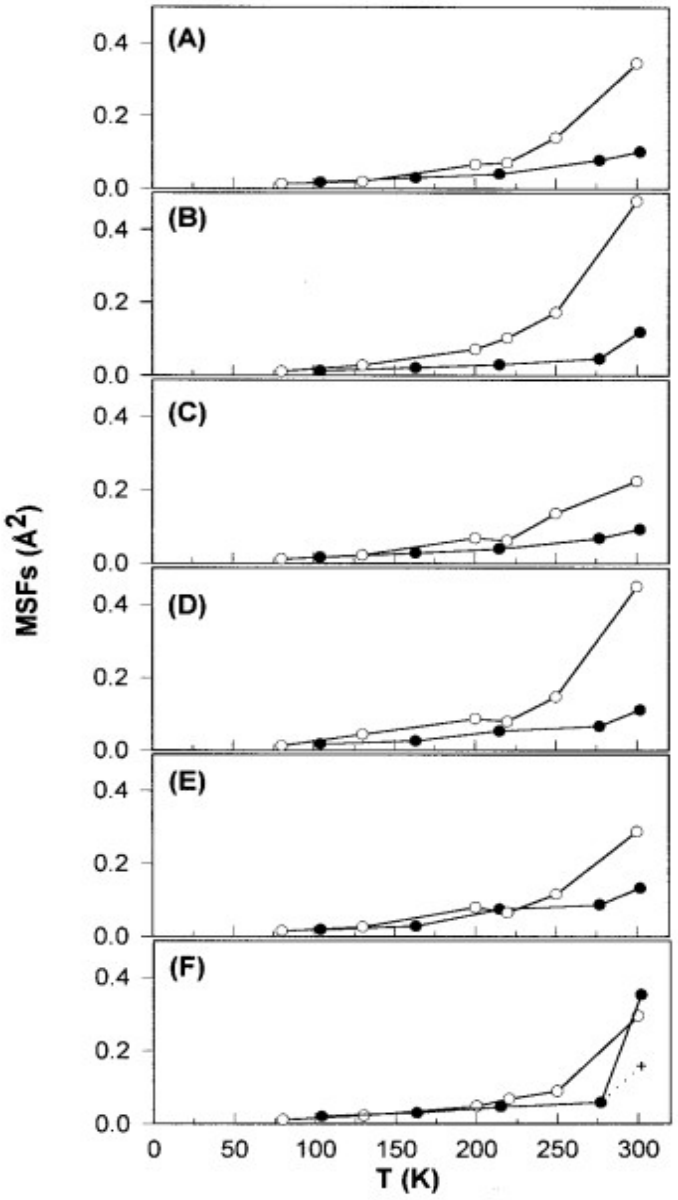
Maltose vs trehalose

Cavities calculated by SURFNET

89% w/w, Cottone et al

Old **MD** simulations: protein dynamics

MbCO/trehalose/water, 89% w/w, 100ps time scale:

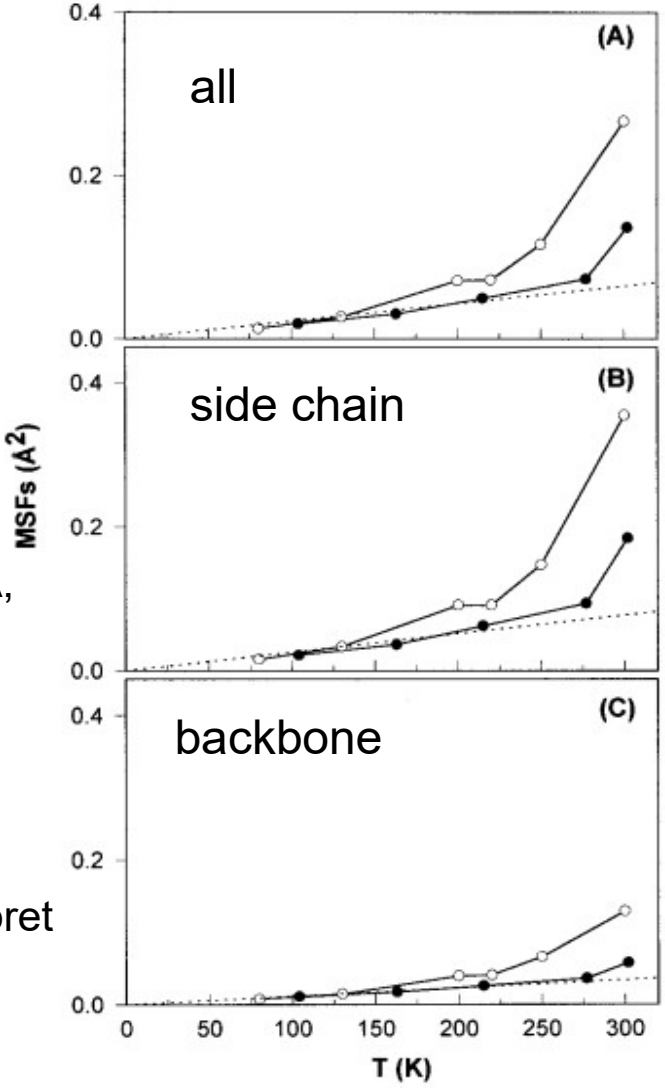


- @89% w/w MSFs averaged on backbone atoms, 300K: 0.04 $Å^2$
- @50% w/w MSFs averaged on all atoms, 300K: 0.24 $Å^2$ (as in water solution)

MD of lysozyme:
@18% w/w, RMFSs Cα atoms 1.0 Å, (Lins et al, Proteins, 55,2004).

@60% w/w
(Corradini et al Sci. Rep. 3, 2013)

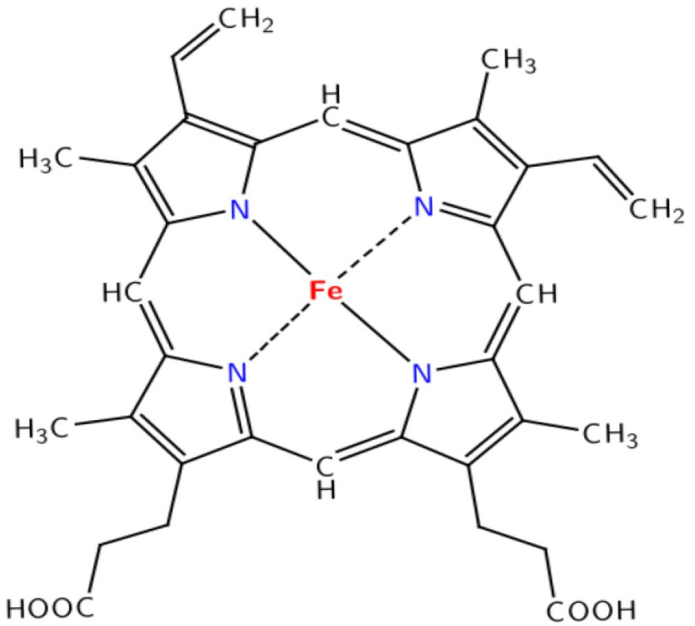
@37-60% w/w (Lerbret et al, JPCB, 109, 2005), 87% w/w, 93%w/w (Lerbret et al, JPCB, 116 2012)

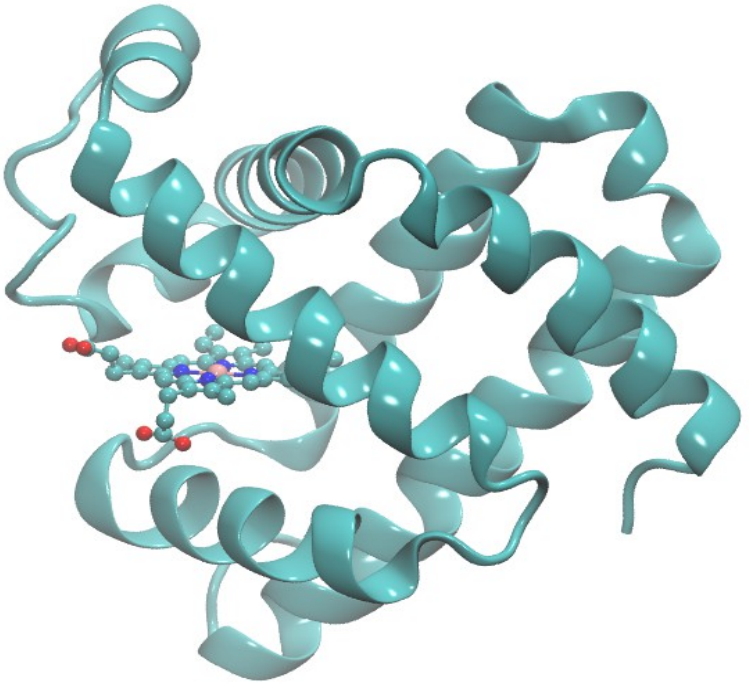


Cottone et al, Biophys. J. ,80,2001

TABLE 1 Room temperature MSFs (in Å^2) of the heme heavy atoms

System	Iron Atom	Porphyrin Nitrogen Atoms	Porphyrin Heavy Atoms	Peripheral Substituents Heavy Atoms
MD on trehalose-coated MbCO (this work)	0.027	0.032	0.039	0.067
MD on H_2O-solvated MbCO (Venturoli, 1998)	0.039	0.043	0.053	0.123





is the heme involved in a direct interaction with the matrix?

See also in Giachini et al, Biophys. J., 92, 2007
XAFS spectroscopy, cytochrome c in dry trehalose matrices

MD: hydrogen bond analysis

TABLE I. Mean number of water and trehalose molecules hydrogen bonded to the protein.[a]

System	Water	Trehalose	OH=1	OH=2	OH=3	OH=4
50%	269±7	22±3	18±3 (82%)	4±1 (18%)	0.1±0.4	0
89%	85±3	55±2	33±3 (60%)	18±3 (33%)	4±1 (7%)	0.9±0.6

TABLE II. Mean number of water molecules forming *n* H-bonds with the protein.

System	*n*=1	*n*=2	*n*=3	*n*=4	Total
50%	209±7 (78%)	51±5 (19%)	8±2 (3%)	0.6±0.7	269±6
89%	57±4 (67%)	23±3 (27%)	4±2 (4%)	1.1±0.8	85±3

TABLE III. Mean number of trehalose OH groups forming *n* H-bonds with the protein.

System	*n*=1	*n*=2	*n*=3	*n*=4	Total
50%	23±3 (88%)	3±1 (12%)	0	0	26±3
89%	79±4 (94%)	5±2 (6%)	0.8±0.6	0	84±4

Sugar-protein interaction does not affect the number of bound water molecules in the first hydration shell

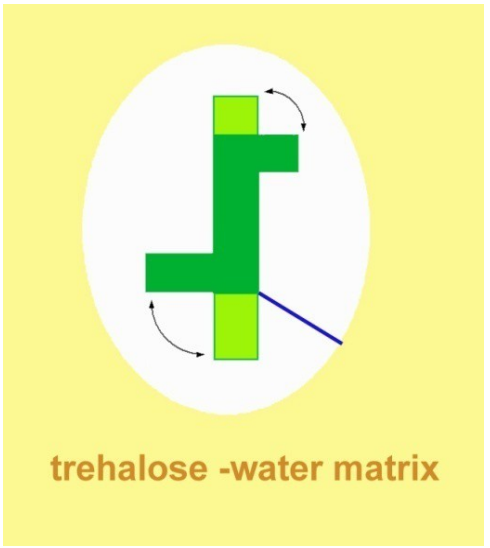
TABLE IV. Mean number of water molecules, shared between trehalose and protein, forming *n* H-bonds with protein and *m* with trehalose.

