Supplementary Information

Stabilization of proteins embedded in sugars and water as studied by dielectric spectroscopy

Christoffer Olsson, Rano Zangana, Jan Swenson*

Department of Physics, Chalmers University of Technology, SE-412 96 Göteborg Sweden

*Corresponding author e-mail: jan.swenson@chalmers.se

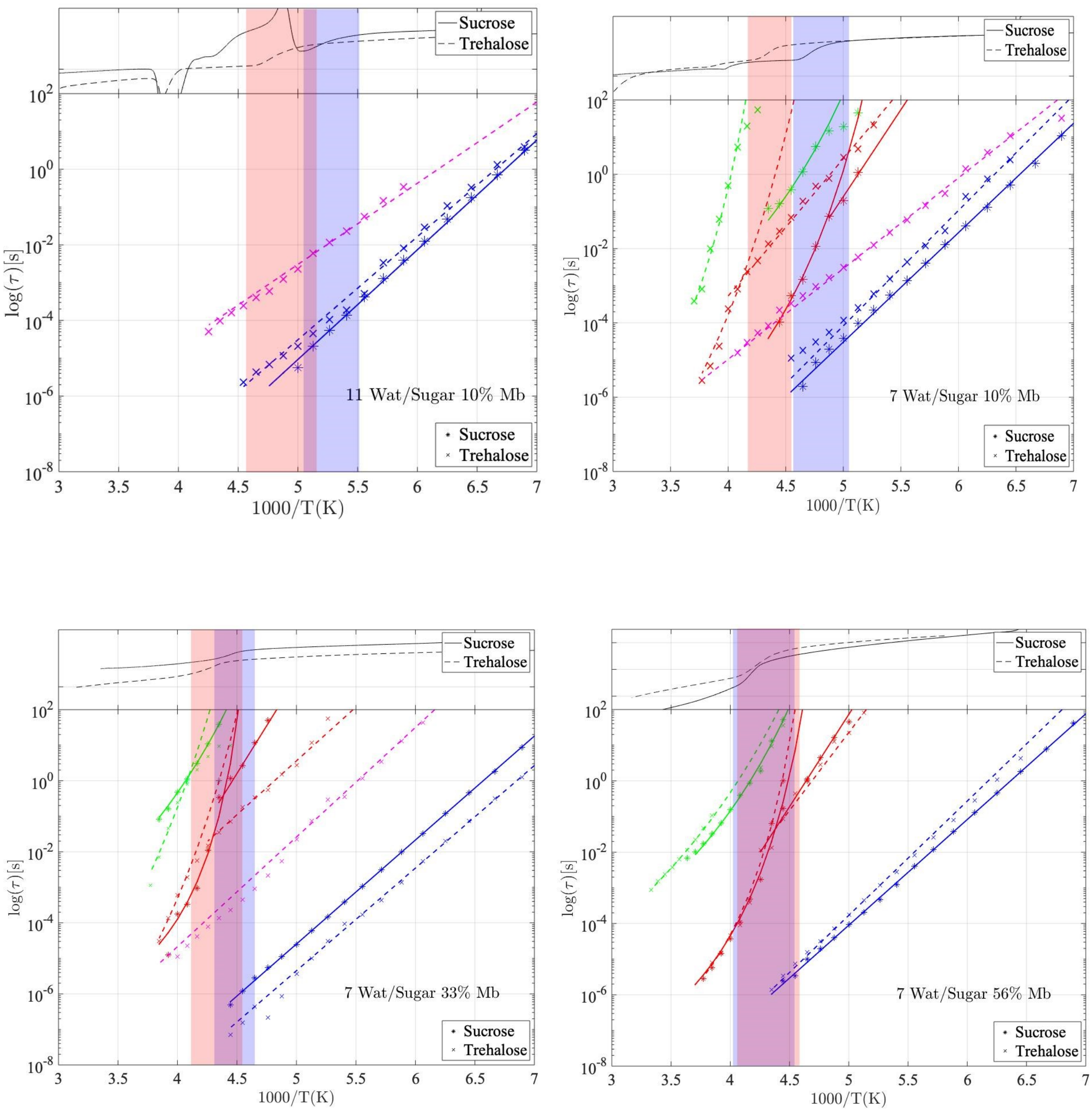

Figure S1. Arrhenius plots for four of the investigated samples compositions. Upper left figure: 11 waters per sugar molecule with 10 wt% myoglobin. Upper right figure: 7 waters per sugar molecule with 10 wt% myoglobin. Lower left figure: 7 water per sugar molecule with 33 wt% myoglobin. Lower right figure: 7 water per sugar molecule with 56 wt% myoglobin. Dashed lines represent fits of trehalose data (marked with x-symbols), and solid lines represents fits to sucrose data (marked with asterisk). Blue, magenta, red, and