Supporting Information

How Heterogeneous Are Trehalose/ Glycerol Cryoprotectant Mixtures? A Combined Time-Resolved Fluorescence and Computer Simulation Investigation

Sandipa Indra and Ranjit Biswas*

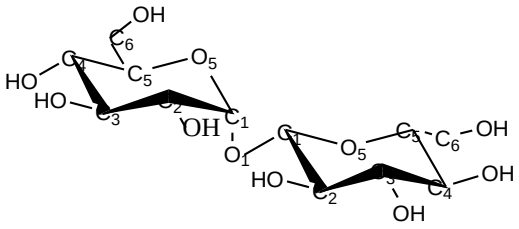
Department of Chemical, Biological and Macromolecular Sciences, S. N. Bose National Centre for Basic Sciences, Block-JD, Salt Lake, Sector-III, Kolkata-700106

*Email: ranjit@bose.res.in; phone: +91 33 2335 5706; Fax: +91 33 2335 3477.

Table S1: Partial Charges and Lennard-Jones parameters for glycerol

Atoms	Charge (e)	ε (kcal/mol)	σ (Å)
C (terminal)	0.182	0.066	3.50
H (attached to terminal C)	0.026	0.030	2.50
O (hydroxyl)	-0.694	0.170	3.12
H (hydroxyl)	0.506	0.000	0.00
C (central)	0.055	0.066	3.50
H (attached to central C)	0.041	0.030	2.50

Table S2: Partial Charges and Lennard-Jones parameters for trehalose. Atomic sites of trehalose are denoted as follows:



Atoms	Charge (e)	ε (kcal/mol)	σ (Å)
O_1	-0.388	0.1700	3.0000
C_1	0.509	0.1094	3.3997
$H(C_1)$	0.000	0.0157	2.2932
C_2	0.246	0.1094	3.3997
$H(C_2)$	0.000	0.0157	2.4714
$O(C_2)$	-0.713	0.2104	3.0665
$H[O(C_2)]$	0.437	0.0300	0.3565
C_3	0.286	0.1094	3.3997
$H(C_3)$	0.000	0.0157	2.4714
$O(C_3)$	-0.699	0.2104	3.0665
$H[O(C_3)]$	0.427	0.0300	0.3565
C_4	0.254	0.1094	3.3997
$H(C_4)$	0.000	0.0157	2.4714
$O(C_4)$	-0.710	0.2104	3.0665
$H[O(C_4)]$	0.436	0.0300	0.3565
C_5	0.283	0.1094	3.3997
$H(C_5)$	0.000	0.0157	2.4714
O5	-0.574	0.1700	3.0000
C_6	0.276	0.1094	3.3997
$H(C_6)$	0.000	0.0157	2.4714
$O(C_6)$	-0.682	0.2104	3.0665
$H[O(C_6)]$	0.418	0.0300	0.3565

Table S3: Multi-exponentialfit parameters for fluorescence intensity decays collected at three different wavelengths ($\lambda_{em.}$) of C153 steady state emission profile at 298 K and 333 K.

$T(K)$	λ_{em} (nm)	a_1	τ_1 (ps)	a_2	τ_2 (ps)	a_3	τ_3 (ps)
298	480	0.58	112	0.25	839	0.17	4395
	550	0.21	34	0.79	4188		
	630	0.41	276	0.59	4029		
333	480	0.77	69	0.10	813	0.13	3707
	550	0.08	755	0.92	3002		
	630	-0.37	76	1.37	3017		