System	Total		*n*=1	*n*=2	*n*=3	*n*=4
50%	108±8 out of 269±6 bound to protein (40%)	with protein	97±7 (90%)	11±3 (10%)	0.6±0.8	0
		with trehalose	84±7 (78%)	23±4 (21%)	1±1	0
89%	62±4 out of 85±3 bound to protein (73%)	with protein	48±4 (77%)	13±2 (21%)	0.8±0.8 (1%)	0
		with trehalose	36±4 (58%)	22±3 (35%)	4±2 (7%)	0

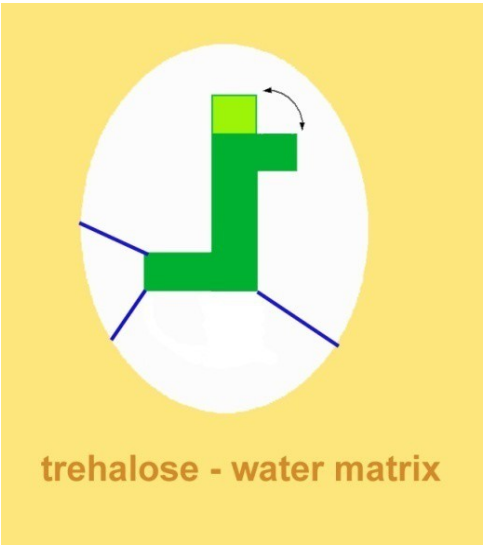
Cottone et al, JCP,117,2002

See also in “Revisiting the conundrum of trehalose stabilization”, Nidhi Katyal and Shashank Deep, Phys. Chem. Chem. Phys., 2014,16, 26746-26761

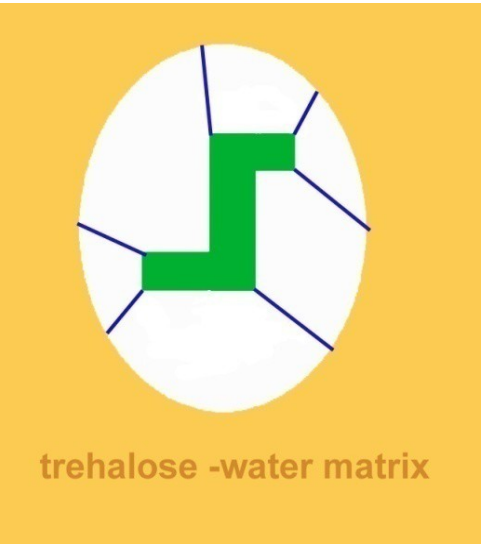
Protein-water-trehalose: a model



wet



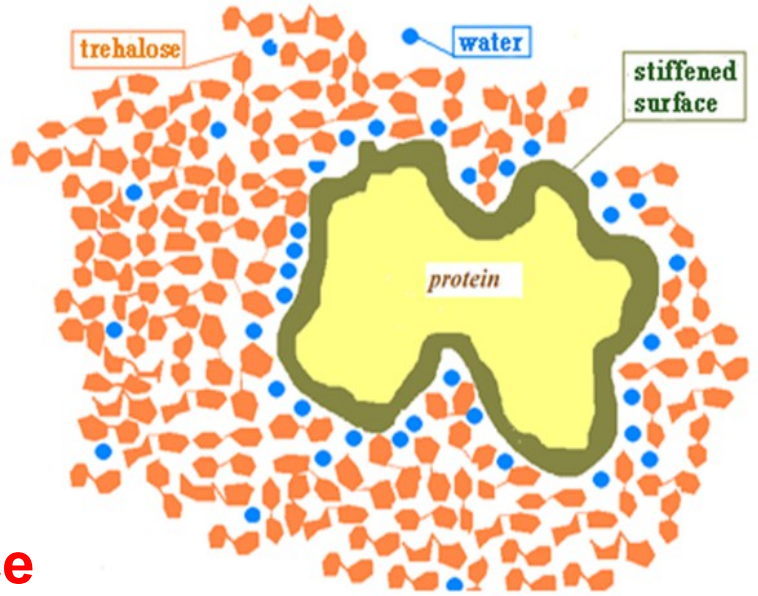
dry



extra-dry

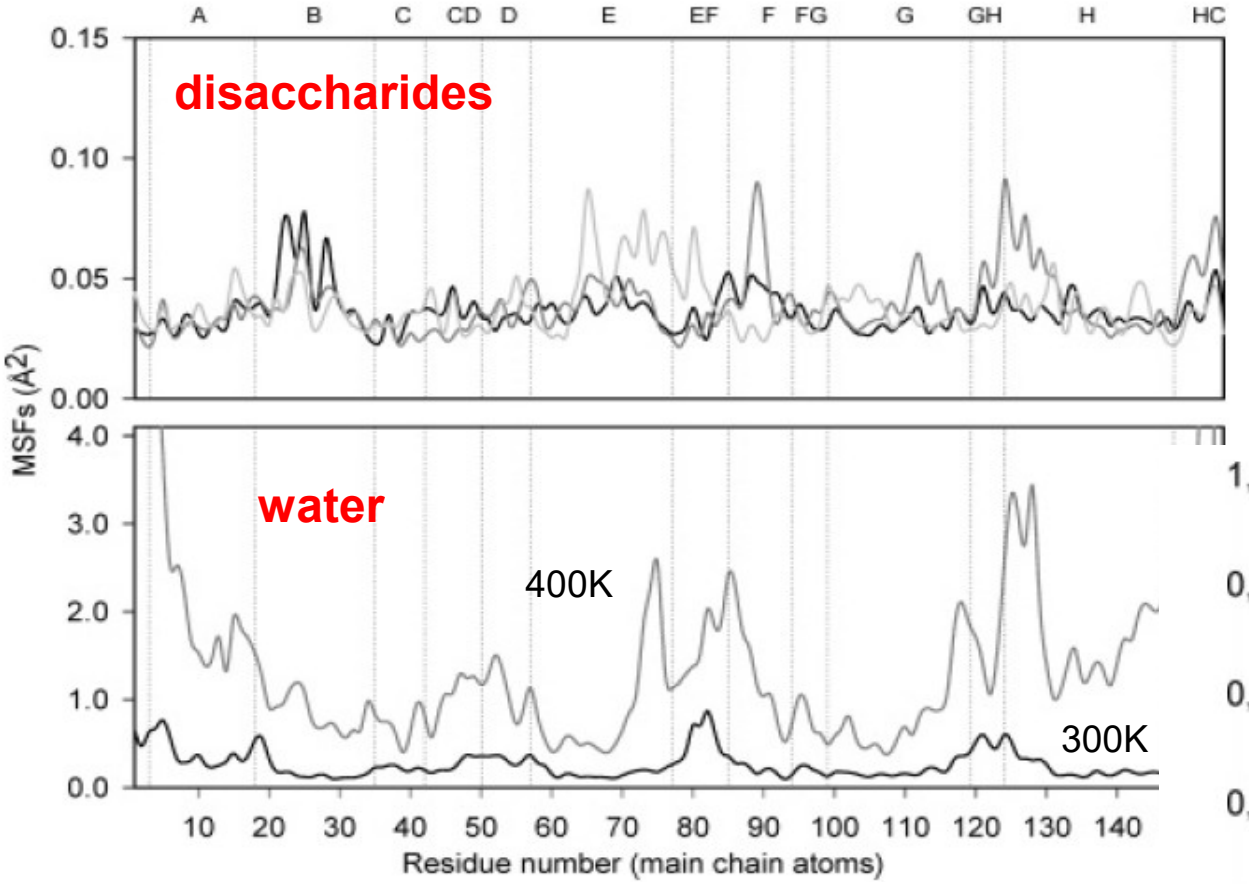
The surface of the protein is confined within a network of H-bonds.
The fraction of water molecules forming multiple H-bonds with both protein and sugar increases upon dehydration.

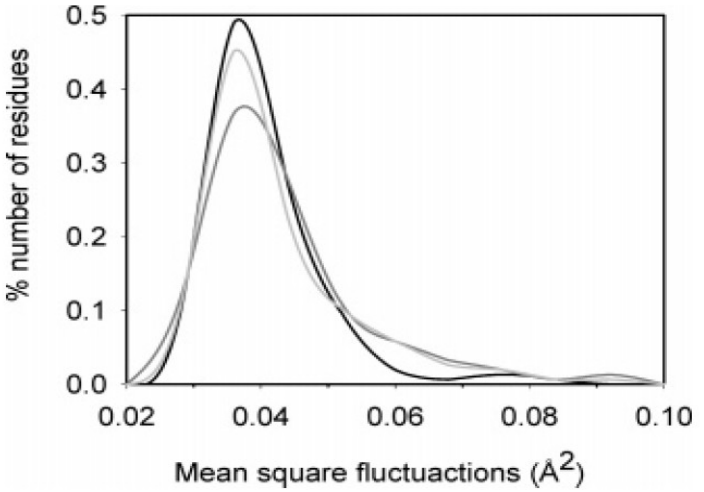
Drastic stiffening of the protein surface

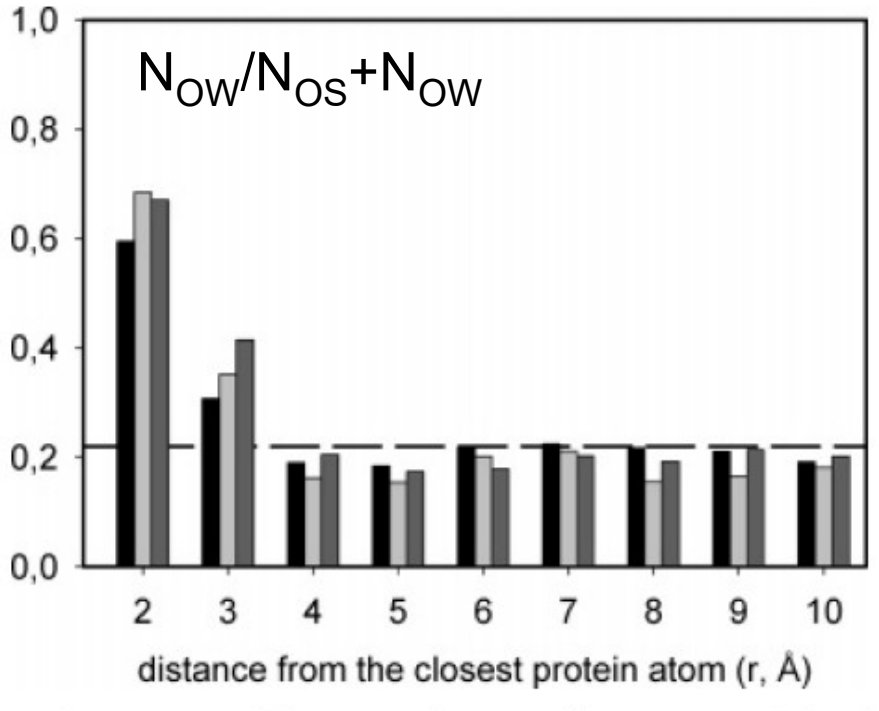


By Marco Malferrari, UNIBO

MD: homologue disaccharides







As in MD of MbCO in water molecules kept at 180K or even fixed around the protein (Vitkup et al, 2000): very low amplitude MSFs, and uniform over the whole chain

G. Cottone , JPCB, 111, 2007

In Lerbret et al, JPCB, 109, 2005; JPCB, 116 2012; Katyal&Deep, Phys. Chem. Chem. Phys., 16, 2014.

