Supplementary Information for "Microscopic mechanism of protein cryopreservation in an aqueous solution with trehalose"

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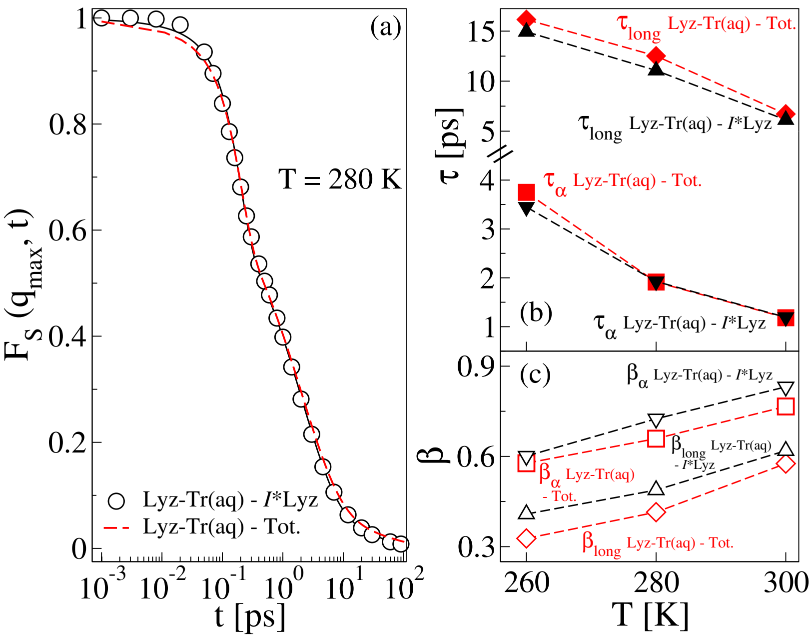

FIG. S1. **SISF for water in Lyz-Tr (aq) with exclusion of the first shell around lysozyme.** (a) $F_S(q_{\rm max}, t)$ for water in Lyz-Tr (aq) excluding the first shell around the lysozyme, "Lyz-Tr(aq) – I*Lyz", at $T = 280$ K (black empty circles). The fit to Eq. 2 (main text) (black solid line) is compared to the fit of the F to Eq. 2 (main text) for water in the Lyz-Tr(aq) with no exclusion, "Lyz-Tr(aq) – Tot.", (red dashed line), replotted from Fig. 1 (main text) for comparison. A similar behaviour is found at $T = 300$ K and $T = 260$ K (not shown). (b) α-relaxation time τ_α and long relaxation time $\tau_{\rm long}$ for water in the Lyz-Tr(aq) excluding the first shell around lysozyme, Lyz-Tr(aq) – I*Lyz (black solid symbols), and for water in the Lyz-Tr(aq) with no exclusions, Lyz-Tr(aq) – Tot. (red solid symbols). (c) β_α and $\beta_{\rm long}$ stretching parameters for water in the Lyz-Tr(aq) excluding the first shell around lysozyme, Lyz-Tr(aq) – I*Lyz (black empty symbols), and for water in the Lyz-Tr(aq) with no exclusions, Lyz-Tr(aq) – Tot. (red empty symbols).

Supplementary Fig. S1(a) shows the behaviour of the $F_S(q_{\rm max}, t)$ when excluding water molecules in the first hydration shell around the lysozyme, "Lyz-Tr(aq) – I*Lyz", at $T =$ 280 K. The behaviour at the other two temperatures is analogous. For defining the first hydration shell around the lysozyme we considered the water molecules hydrogen bonded to oxygen or nitrogen atoms of the protein with the same criterion used for water hydrogen bonded to trehalose (see description of Fig. 3 in the main text).

We can see that the $F_S(q_{\rm max}, t)$ is similar to Lyz-Tr(aq) case with no exclusions, "Lyz-