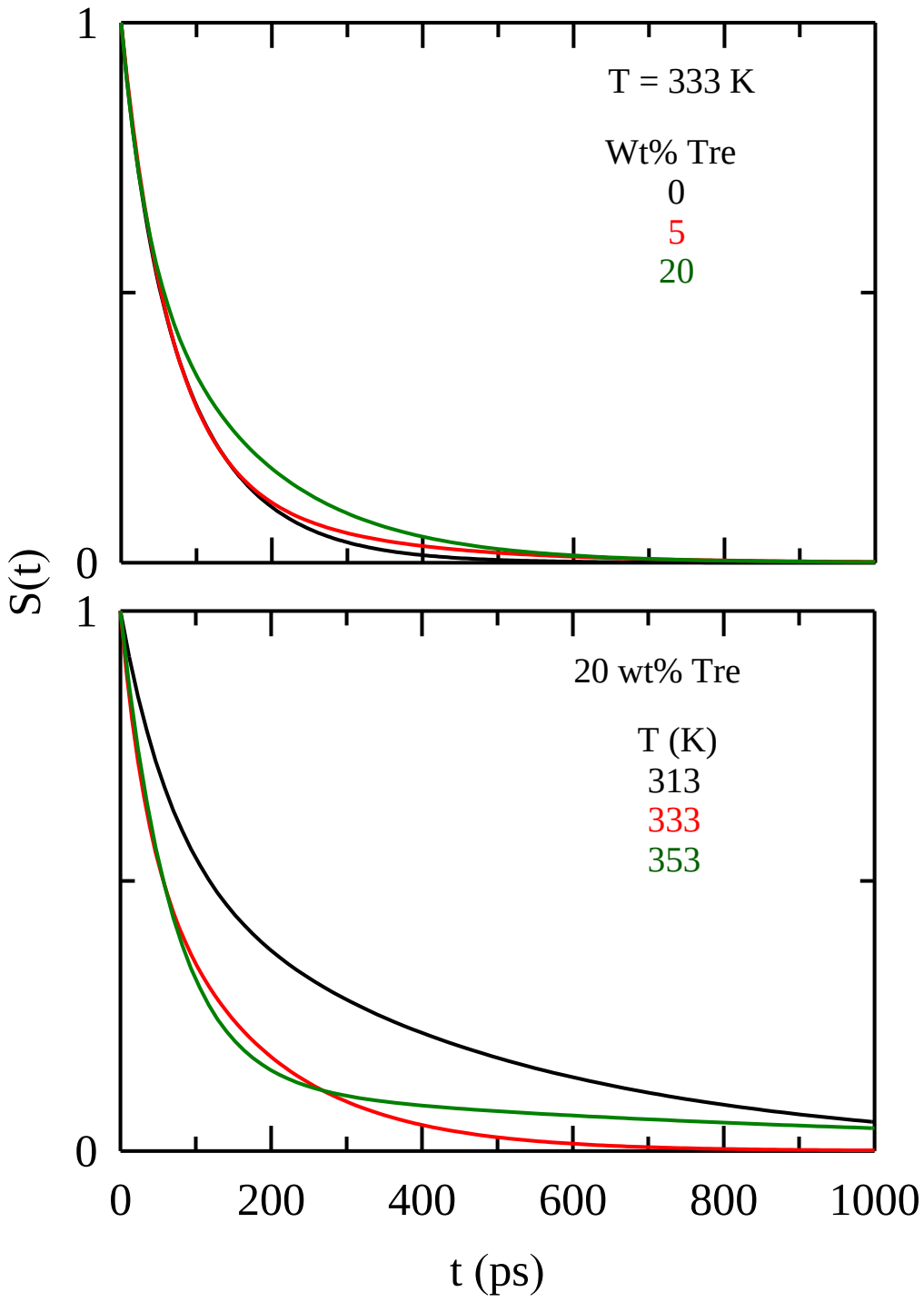


Figure S4: Solvation response functions, $S(t)$, at three different trehalose–glycerol compositions (upper panel) and at three different temperatures (lower panel). Representations are color-coded.

