Correction notice

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Microscopic mechanism of protein cryopreservation in an aqueous solution with trehalose

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In the version of this Article initially published online, there were several typographical errors. These have now been corrected.

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

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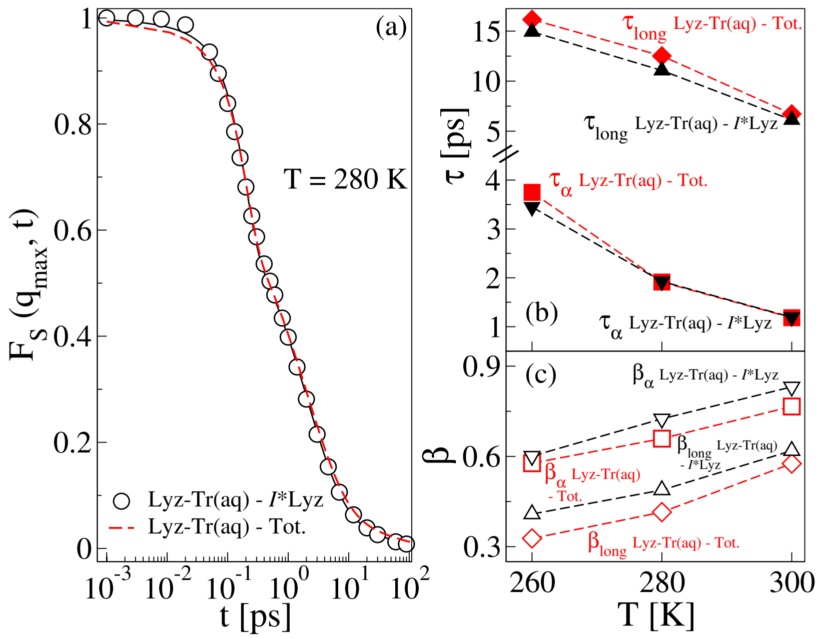

FIG. S1. **SISF for water in Lyz-Tr(aq) with exclusion of the first shell around lysozyme.** (a) $F_S(q_{\rm max}, t)$ for water in Lyz-Tr(aq) excluding the first shell around the lysozyme, "Lyz-Tr(aq) – I*Lyz", at $T = 280$ K (black empty circles). The fit to Eq. 2 (main text) (black solid line) is 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 consider 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) $F_S(q_{\max}, t) = [1 - f_{q_{\max}} - f'_{q_{\max}}] \exp\left[-\left(\frac{t}{\tau_{\text{short}}}\right)^2\right] + f_{q_{\max}} \exp\left[-\left(\frac{t}{\tau_\alpha}\right)^{\beta_\alpha}\right] + f'_{q_{\max}} \exp\left[-\left(\frac{t}{\tau_{\text{long}}}\right)^{\beta_{\text{long}}}\right]$ 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.	0.584	0.112	0.190	1.184	6.716	0.766	0.576
	Lyz-Tr(aq) – I^*Lyz	0.535	0.129	0.189	1.193	6.134	0.831	0.619
	Lyz-Tr(aq) – I^*	0.652	–	0.202	1.172	–	0.798	–
	Lyz-Tr(aq) – II^*	0.666	–	0.200	0.961	–	0.869	–
	Bulk	0.665	–	0.193	0.857	–	0.910	–
$T = 280$ K	Lyz-Tr(aq) – Tot.	0.592	0.135	0.203	1.914	12.517	0.659	0.415
	Lyz-Tr – I^*Lyz	0.559	0.134	0.196	1.926	11.069	0.725	0.488
	Lyz-Tr – I^*	0.682	–	0.215	1.813	–	0.691	–
	Lyz-Tr – II^*	0.684	–	0.216	1.472	–	0.747	–
	Bulk	0.685	–	0.191	1.278	–	0.836	–
$T = 260$ K	Lyz-Tr(aq) – Tot.	0.582	0.192	0.204	3.748	16.159	0.577	0.327
	Lyz-Tr(aq) – I^*-Lyz	0.558	0.208	0.189	3.448	14.939	0.601	0.408
	Lyz-Tr(aq) – I^*	0.737	–	0.198	3.203	–	0.617	–
	Lyz-Tr(aq) – II^*	0.710	–	0.194	2.564	–	0.699	–
	Bulk	0.678	–	0.179	2.442	–	0.815	–