Tr(aq) – Tot.”. In fact it retains a two relaxations behaviour and it can be fitted to Eq. 2 (main text). Nonetheless water molecules in the first hydration shell of lysozyme bring some contribution to the global slow behaviour of the F in the Lyz-Tr(aq). This can be better appreciated looking at parameters of the fits to Eq. 2 (main text) in Supplementary Fig. S1(b) and Supplementary Fig. S1(c). While the α relaxation times remain similar in the two cases $\tau_\alpha^{\mathrm{Lyz-Tr(aq)}-I^*\mathrm{Lyz}} \simeq \tau_\alpha^{\mathrm{Lyz-Tr(aq)-Tot.}}$, a difference can be noted for the long relaxation for which $\tau_{\mathrm{long}}^{\mathrm{Lyz-Tr(aq)}-I^*\mathrm{Lyz}}/\tau_{\mathrm{long}}^{\mathrm{Lyz-Tr(aq)-Tot.}} \simeq 0.9$ at all temperatures. At the same time the stretching parameters β_α and β_{long} increase slightly with respect to the Lyz-Tr(aq) with no exclusion case. The parameters of the fits to Eq. 2 (main text) for the Lyz-Tr(aq) – I*Lyz case are reported in Supplementary Table S1. The behaviour of the SISF when the first hydration shell around the lysozyme is excluded suggests that slow water is in contact with the protein. In fact while the two relaxations behaviour can be mostly attributed to the water molecules in the hydration shells of trehalose (see Fig. 3 in the main text), the behaviour of the SISF shows a faster relaxation when water around the lysozyme is excluded.

TABLE S1. List of the parameters of the fits to Eq. 2 (main text) $F_S(q_{\max}, t) = \left[1 - f_{q_{\max}} - f'_{q_{\max}}\right] \exp\left[-\left(\frac{t}{\tau_{\text{short}}}\right)^2\right] + f_{q_{\max}} \exp\left[-\left(\frac{t}{\tau_\alpha}\right)^{\beta_\alpha}\right] + f'_{q_{\max}} \exp\left[-\left(\frac{t}{\tau_{\text{long}}}\right)^{\beta_{\text{long}}}\right]$ for Lyz-Tr(aq) – Tot. and Lyz-Tr(aq) – I*Lyz and of the fits to Eq. 1 (main text) $F_S(q_{\max}, t) = [1 - f_{q_{\max}}] \exp\left[-\left(\frac{t}{\tau_{\text{short}}}\right)^2\right] + f_{q_{\max}} \exp\left[-\left(\frac{t}{\tau_\alpha}\right)^{\beta_\alpha}\right]$ for Lyz-Tr(aq) – I* (trehalose), Lyz-Tr(aq) – II* (trehalose) and Bulk.

		$f_{q_{\max}}$	$f'_{q_{\max}}$	τ_{short}	τ_α	τ_{long}	β_α	β_{long}
$T = 300$ K	Lyz-Tr(aq) – Tot.	0.584359	0.111535	0.190230	1.18387	6.71622	0.766115	0.576114
	Lyz-Tr(aq) – I*Lyz	0.535120	0.129061	0.189303	1.19341	6.13365	0.831296	0.618946
	Lyz-Tr(aq) – I*	0.652241	–	0.202432	1.17182	–	0.798041	–
	Lyz-Tr(aq) – II*	0.665936	–	0.199643	0.96099	–	0.869277	–
	Bulk	0.664717	–	0.193461	0.85728	–	0.910156	–
$T = 280$ K	Lyz-Tr(aq) – Tot.	0.591952	0.134828	0.203097	1.91389	12.51730	0.659084	0.414610
	Lyz-Tr – I*Lyz	0.558884	0.133505	0.195751	1.92569	11.06920	0.724976	0.488304
	Lyz-Tr – I*	0.682218	–	0.214967	1.81278	–	0.690843	–
	Lyz-Tr – II*	0.683894	–	0.215981	1.47241	–	0.746518	–
	Bulk	0.684976	–	0.191378	1.27801	–	0.835559	–
$T = 260$ K	Lyz-Tr(aq) – Tot.	0.582011	0.191664	0.204441	3.74828	16.15930	0.577371	0.327369
	Lyz-Tr(aq) – I*-Lyz	0.557836	0.207728	0.189253	3.44751	14.93850	0.601019	0.408345
	Lyz-Tr(aq) – I*	0.737366	–	0.197649	3.20290	–	0.616682	–
	Lyz-Tr(aq) – II*	0.710196	–	0.194428	2.56413	–	0.699302	–
	Bulk	0.677822	–	0.179273	2.44198	–	0.815086	–