MD: homologue disaccharides

TABLE 1: Mean Number of Different Water and Sugar Molecules Hydrogen-Bonded to the Protein[a]

sugar/ (sugar + water) w/w	water	sugar	OH = 1	OH = 2	OH = 3	OH = 4
89% trehalose	88 ± 2	55 ± 2	32 ± 2	17 ± 3 (58%)	4 ± 1 (31%)	0.9 ± 0.1 (7%)
89% sucrose	128 ± 3	49 ± 1	31 ± 2	13 ± 1 (63%)	5 ± 1 (26%)	0.1 ± 0.1 (10%)
89% maltose	140 ± 1	57 ± 1	33 ± 2	18 ± 1 (58%)	4 ± 1 (31%)	2.0 ± 1 (7%)

TABLE 2: Mean Number of Different Water Molecules Forming *n* H-bonds with the Protein[a]

system	*n* = 1	*n* = 2	*n* = 3	*n* = 4	total
89% w/w trehalose	58 ± 2 (66%)	24 ± 1 (27%)	5 ± 1 (6%)	0.9 ± 0.4	88 ± 2
89% w/w sucrose	85 ± 3 (66%)	32 ± 1 (25%)	10 ± 1 (8%)	2 ± 1	128 ± 3
89% w/w maltose	95 ± 1 (68%)	36 ± 1 (26%)	8 ± 1 (6%)	0.7 ± 0.2	140 ± 1

[a] The percentages are relative to the total number of water molecules bound to the protein.

TABLE 3: Mean Number of Different Sugar OH Groups Forming *n* H-bonds with the Protein[a]

system	*n* = 1	*n* = 2	*n* = 3	*n* = 4	total
89% w/w trehalose	79 ± 1 (93%)	5 ± 1 (6%)	0.6 ± 0.1	0	85 ± 2
89% w/w sucrose	64 ± 2 (90%)	7 ± 1 (10%)	0.3 ± 0.1	0	71 ± 2
89% w/w maltose	80 ± 2 (89%)	9 ± 1 (10%)	0.5 ± 0.2	0	90 ± 2

[a] The percentages are relative to the total number of OH groups bound to the protein.

G. Cottone , JPCB, 111, 2007

TABLE 4: Mean Number of Water Molecules, Shared between Sugar Molecules and the Protein, Forming *n* H-bonds with Protein and *m* with Sugar[a]

system	shared water		*n* = 1	*n* = 2	*n* = 3
89% w/w trehalose	64 ± 1 out of 88 ± 1 bound to protein (73%)	with protein	48 ± 2 (75%)	14 ± 1 (22%)	1 ± 1 (1%)
		with trehalose	37 ± 1 (58%)	22 ± 1 (34%)	5 ± 1 (8%)
89% w/w sucrose	84 ± 2 out of 128 ± 3 bound to protein (66%)	with protein	66 ± 2 (74%)	16 ± 1 (19%)	1 ± 1 (1%)
		with sucrose	51 ± 2 (61%)	28 ± 1 (33%)	4 ± 1 (5%)
89% w/w maltose	95 ± 1 out of 140 ± 1 bound to protein (68%)	with protein	72 ± 1 (76%)	20 ± 1 (21%)	3 ± 1 (3%)
		with maltose	57 ± 1 (60%)	32 ± 1 (34%)	6 ± 1 (6%)

[a] The percentages in columns 4–6 are relative to the total number of shared water molecules.

The fraction of water molecules bridging different sugar molecules, *irrespective of their interaction with protein*:

70% trehalose, 66% maltose, 63% sucrose

TABLE 5: Total Number of H-Bonds between Protein Secondary Structure Elements (Helices and Loops) and Water Molecules (Acting as Hydrogen Bond Donor)[a]

	secondary structure elements[b]											
system	A	B	C	CD	D	E	EF	F	FG	G	GH	H
89% w/w trehalose	72	37	68	26	23	53	22	25	20	41	30	62
89% w/w sucrose	91	72	32	27	48	59	38	25	14	57	46	92
89% w/w maltose	104	76	72	42	38	66	37	36	13	54	42	97

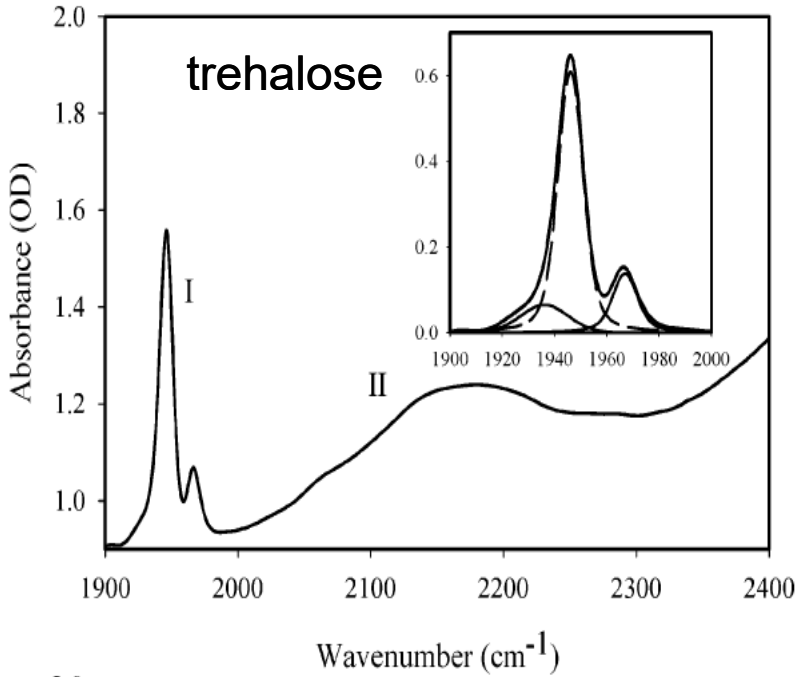
Water excess in sucrose and maltose does not imply more rigid hydrogen bond networks at the protein surface:

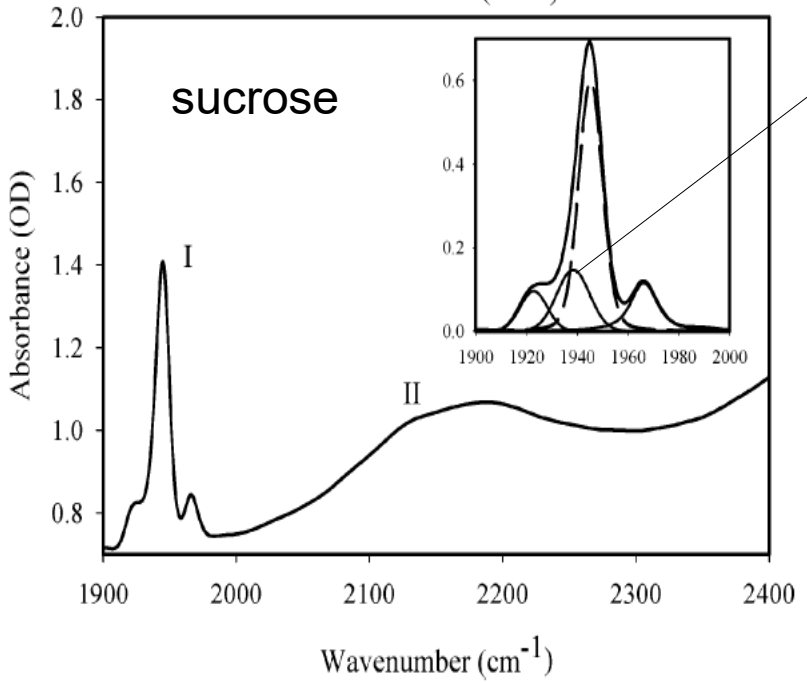
the main contribution to water excess comes from the subset of water molecules bound with single HB, e.e. weaker interaction with the protein;

even for bridging water, water excess does not shift the distribution towards multiple HB

No clear cut correlation between residue fluctuations and the overall number of HB

FTIR : the CO band

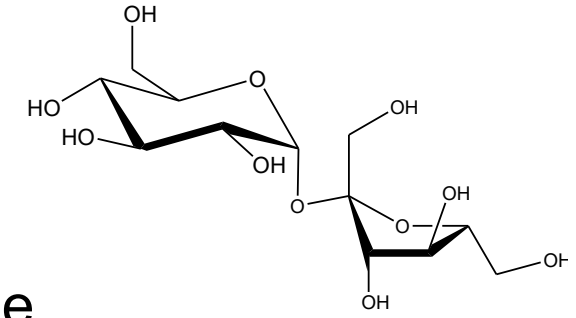




Three sub–bands:
A0 (1966 cm^{-1}), A1 (1951 cm^{-1}), A3 (1938 cm^{-1})
-parameters dependent on temperature, pressure, pH;
-different local electric field within the heme pocket

a fourth sub-band at 1925 cm^{-1}

sucrose-like units? the lower flexibility of the penta-atomic might constrain the protein structure in a new substate...