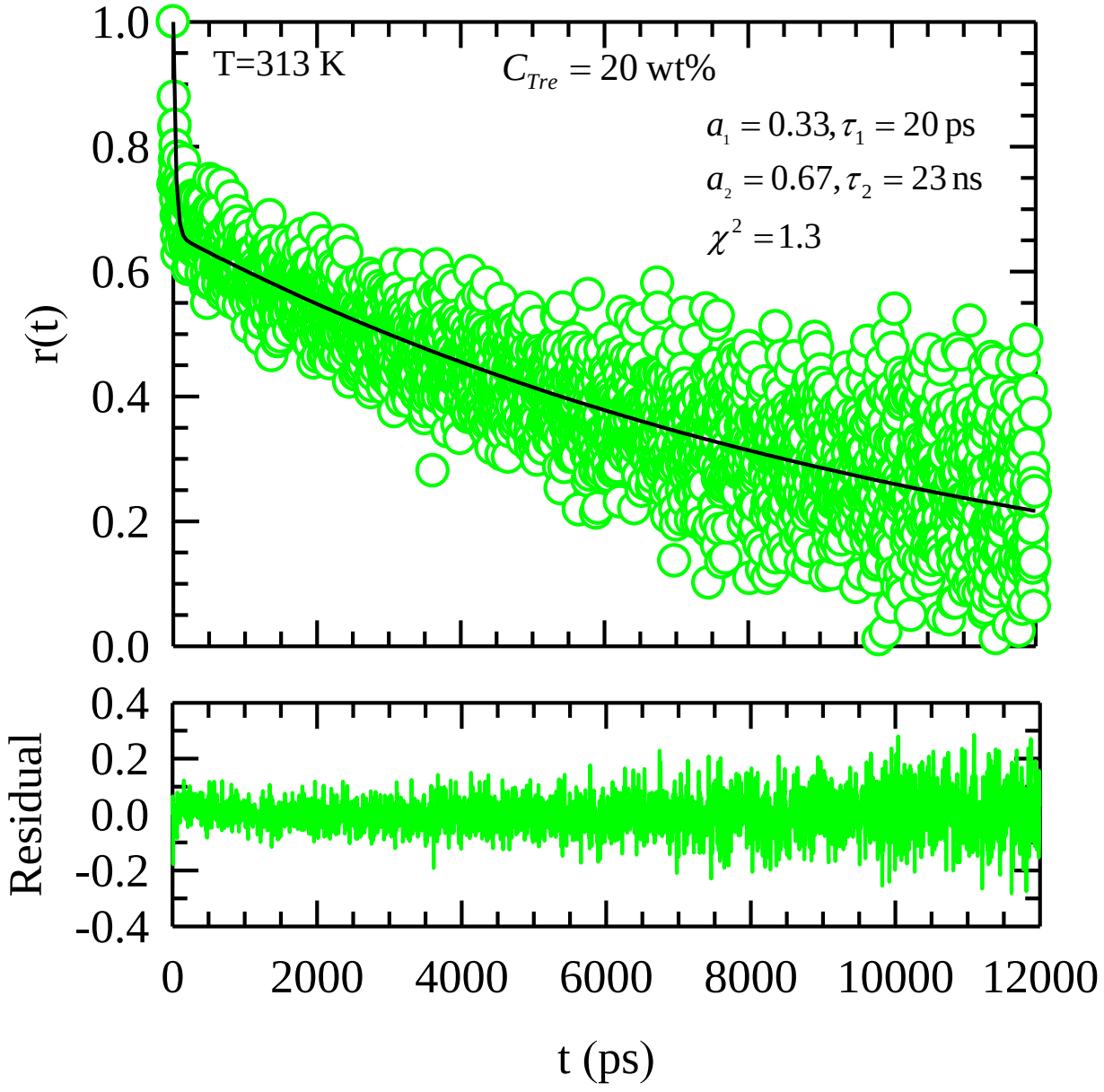


Figure S5: Representative fluorescence anisotropy decay, $r(t)$, for C153 in 20 wt% trehalose at 313K (upper panel). While open circles represent the data, the solid line shows the fit through the data. Parameters obtained from the bi-exponential fit to the data are also listed in the upper panel. The *goodness of fit parameter* (reduced χ^2) is also shown. Residualis represented in the lower panel.

Table S6: Average rotational times, $\langle \tau_r \rangle$, for C153 in trehalose/glycerol mixtures obtained from dynamic anisotropy measurements, and predictions from the SED relation using the stick and the slip boundary conditions at different trehalose concentrations (C_{Tre}) and temperatures.

C_{Tre}(wt%)	T(K)	η(cP)	Measured $\langle \tau_r \rangle$ (ns)	Predicted $\langle \tau_r \rangle$ from the SED relation	
				Slip (ns)	Stick (ns)
0	298	861	82.18	21.26	88.68
	313	273	5.55	6.36	26.51
	333	82	2.12	1.81	7.57
	353	33	0.76	0.69	2.87
5	298	937	96.95	23.17	96.62
	313	298	4.60	7.01	29.26
	333	89	2.29	1.97	8.21
	353	36	0.80	0.75	3.13
20	298	2258	229.5	55.83	232.85
	313	631	15.45	14.85	61.95
	333	167	3.82	3.70	15.41
	353	61	0.98	1.28	5.34

Table S7: Comparisonofsimulated densities (ρ_{sim}) with the experimental values[a]($\rho_{\exp}$) at 298K.

C_{Tre}(wt%)	ρ_{sim} (g/cc)	$\rho_{\exp}$ (g/cc)
0	1.223	1.257
5	1.225	1.266
20	1.279	1.297

a. This work.