TABLE S2. List of the parameters of the fits to Eq. 2 (main text) $F_S(q_{\max},t) = \left[1 - f_{q_{\max}} - f'_{q_{\max}}\right] \exp\left[-\left(\frac{t}{\tau_{\text{short}}}\right)^2\right] + f_{q_{\max}} \exp\left[-\left(\frac{t}{\tau_\alpha}\right)^{\beta_\alpha}\right] + f'_{q_{\max}} \exp\left[-\left(\frac{t}{\tau_{\text{long}}}\right)^{\beta_{\text{long}}}\right]$ for Lyz-Tr(aq) – Tot.; Tr 4 Å [Lyz-Tr(aq)] at $T = 300$ K and $T = 280$ K; Lyz 4 Å [Lyz(aq)] at $T = 300$ K and $T = 280$ K; Tr 6 Å [Lyz-Tr(aq)]; Lyz 6 Å [Lyz(aq)], and of the fits to Eq. 1 (main text) $F_S(q_{\max},t) = \left[1 - f_{q_{\max}}\right] \exp\left[-\left(\frac{t}{\tau_{\text{short}}}\right)^2\right] + f_{q_{\max}} \exp\left[-\left(\frac{t}{\tau_\alpha}\right)^{\beta_\alpha}\right]$ for Lyz(aq) – Tot.; Bulk; Tr 4 Å [Lyz-Tr(aq)] at $T = 260$ K; Lyz 4 Å [Lyz-Tr(aq)]; Lyz 4 Å [Lyz(aq)] at $T = 260$ K and Lyz 6 Å [Lyz-Tr(aq)].

		$f_{q_{\max}}$	$f'_{q_{\max}}$	τ_{short}	τ_α	τ_{long}	β_α	β_{long}
$T = 300$ K	Lyz-Tr(aq) – Tot.	0.584359	0.111535	0.190230	1.18387	6.71622	0.766115	0.576114
	Lyz(aq) – Tot.	0.689111	–	0.198597	0.82475	–	0.877819	–
	Bulk	0.664717	–	0.193461	0.85728	–	0.910156	–
	Tr 4 Å [Lyz-Tr(aq)]	0.571267	0.106773	0.204395	1.94960	29.49980	0.649551	0.452266
	Lyz 4 Å [Lyz-Tr(aq)]	0.698100	–	0.153800	8.38500	–	0.556300	–
	Lyz 4 Å [Lyz(aq)]	0.569766	0.106662	0.181084	1.75068	29.09700	0.715022	0.664794
	Tr 6 Å [Lyz-Tr(aq)]	0.604741	0.095060	0.214644	1.42628	8.35204	0.682437	0.406320
	Lyz 6 Å [Lyz-Tr(aq)]	0.676900	–	0.177400	4.77500	–	0.578100	–
	Lyz 6 Å [Lyz(aq)]	0.551527	0.112901	0.185124	1.25059	6.24917	0.804940	0.566776
$T = 280$ K	Lyz-Tr(aq) – Tot.	0.591952	0.134828	0.203097	1.91389	12.51730	0.659084	0.414610
	Lyz(aq) – Tot.	0.709957	–	0.196084	1.17256	–	0.812877	–
	Bulk	0.684976	–	0.191378	1.27801	–	0.835559	–
	Tr 4 Å [Lyz-Tr(aq)]	0.530068	0.229836	0.182635	1.91433	41.39240	0.611325	0.527808
	Lyz 4 Å [Lyz-Tr(aq)]	0.722800	–	0.142300	19.0600	–	0.548700	–
	Lyz 4 Å [Lyz(aq)]	0.483972	0.228089	0.159823	1.81168	39.02960	0.739354	0.727756
	Tr 6 Å [Lyz-Tr(aq)]	0.561877	0.163991	0.195753	1.96479	16.78740	0.666699	0.461131
	Lyz 6 Å [Lyz-Tr(aq)]	0.714300	–	0.163100	11.8300	–	0.521200	–
	Lyz 6 Å [Lyz(aq)]	0.524932	0.174574	0.168871	1.39659	13.17730	0.800175	0.872130
$T = 260$ K	Lyz-Tr(aq) – Tot.	0.582011	0.191664	0.204441	3.74828	16.15930	0.577371	0.327369
	Lyz(aq) – Tot.	0.731959	–	0.189956	1.97533	–	0.729689	–
	Bulk	0.677822	–	0.179273	2.44198	–	0.815086	–
	Tr 4 Å [Lyz-Tr(aq)]	0.723200	–	0.173600	12.9300	–	0.500700	–
	Lyz 4 Å [Lyz-Tr(aq)]	0.741000	–	0.128200	27.1600	–	0.561700	–
	Lyz 4 Å [Lyz(aq)]	0.732600	–	0.177400	11.4500	–	0.522000	–
	Tr 6 Å [Lyz-Tr(aq)]	0.494265	0.245038	0.183916	4.74424	23.86400	0.616644	0.366783
	Lyz 6 Å [Lyz-Tr(aq)]	0.735300	–	0.160600	23.2800	–	0.47850	–
	Lyz 6 Å [Lyz(aq)]	0.580614	0.167992	0.212598	4.12236	19.80210	0.562879	0.334729