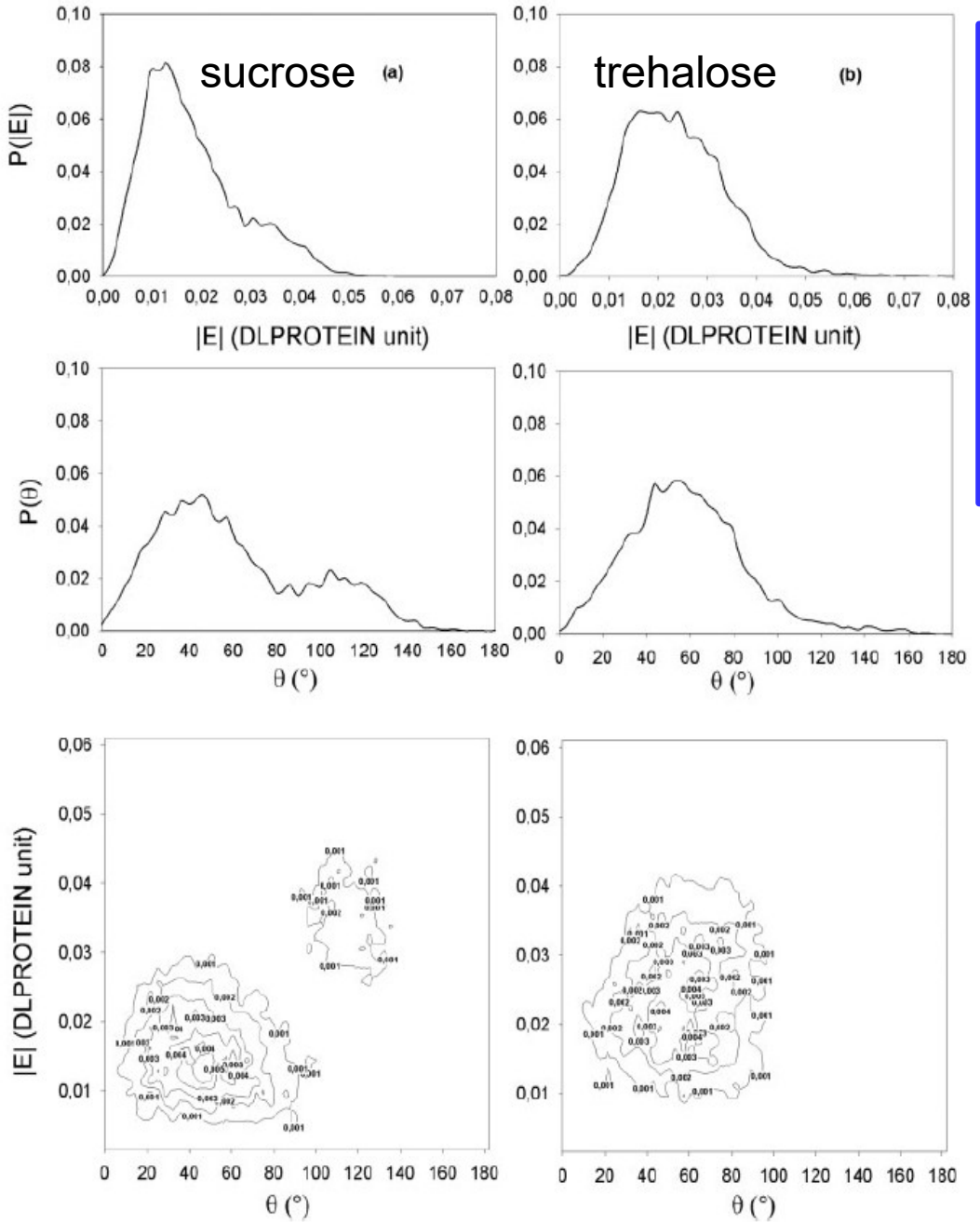


Found also in raffinose..

Giuffrida et al, JPCB, 108, 2004

MD simulations: the CO band

From |E| and θ distributions > CO stretching frequency shift induced by Stark effect,
(Ma et al., 1997; Meller and Elber, 1998; Nutt and Meuwly, 2003, 2006…)

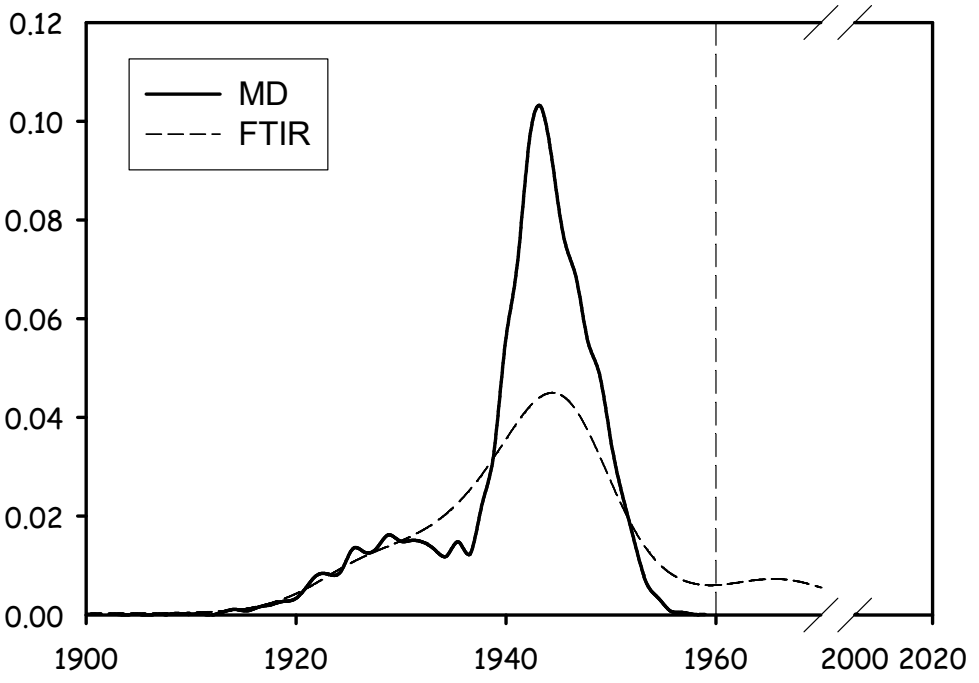


$$\Delta\nu = -\frac{1}{\hbar}\Delta\vec{\mu}\cdot\vec{E}\,, \quad \Delta\vec{\mu} = -0.0162\,D\,\hat{u}_{CO}$$

$$P(\Delta\nu) = \langle\delta[\Delta\nu + {}^{1}/_{h}\,|\Delta\vec{\mu}|\,|\vec{E}|\,\cos\theta]\rangle$$

where the average is defined as

$$\langle\ldots\rangle = \int_0^{\pi} d\theta\,\sin\theta \int_0^{\infty} d|\vec{E}|\,\ldots\,g(\theta,|\vec{E}|)$$