green lines represent the water β-relaxation, trehalose β-relaxation, solvent α-relaxation/solvent low-T relaxation, and protein α-relaxation respectively. The heat-flow from a DSC scan (heating cycle) for each sample is shown in the upper part of each panel, and the temperature region of the glass transition is highlighted by the red and blue shaded area for trehalose and sucrose samples respectively. Solvent and protein α-relaxations have been omitted for the 11 Wat/Sugar 10%Mb samples due to cold crystallization, which makes these processes difficult to interpret.

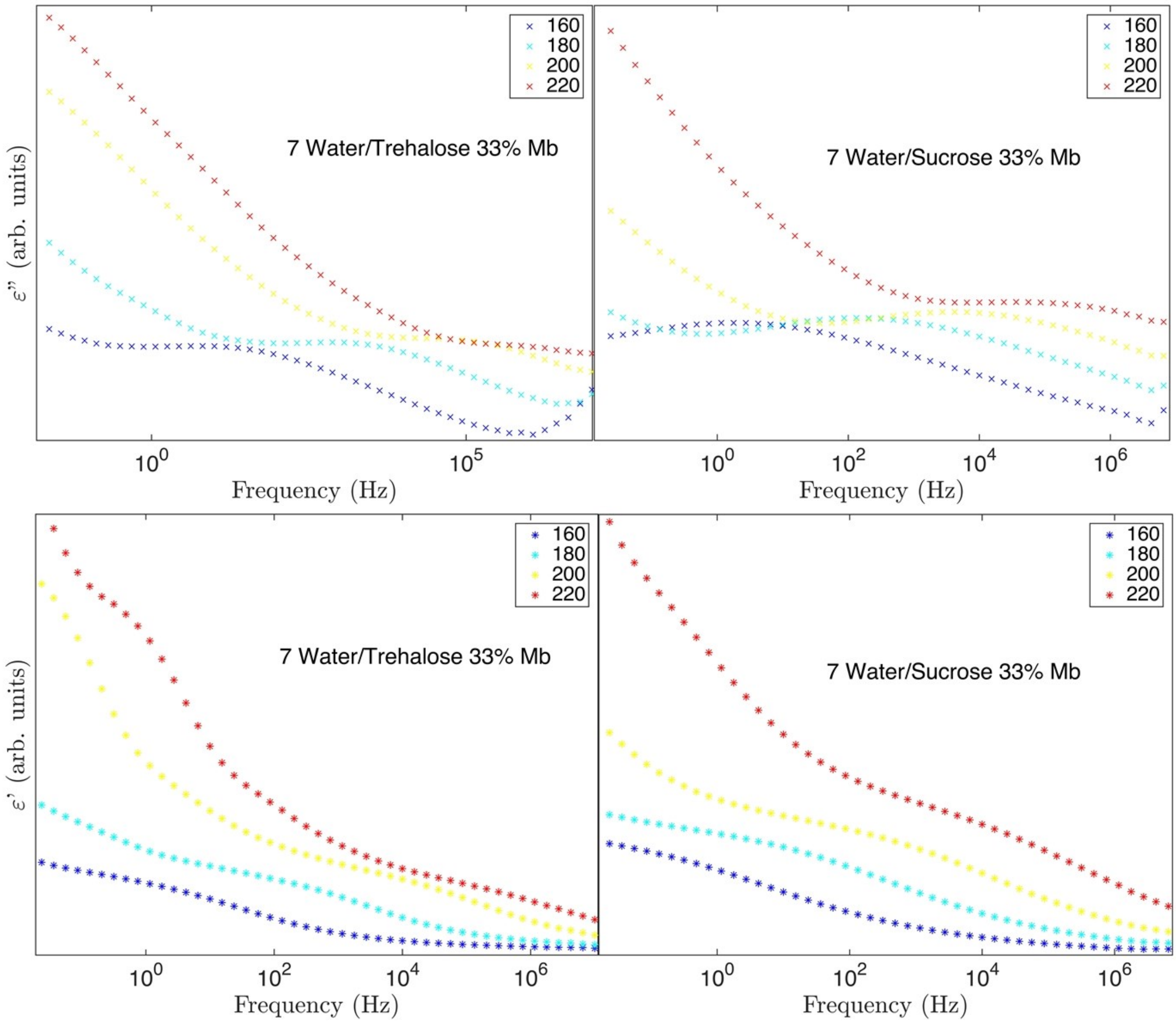

Figure S2. Dielectric data from samples of 7 water per sugar molecule and 33wt% myoglobin. Left panels show samples with trehalose, right panels for sucrose. Upper panels show imaginary permittivity and lower panels show real permittivity.