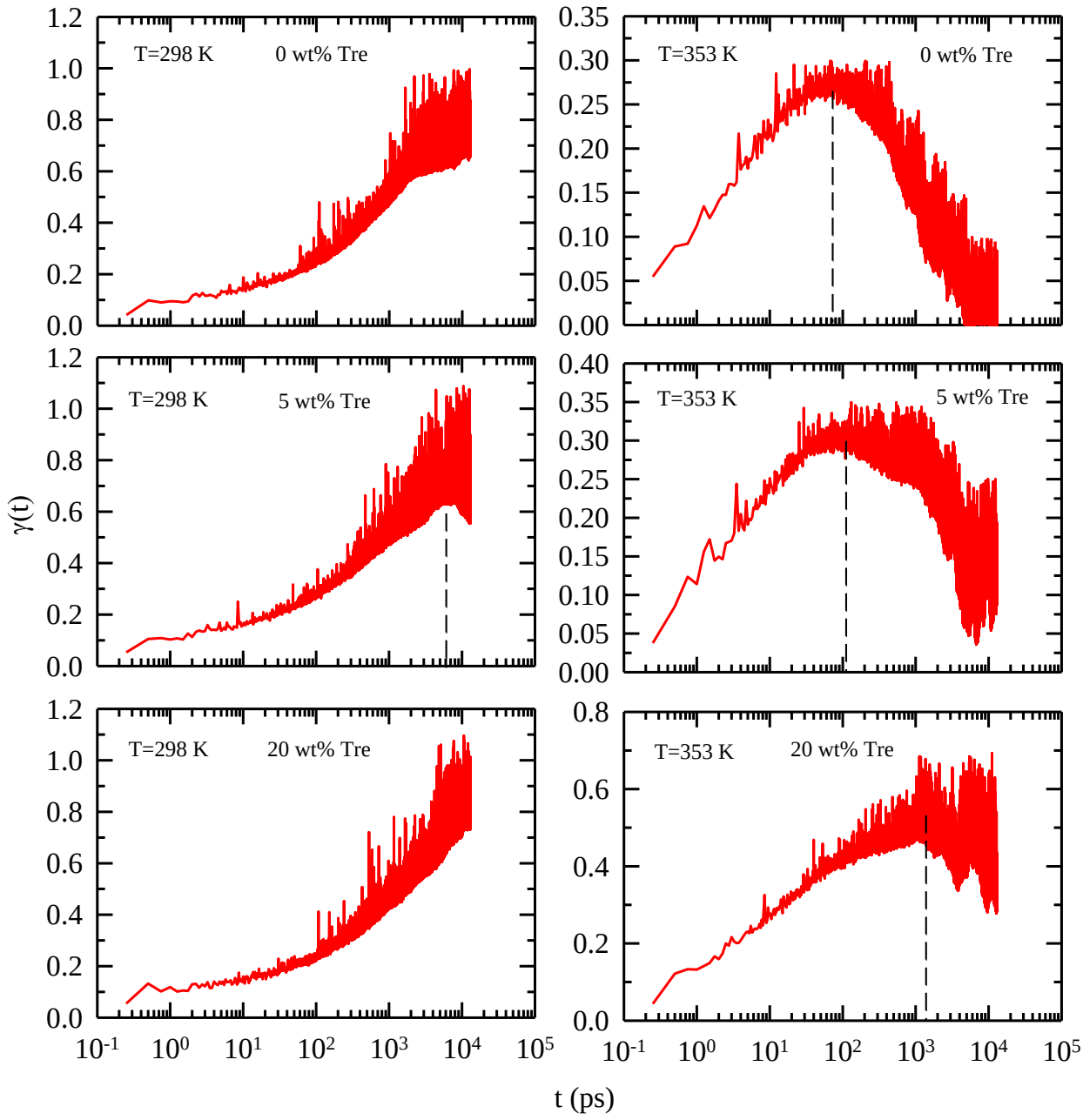


Figure S8: Simulated new non-Gaussian $\gamma(t)$ parameters for glycerol in trehalose/glycerol mixtures at three different trehalose concentrations. Results at 298 K are shown in the left panels while those at 353K are in the right panels. Vertical dashed lines represent peak times, τ_{NNG}, of $\gamma(t)$.

Table S9: Stretched exponentialfit parameters to glycerol–glycerol H-bond lifetime relaxation, $S_{HB}(t)$ at different trehalose concentrations in the trehalose/glycerol mixtures at 298K and 353K.

C_{Tre}(wt%)	T (K)	a	τ (ps)	β	$\langle\tau\rangle$ (ps)
0	298	1.00	5.28	0.42	15.43
	353	1.00	1.81	0.57	2.92
5	298	1.00	3.72	0.40	12.37
	353	1.00	1.80	0.57	2.91
20	298	1.00	3.17	0.39	11.30
	353	1.00	1.92	0.53	3.46

Table S10: (Stretched + single exponential) fit parameters to glycerol–glycerol H-bond structural relaxation, $C_{HB}(t)$ at different concentrations of trehalose at 298K and 353K.