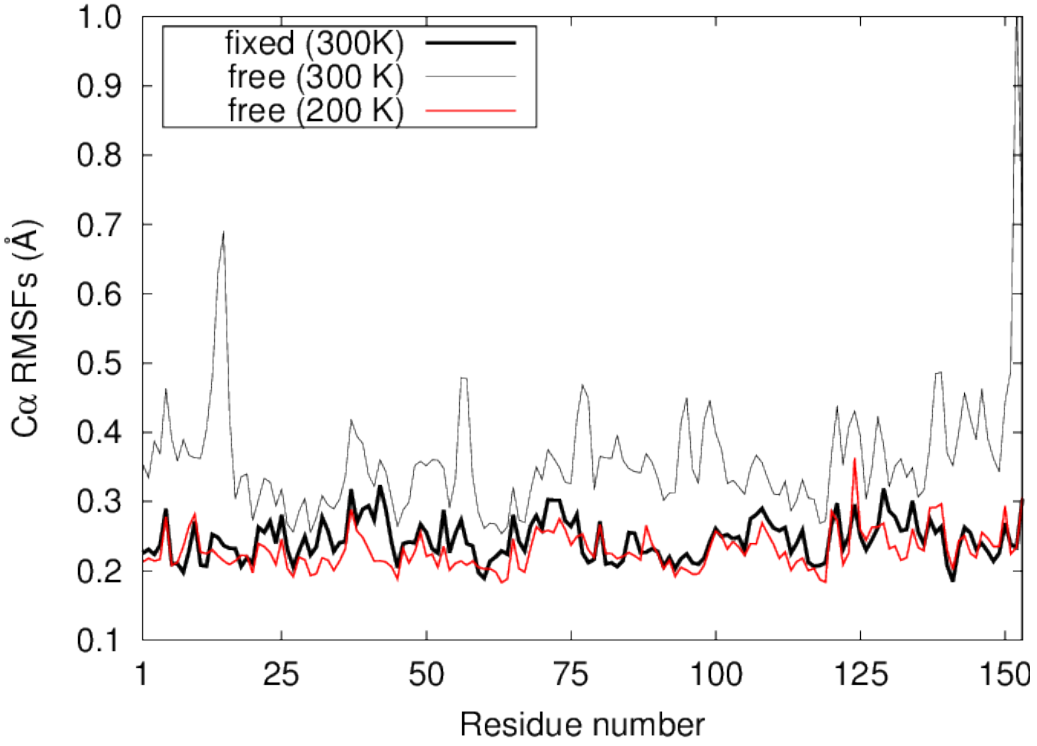
Cottone et al, Proteins, 59, 2005

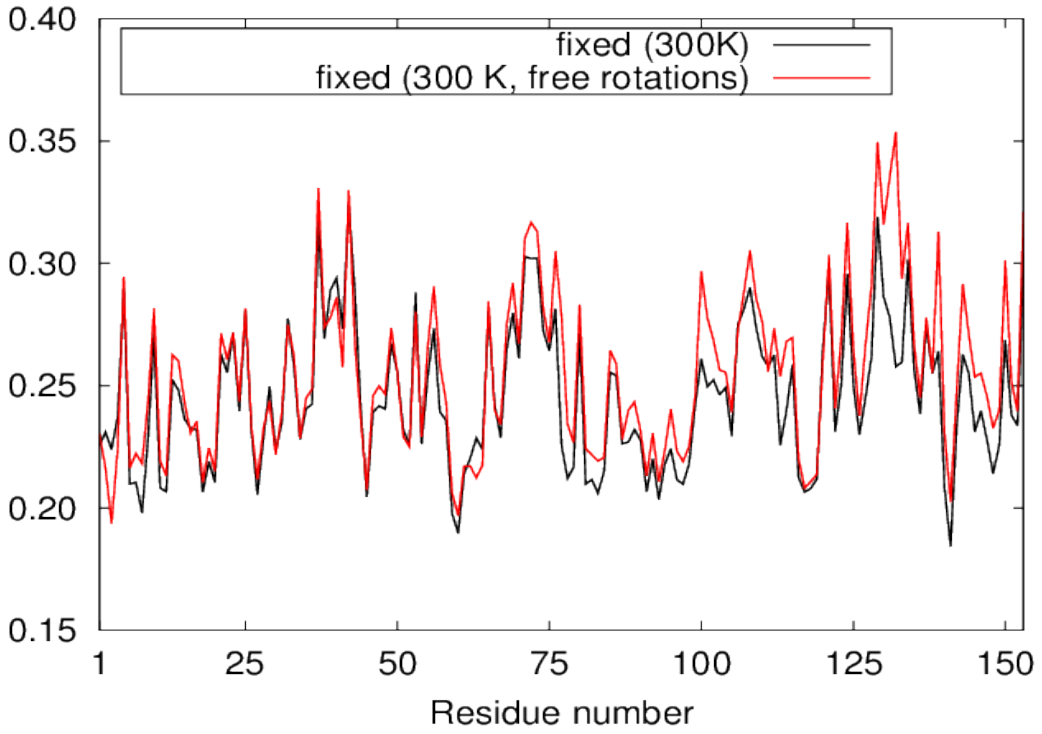
MD: the role of residual water

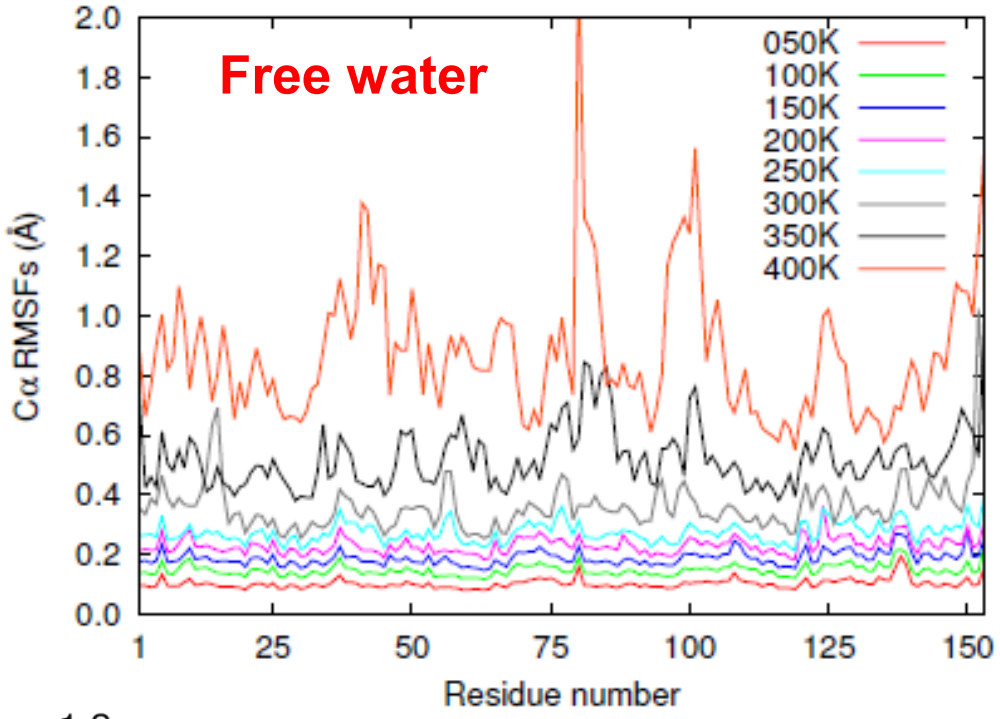
MbCO/trehalose/water @ 89% w/w by blocking water translational and/or rotational motions, 50-400K, ns time scale

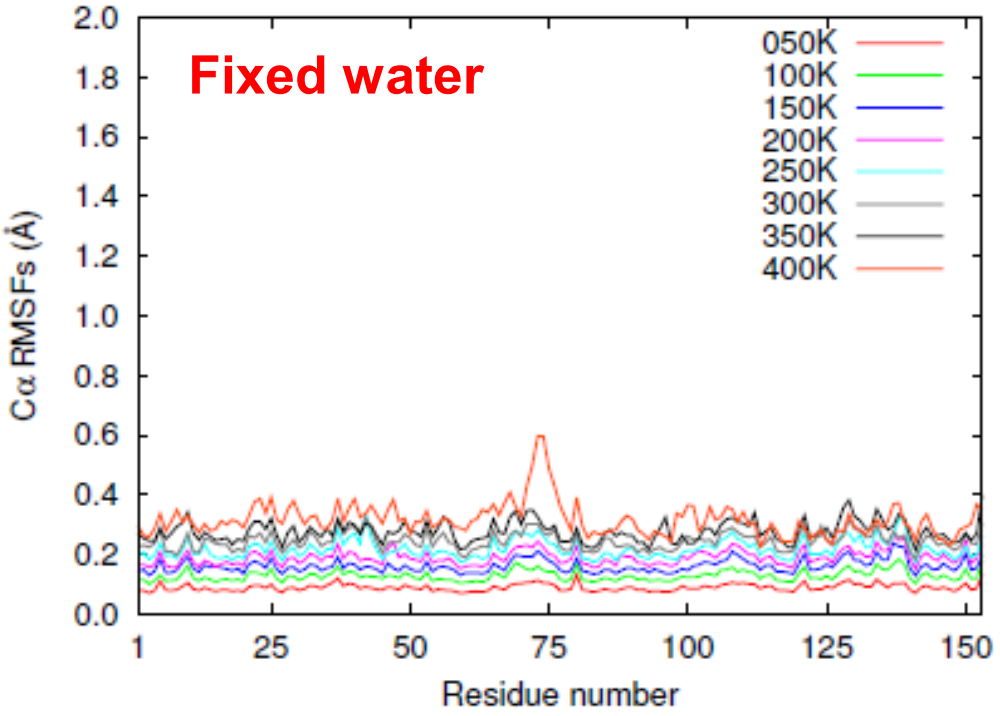
RMSFs vs protein chain ~0.25Å (slow down the ns dynamics to the 100 ps time scale)

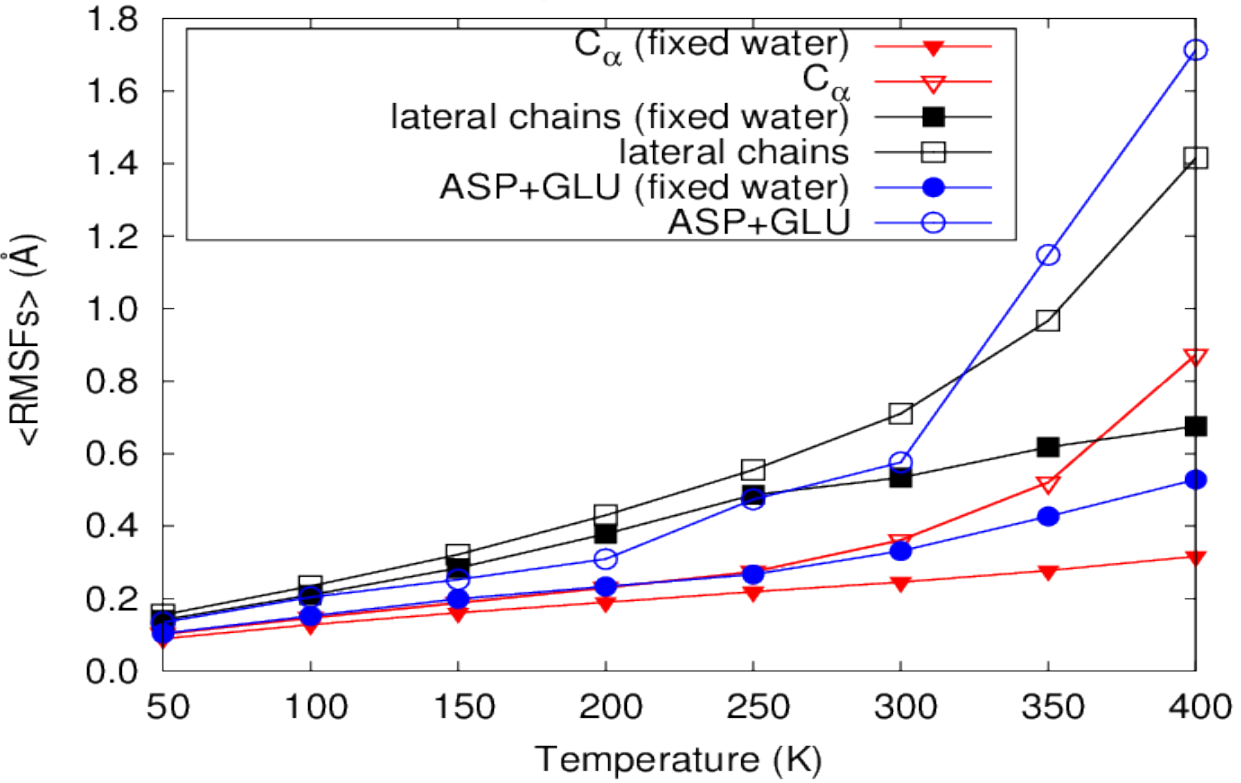
Hindering of water translation is the primary mechanism even in ternary systems