Table S1. Arrhenius parameters (see equation 2 in the main paper) of water β-relaxation, sugar β-relaxation, and solvent low-T β-relaxation (Low-T) for all samples. All two-component systems with trehalose are taken from Ref. 1, and the two-component systems with sucrose are made by fitting the dielectric data which were presented in Ref. 2. Values are given for samples containing trehalose/sucrose.

Sample	Water-β Ea (eV)	Water-β τ_0 (s•10^{-20})	Sugar-β Ea (eV)	Sugar-β τ_0 (s)	Low-T Ea (eV)	Low-T τ_0 (s)*
11 Wat/Sug Mb 10 wt%	0.54/0.57	71/2.9	0.43	5.6•10^{-14}	–/–	–/–
11 Wat/Sug Mb 33 wt%	0.55/0.59	7.8/1.1	0.44	4.3•10^{-14}	0.74/0.67	2•10^{-18}/2•10^{-17}
11 Wat/Sug Mb 56 wt%	0.59/0.58	3.6/8.2	–/–	–/–	0.66/1.1	2•10^{-24}/5•10^{-16}
7 Wat/Sug Mb 10 wt%	0.56/0.58	106/5.7	0.47	3.1•10^{-15}	0.73/0.93	9•10^{-19}/1•10^{-24}
7 Wat/Sug Mb 33 wt%	0.59/0.58	3.6/5.9	0.61	9.8•10^{-18}	0.60/0.68	3•10^{-15}/1•10^{-24}
7 Wat/Sug Mb 56 wt%	0.64/0.59	1.6/13.6	–/–	–/–	0.87/1.0	3•10^{-21}/1•10^{-24}
4.7 Wat/Tre[1]/Suc[2]	0.61/0.58	63.1/5	0.66	3.98•10^{-19}	–/–	–/–
8.1 Wat/Tre[1]/Suc[2]	0.57/0.60	12.5/5	0.53	1.58•10^{-16}	–/–	–/–
12.7 Wat/Tre[1]	0.53	20.0/–	0.41	3.16•10^{-14}	–/–	–/–

* τ_0 for solvent low-T process was fixed between 10^{-24} and 10^{-13} seconds.

Table S2. VFT parameters (see equation 3) of solvent and protein α-relaxation for all samples. 11Wat/Tre was fitted as well, although these processes have been omitted from figure 2 for clarity. Values are given for samples containing trehalose/sucrose.

Sample	Solvent α τ_0 (s)	Solvent α T_0 (K)	Solvent α D	Protein α τ_0 (s)	Protein a T_0 (K)	Protein α D
11 Wat/Sug Mb 33 wt%	$10^{-13}/10^{-8}$	171/149	9.5/8	$10^{-10}/10^{-6}$	159/169	13/4
11 Wat/Sug Mb 56 wt%	$10^{-8}/10^{-10}$	195/186	3/4	$10^{-10}/10^{-6}$	138/177	17/5
7 Wat/Sug Mb 10 wt%	$10^{-14}/10^{-8}$	179/149	9/7.5	$10^{-10}/10^{-6}$	197/163	6/5
7 Wat/Sug Mb 33 wt%	$10^{-14}/10^{-14}$	154/155	15/14	$10^{-10}/10^{-10}$	185/129	7/21
7 Wat/Sug Mb 56 wt%	$10^{-8}/10^{-8}$	212/211	1.5/1.5	$10^{-8}/10^{-6}$	170/181	8/5
4.7 Wat/Tre*	10^{-11}/–	140.8/–	20.9/–	–/–	–/–	–/–

*Fit parameters for binary trehalose-water solution obtained from Ref. 1.

References – Supplementary Information

1 S. E. Pagnotta, A. Alegría and J. Colmenero, *Phys. Chem. Chem. Phys.*, 2012, **14**, 2991–2996.

2 H. Jansson, R. Bergman and J. Swenson, *J. Non. Cryst. Solids*, 2005, **351**, 2858–2863.