TABLE S3. List of the parameters of the fits to Eq. 2 (main text) $F_S(q_{\max}, t) = \left[1 - f_{q_{\max}} - f'_{q_{\max}}\right] \exp\left[-\left(\frac{t}{\tau_{\text{short}}}\right)^2\right] + f_{q_{\max}} \exp\left[-\left(\frac{t}{\tau_\alpha}\right)^{\beta_\alpha}\right] + f'_{q_{\max}} \exp\left[-\left(\frac{t}{\tau_{\text{long}}}\right)^{\beta_{\text{long}}}\right]$ for Lyz-Tr(aq) – Tot.; Lyz COG – OW < 30 Å [Lyz-Tr(aq)] and Lyz COG – OW > 30 Å [Lyz-Tr(aq)] and of the fits to Eq. 1 (main text) $F_S(q_{\max}, t) = [1 - f_{q_{\max}}] \exp\left[-\left(\frac{t}{\tau_{\text{short}}}\right)^2\right] + f_{q_{\max}} \exp\left[-\left(\frac{t}{\tau_\alpha}\right)^{\beta_\alpha}\right]$ for Lyz(aq) – Tot.; Lyz COG – OW < 30 Å [Lyz(aq)] and Lyz COG – OW > 30 Å [Lyz(aq)].

		$f_{q_{\max}}$	$f'_{q_{\max}}$	τ_{short}	τ_α	τ_{long}	β_α	β_{long}
$T = 300$ K	Lyz-Tr(aq) – Tot.	0.584359	0.111535	0.190230	1.18387	6.71622	0.766115	0.576114
	Lyz(aq) – Tot.	0.689111	–	0.198597	0.82475	–	0.877819	–
	Lyz COG – OW < 30 Å [Lyz-Tr(aq)]	0.504875	0.174221	0.183818	1.37205	16.36800	0.753093	0.587430
	Lyz COG – OW > 30 Å [Lyz-Tr(aq)]	0.554536	0.116388	0.187745	1.20168	6.08090	0.813751	0.585492
	Lyz COG – OW < 30 Å [Lyz(aq)]	0.641697	–	0.214730	1.02087	–	0.813798	–
	Lyz COG – OW > 30 Å [Lyz(aq)]	0.671666	–	0.193427	0.82197	–	0.945596	–
$T = 280$ K	Lyz-Tr(aq) Tot.	0.591952	0.134828	0.203097	1.91389	12.51730	0.659084	0.414610
	Lyz(aq) – Tot.	0.709957	–	0.196084	1.17256	–	0.812877	–
	Lyz COG – OW < 30 Å [Lyz-Tr(aq)]	0.495345	0.214257	0.187461	2.28361	37.28530	0.662217	0.548706
	Lyz COG – OW > 30 Å [Lyz-Tr(aq)]	0.578246	0.114585	0.198959	1.89661	12.03400	0.723795	0.466510
	Lyz COG – OW < 30 Å [Lyz(aq)]	0.673526	–	0.226589	1.47433	–	0.726963	–
	Lyz COG – OW > 30 Å [Lyz(aq)]	0.687277	–	0.190699	1.17371	–	0.867815	–
$T = 260$ K	Lyz-Tr(aq) – Tot.	0.582011	0.191664	0.204441	3.74828	16.15930	0.577371	0.327369
	Lyz(aq) – Tot.	0.731959	–	0.189956	1.97533	–	0.729689	–
	Lyz COG – OW < 30 Å [Lyz-Tr(aq)]	0.569052	0.149054	0.167184	4.20910	92.90600	0.648069	0.989989
	Lyz COG – OW > 30 Å [Lyz–Tr(aq)]	0.510947	0.207283	0.186237	3.50107	19.63330	0.679091	0.468595
	Lyz COG – OW < 30 Å [Lyz(aq)]	0.724274	–	0.283457	2.61937	–	0.553157	–
	Lyz COG – OW > 30 Å [Lyz(aq)]	0.697309	–	0.187282	2.02271	–	0.796245	–