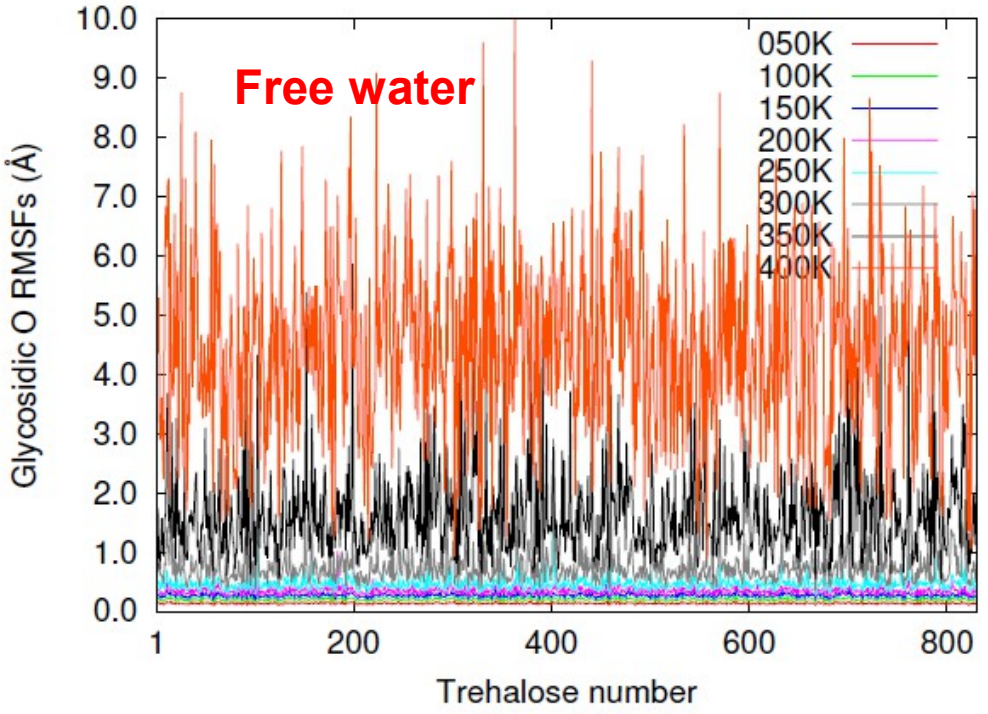


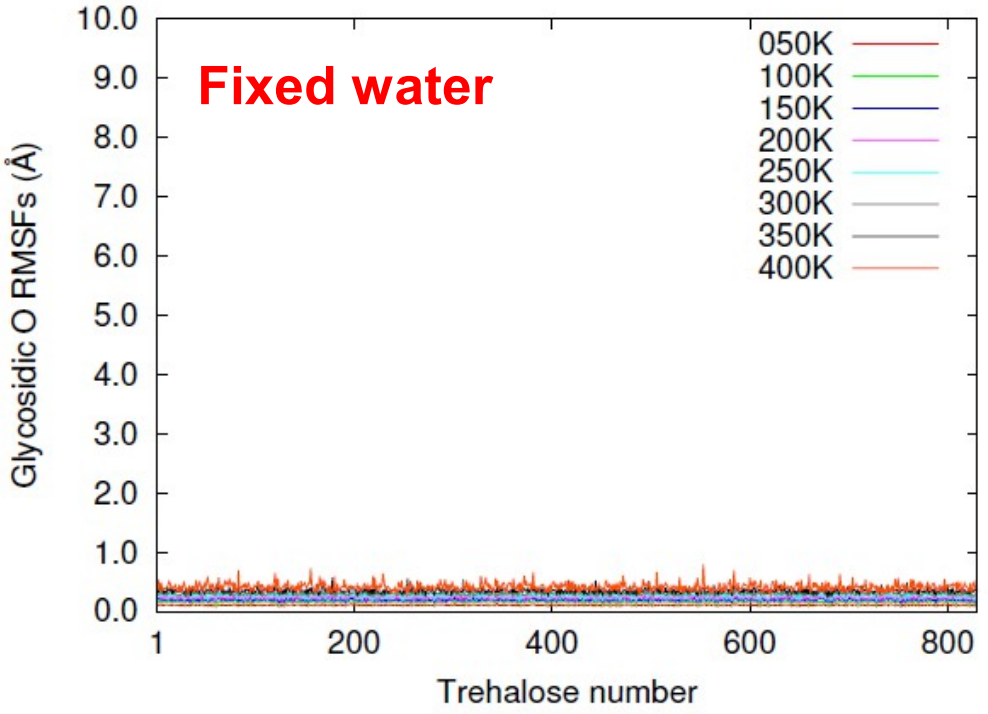


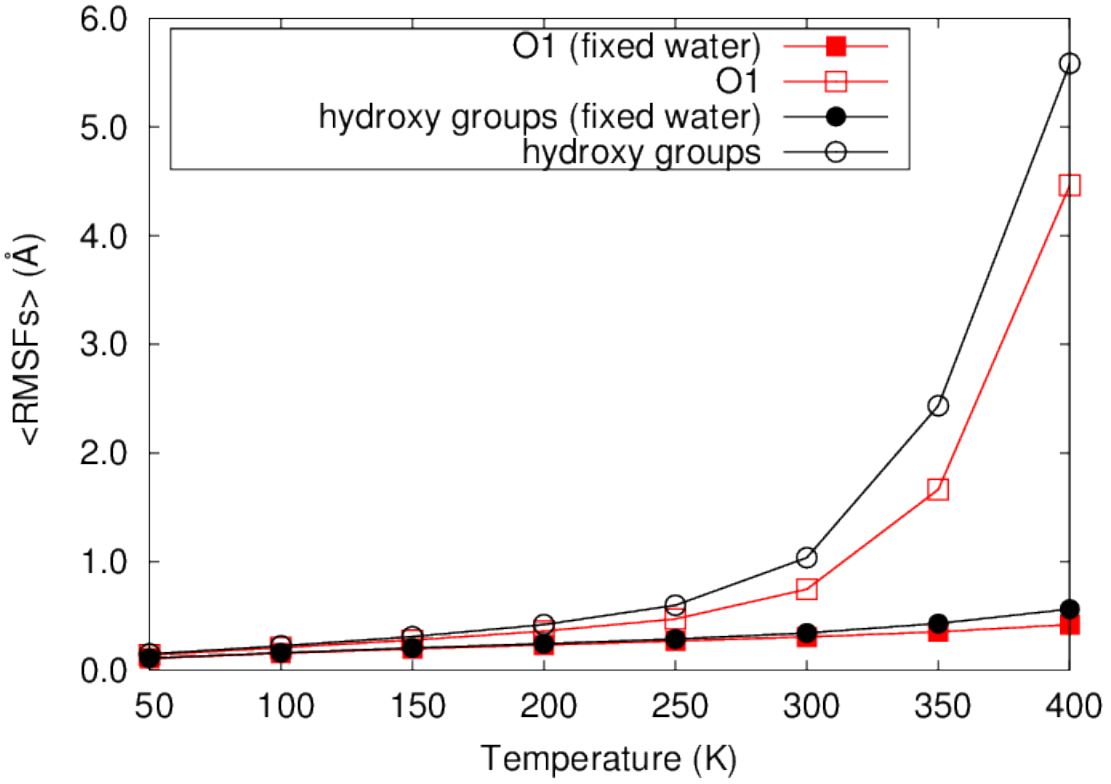


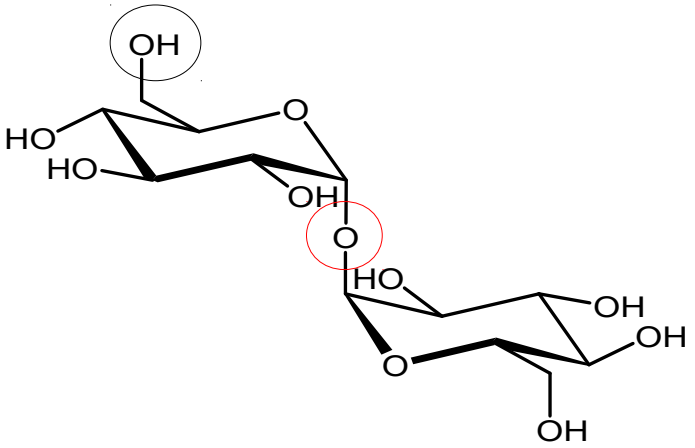
NO transition at the protein dynamical transition T or at the matrix Tg

ASP & GLU: the reduction is larger than other protein atoms classes (see in Allison et al, Arch. Biochem.Biophys, 1999)









Same scale of protein dynamics if water translations are blocked

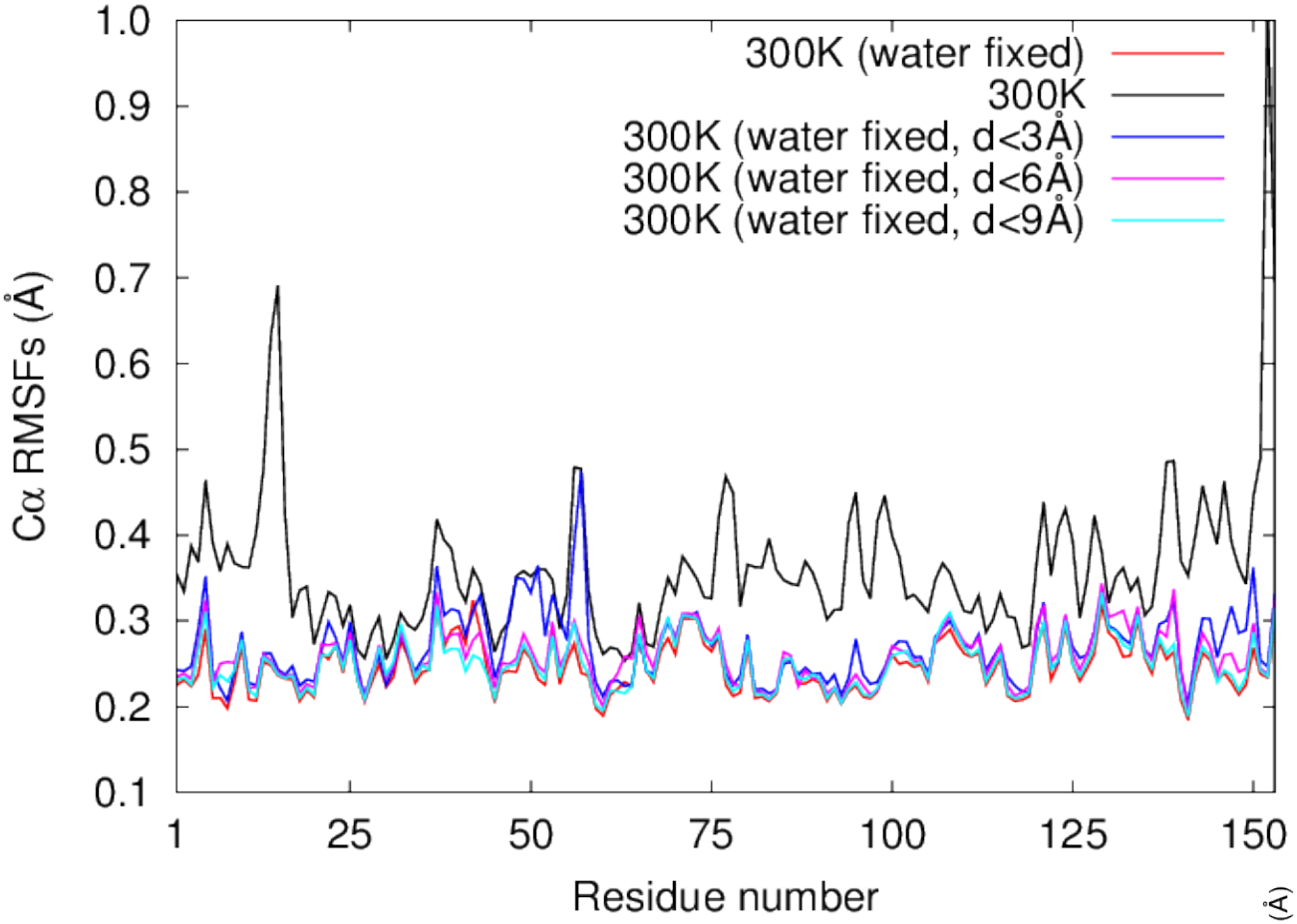
"Bioprotection offered by trehalose is a non specific interaction whereby the protein is selectively hydrated by a thin, immobilized layer of water. "

"It is an important and challenging goal to understand the effect of varying thickness of confined solvent on protein structure and dynamics."

A. M. Massari et al., JACS, 127, 2005; JACS, 128, 2006

vibrational echo spectroscopy on heme proteins in trehalose glasses

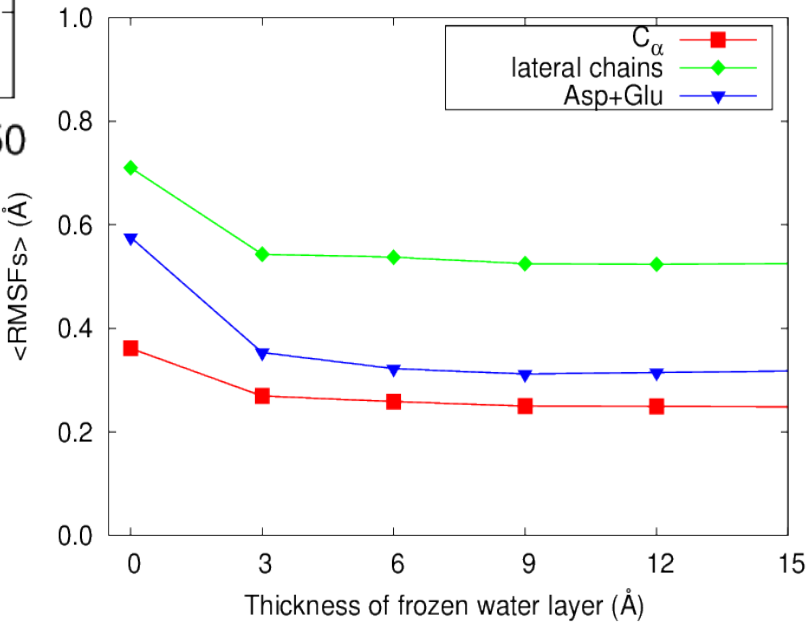
Fixed water only up to a definite distance from the protein surface @300K: protein dynamics



Only the first layers of water are really needed to modulate protein dynamics

@3Å: the only region with larger flexibility is the CD loop/D helix.

Protein atoms RMSFs are constant if water beyond 3Å is fixed, same value they would have if all the water molecules were fixed



Conclusions

From MD and FTIR: a description of solid amorphous sugar matrices, mechanism of preservation and trehalose peculiarity, at molecular level**.**

Water and CO IR bands: explore, at one and the same time, properties of matrix and protein.