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.	0.584	0.112	0.190	1.184	6.716	0.766	0.576
	Lyz(aq) – Tot.	0.689	–	0.199	0.825	–	0.878	–
	Bulk	0.665	–	0.193	0.857	–	0.910	–
	Tr 4 Å [Lyz-Tr(aq)]	0.571	0.107	0.204	1.950	29.500	0.650	0.452
	Lyz 4 Å [Lyz-Tr(aq)]	0.698	–	0.154	8.385	–	0.556	–
	Lyz 4 Å [Lyz(aq)]	0.570	0.107	0.181	1.751	29.097	0.715	0.665
	Tr 6 Å [Lyz-Tr(aq)]	0.605	0.095	0.215	1.426	8.352	0.682	0.406
	Lyz 6 Å [Lyz-Tr(aq)]	0.677	–	0.177	4.775	–	0.578	–
	Lyz 6 Å [Lyz(aq)]	0.552	0.113	0.185	1.251	6.249	0.805	0.567
$T = 280$ K	Lyz-Tr(aq) – Tot.	0.592	0.135	0.203	1.914	12.517	0.659	0.415
	Lyz(aq) – Tot.	0.710	–	0.196	1.173	–	0.813	–
	Bulk	0.685	–	0.191	1.278	–	0.836	–
	Tr 4 Å [Lyz-Tr(aq)]	0.530	0.230	0.183	1.914	41.392	0.611	0.528
	Lyz 4 Å [Lyz-Tr(aq)]	0.723	–	0.142	19.060	–	0.549	–
	Lyz 4 Å [Lyz(aq)]	0.484	0.228	0.160	1.812	39.030	0.739	0.728
	Tr 6 Å [Lyz-Tr(aq)]	0.562	0.164	0.196	1.965	16.787	0.667	0.461
	Lyz 6 Å [Lyz-Tr(aq)]	0.714	–	0.163	11.830	–	0.521	–
	Lyz 6 Å [Lyz(aq)]	0.525	0.175	0.169	1.397	13.177	0.800	0.872
$T = 260$ K	Lyz-Tr(aq) – Tot.	0.582	0.192	0.204	3.748	16.159	0.577	0.327
	Lyz(aq) – Tot.	0.732	–	0.190	1.975	–	0.730	–
	Bulk	0.678	–	0.179	2.442	–	0.815	–
	Tr 4 Å [Lyz-Tr(aq)]	0.723	–	0.174	12.930	–	0.501	–
	Lyz 4 Å [Lyz-Tr(aq)]	0.741	–	0.128	27.160	–	0.562	–
	Lyz 4 Å [Lyz(aq)]	0.733	–	0.177	11.450	–	0.522	–
	Tr 6 Å [Lyz-Tr(aq)]	0.494	0.245	0.184	4.744	23.864	0.617	0.367
	Lyz 6 Å [Lyz-Tr(aq)]	0.735	–	0.161	23.280	–	0.479	–
	Lyz 6 Å [Lyz(aq)]	0.581	0.168	0.213	4.122	19.802	0.563	0.335

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 $\leq$ 30 Å [Lyz-Tr(aq)] and Lyz COG – OW > 30 Å [Lyz-Tr(aq)] and of the fits to Eq. 1 (main text) $F_S(q_{\max}, t) = \left[1 - f_{q_{\max}}\right] \exp\left[-\left(\frac{t}{\tau_{\text{short}}}\right)^2\right] + f_{q_{\max}} \exp\left[-\left(\frac{t}{\tau_\alpha}\right)^{\beta_\alpha}\right]$ for Lyz(aq) – Tot.; Lyz COG – OW $\leq$ 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.	0.584	0.112	0.190	1.184	6.716	0.766	0.576
	Lyz(aq) – Tot.	0.689	–	0.199	0.825	–	0.878	–
	Lyz COG – OW $\leq$ 30 Å [Lyz-Tr(aq)]	0.505	0.174	0.184	1.372	16.368	0.753	0.587
	Lyz COG – OW > 30 Å [Lyz-Tr(aq)]	0.555	0.116	0.188	1.202	6.081	0.814	0.585
	Lyz COG – OW $\leq$ 30 Å [Lyz(aq)]	0.642	–	0.215	1.021	–	0.814	–
	Lyz COG – OW > 30 Å [Lyz(aq)]	0.672	–	0.193	0.822	–	0.946	–
$T = 280$ K	Lyz-Tr(aq) Tot.	0.592	0.135	0.203	1.914	12.517	0.659	0.415
	Lyz(aq) – Tot.	0.710	–	0.196	1.173	–	0.813	–
	Lyz COG – OW $\leq$ 30 Å [Lyz-Tr(aq)]	0.495	0.214	0.187	2.284	37.285	0.662	0.549
	Lyz COG – OW > 30 Å [Lyz-Tr(aq)]	0.578	0.115	0.199	1.897	12.034	0.724	0.467
	Lyz COG – OW $\leq$ 30 Å [Lyz(aq)]	0.674	–	0.227	1.474	–	0.727	–
	Lyz COG – OW > 30 Å [Lyz(aq)]	0.687	–	0.191	1.174	–	0.868	–
$T = 260$ K	Lyz-Tr(aq) – Tot.	0.582	0.192	0.204	3.748	16.159	0.577	0.327
	Lyz(aq) – Tot.	0.732	–	0.190	1.975	–	0.730	–
	Lyz COG – OW $\leq$ 30 Å [Lyz-Tr(aq)]	0.569	0.149	0.167	4.209	92.906	0.648	0.990
	Lyz COG – OW > 30 Å [Lyz–Tr(aq)]	0.511	0.207	0.186	3.501	19.633	0.679	0.469
	Lyz COG – OW $\leq$ 30 Å [Lyz(aq)]	0.724	–	0.283	2.619	–	0.553	–
	Lyz COG – OW > 30 Å [Lyz(aq)]	0.697	–	0.187	2.023	–	0.796	–