C_{Tre}(wt%)	T (K)	a_1	τ_1 (ns)	β	a_2	τ_2 (ns)	$\langle\tau\rangle$ (ns)
0	298	0.67	6.37	0.32	0.33	15.48	35.12
	353	0.51	0.03	0.33	0.49	0.14	0.16
5	298	0.59	3.58	0.32	0.41	11.99	19.78
	353	0.57	0.04	0.35	0.43	0.17	0.19
20	298	0.82	8.34	0.36	0.18	26.77	36.08
	353	0.67	0.08	0.38	0.33	0.27	0.29

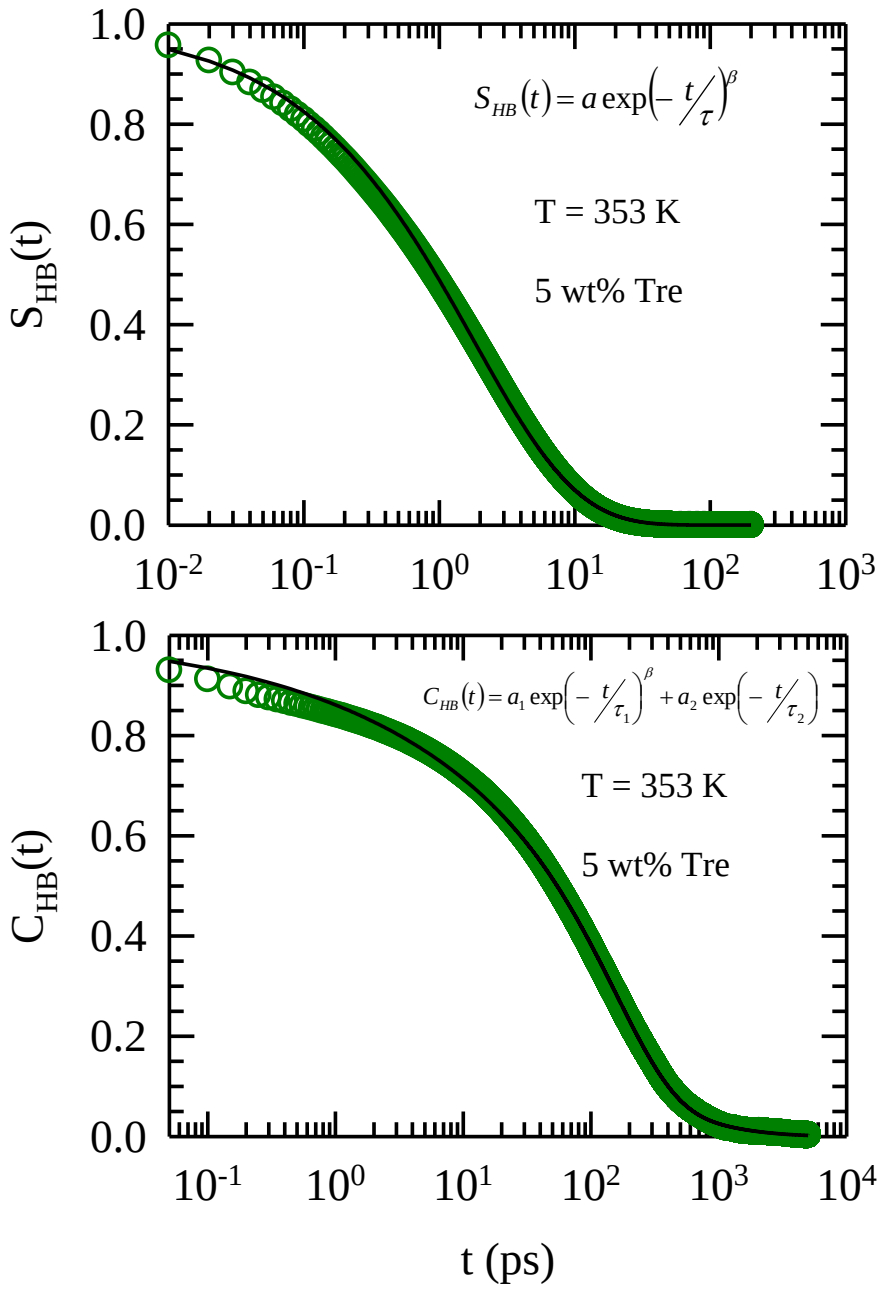


Figure S11: Simulated (symbols) relaxation decays of $S_{HB}(t)$ (upper panel) and $C_{HB}(t)$ (lower panel) with corresponding fits (solid lines) for glycerol–glycerolH-bonds in 5 wt% trehalose into glycerol at ~353 K. Fitting function is provided in the respective panel. This shows the typical quality of fits obtained in the present work.