Different saccharide matrices react differently to myoglobin introduction:

- *Trehalose, maltose*: the chaotropic effect of protein change the population of the sub-components (i.e. different water environments), by increasing the low frequency ones
- *Sucrose*: more a perturbation of a single, bulk–like, water population; conversely, protein undergoes stronger stress conditions

Conclusions

- **MD in trehalose/water systems:** disentangling the effects of the two solvent components

Water dynamics is the **key** factor in ternary systems

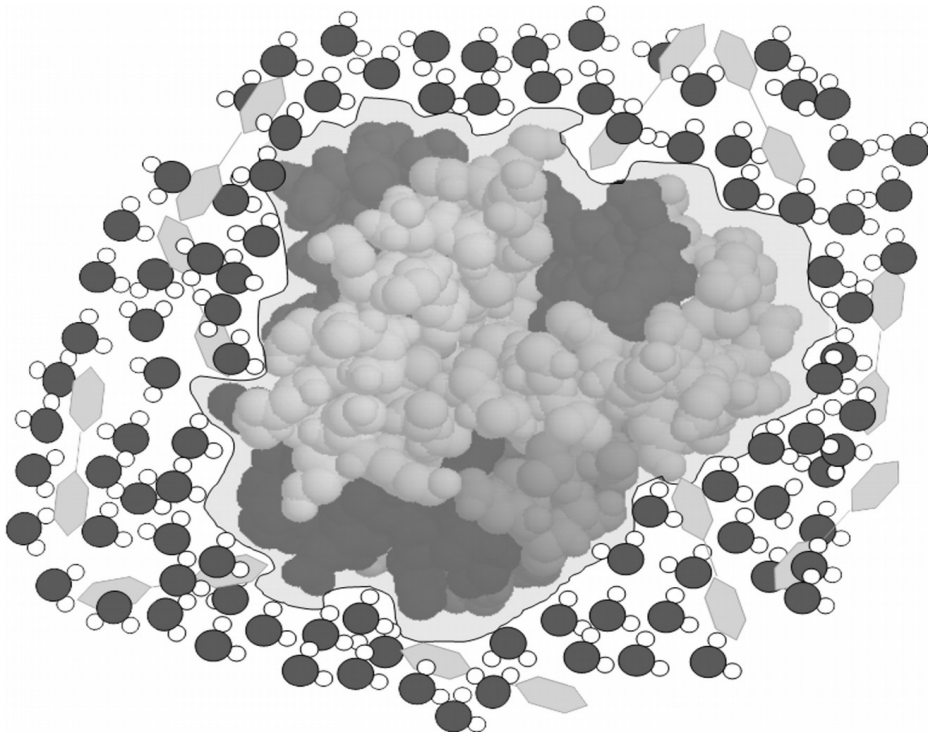
- *Shutting down water traslations*: the entire system behaves like a harmonic solid
- *By restricting the water traslations in specific shells around the protein*: water molecules up to 6 Å from the protein surface mostly control protein dynamics

In Corradini et al, Sci Rep, 3, 2013

> water ≤ 6 Å from the surface of the lysozyme in Lyz-Tr(aq) is approximately 4 times slower than when no trehalose is present at $T = 300$ K and it becomes approximately 6 times slower at $T = 260$ K.

Bioprotection by saccharides...

Saccharides protect not simply by preserving proteins native solvation bur rather locking their surface through constrained water molecules.



In Corradini et al, Sci. Rep., 3, 2013:

"*a layer of water packed between the lysozyme and an approximate shell of trehalose molecules. Dynamically, water molecules in the layer between the trehalose cage and the lysozyme are slower than in bulk water.*"

In Olsson et al , JPCB, 120,2016

"…*our results suggest that the protein surface is mainly covered by 1-2 molecular layers of water and that the stabilizing trehalose molecules are generally located outside this hydration layer...*",

from DSC and viscosity measurements

Acknowledgments

Sergio Giuffrida (FTIR, Flash Photolyis, SAXS)

Giuseppe Bellavia (DSC)

Lorenzo Cordone, Giovanni Ciccotti, Antonio Cupane

XAFS

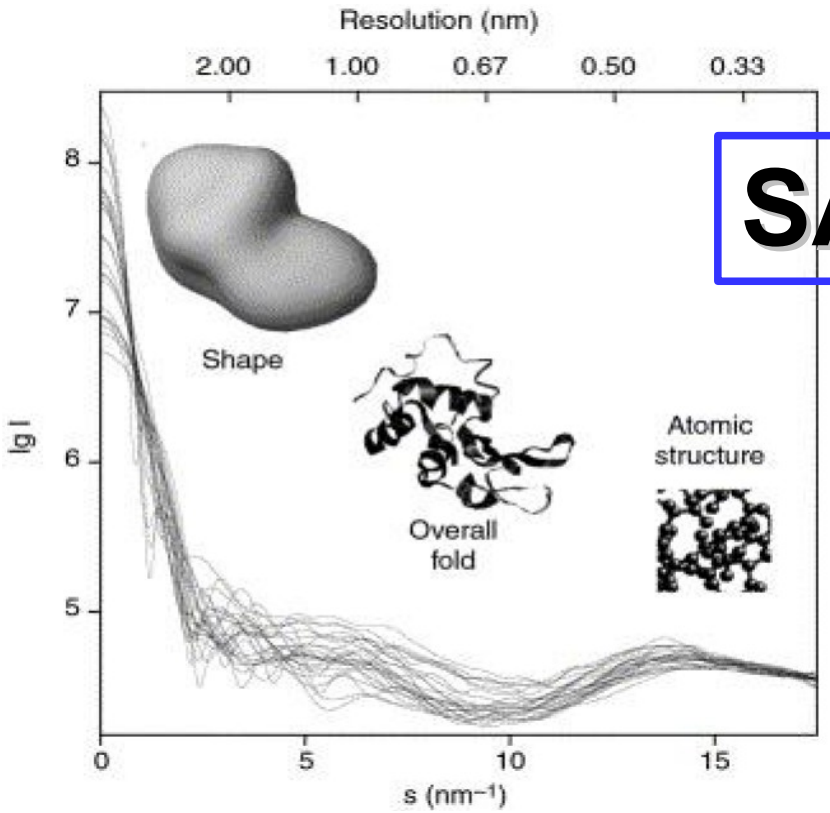
study of cytochrome c in trehalose glasses at different content of residual water:

-reduction of the mean square relative displacements of the central Fe with respect the first coordination shell;

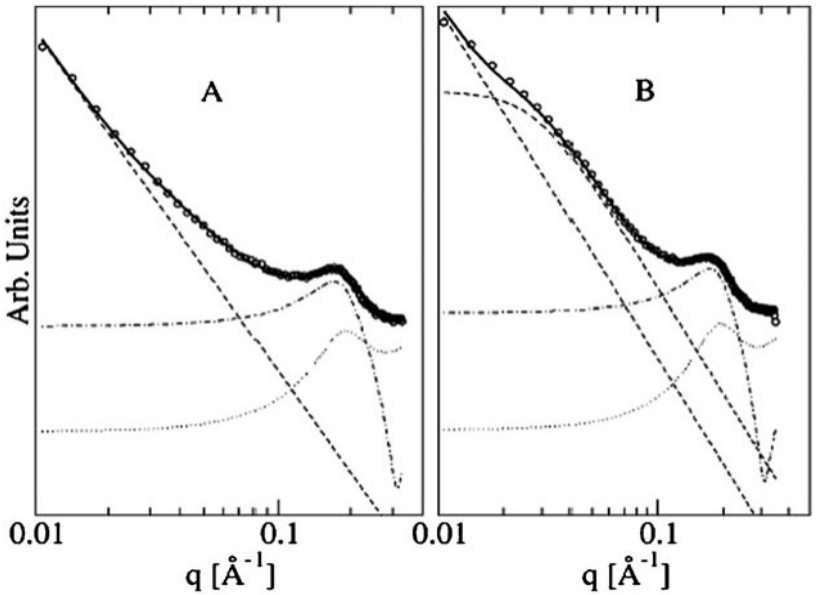
-distortion of the porphirin group.

"...the matrix induces conformational changes in the protein which in turn result in structural distortions of the heme group at the local atomic scale..... the heme could be involved in a direct interaction with the matrix."

Giachini et al, Biophys. J., 92, 2007

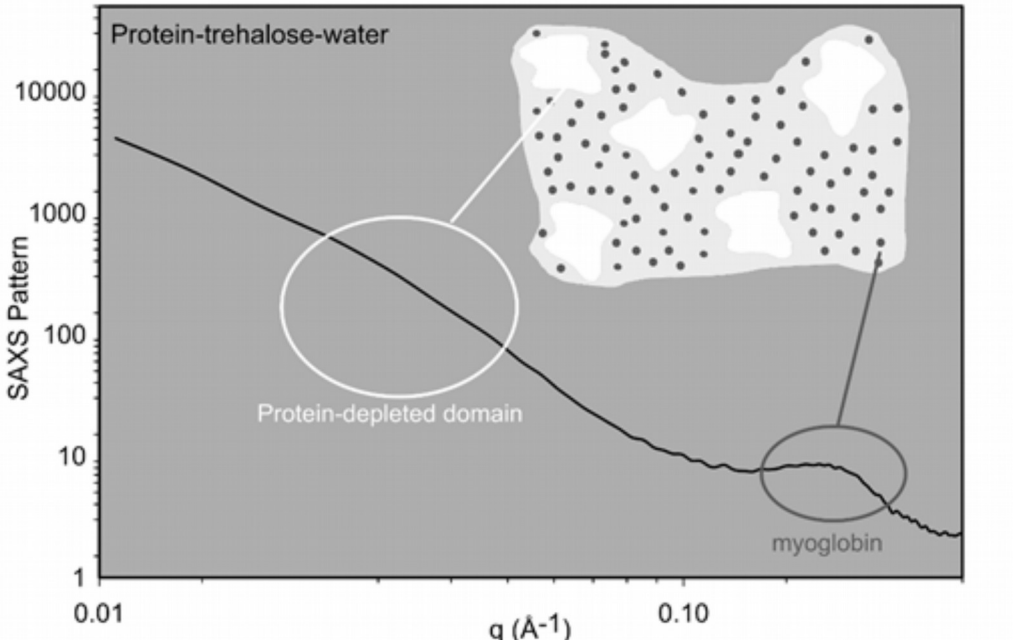


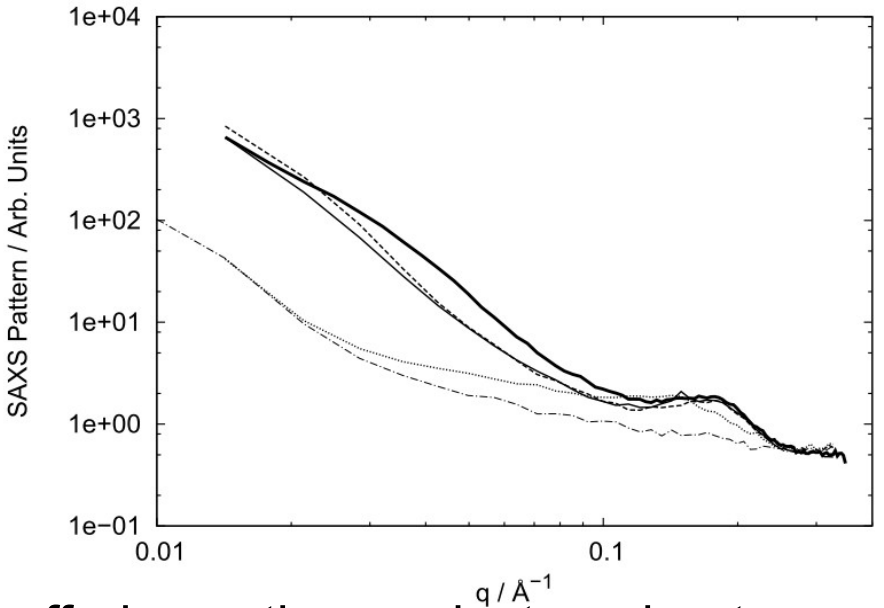
SAXS



Water-dependent domains evidenced by small angle X-ray scattering

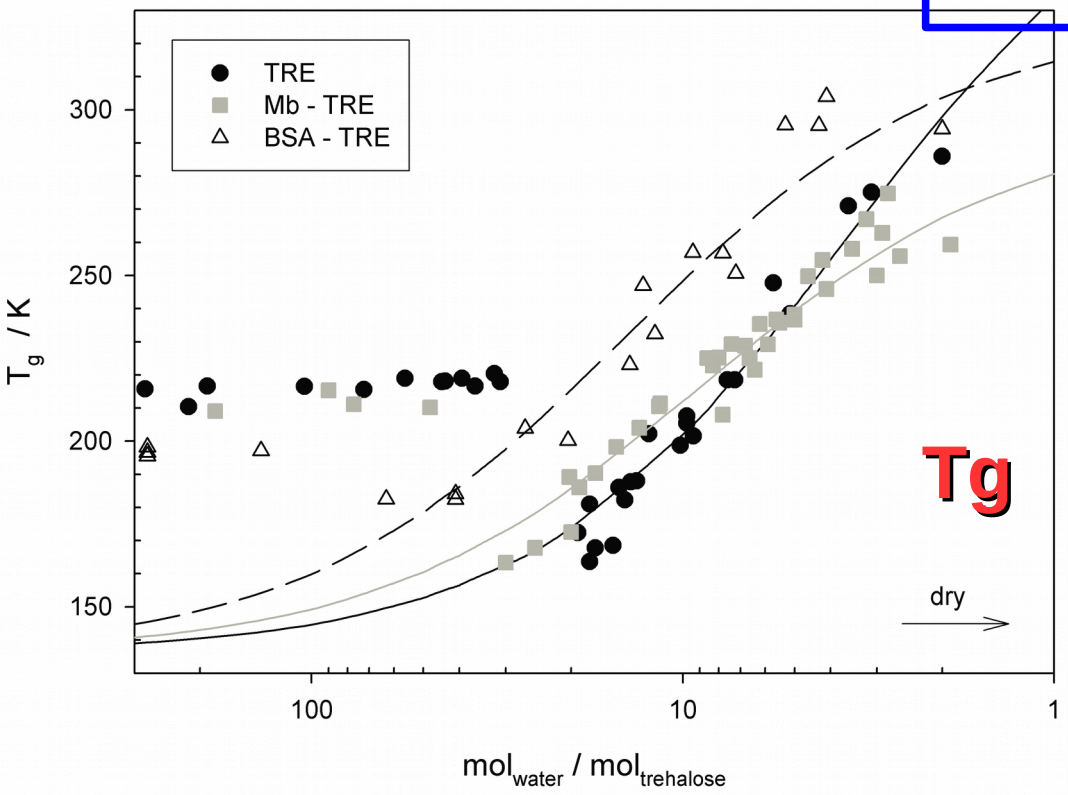
Longo et al.,PCCP, 12, 2010;
Giuffrida et al, Eur. Phys. J. 34,2011

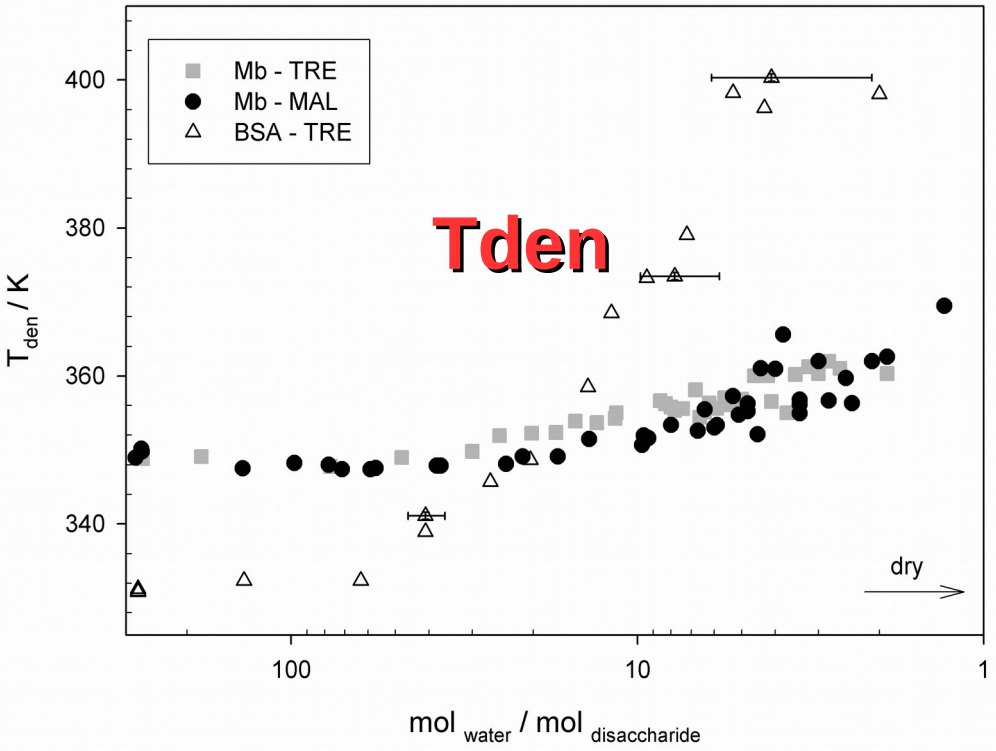




A buffering action against moderate variation of the sample hydration, contributing to the protein preservation

DSC

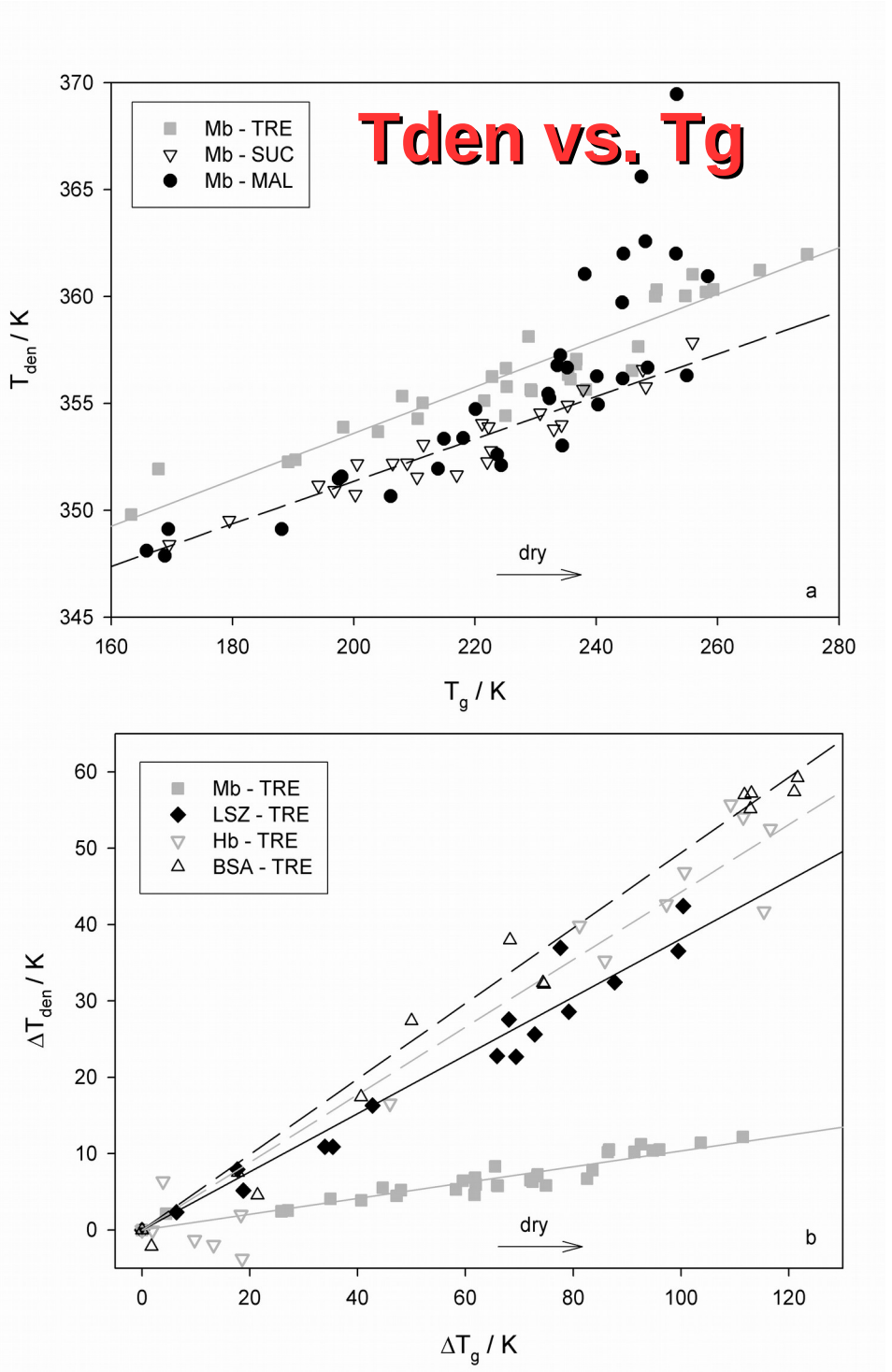




<u>At low hydration</u>: Tg marks the transition between a liquid/rubbery state and an homogeneous glassy state (Gordon-Taylor applies);
<u>At high hydration</u>, between an homogeneous liquid and an heterogeneous solid, a mixture of ice and glass (Tg constant).

- Proteins decreases the Tg extrapolated at zero water, hinting for looser water/sugar interactions: the protein, competing for water with sugar, reduces the strength of the HB network. A strong effect of the protein size was found on *Tg* values, while both protein size and charge do affect *T*den for given *Tg* values.

DSC


Tden vs. Tg
Mb - TRE
Mb - SUC
Mb - MAL
T_{den} / K
T_g / K
dry
a
Mb - TRE
LSZ - TRE
Hb - TRE
BSA - TRE
ΔT_{den} / K
ΔT_g / K
dry
b