Supplemental Information to "The effect of cholesterol on the bending modulus of DOPC bilayers: Re-analysis of NSE Data"

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S1. Detailed results of NSE data analysis

Neutron Spin Echo (NSE) data (1) was re-analyzed using different models, as indicated in the manuscript's main text. For all datasets (0%—50% cholesterol) and models, plots of fits of the experimentally determined $S(q,t)/S(q,0)$, best-fit parameters and goodness of fit values, χ^2, are provided here. Tables contain, if applicable, fit parameter limits, median fit parameter values, and 68% confidence intervals on both sides of the median. Fits to the data were obtained using a Monte Carlo Markov Chain-based global optimizer as provided by the Bumps data analysis Python package. (2) Error bars on plots of experimentally determined $S(q,t)/S(q,0)$ indicate 68% confidence limits. The q-values denoted in the figure legends are in units of $Å^{-1}$.

0% cholesterol

Two-parameter fit of K_C and η_m

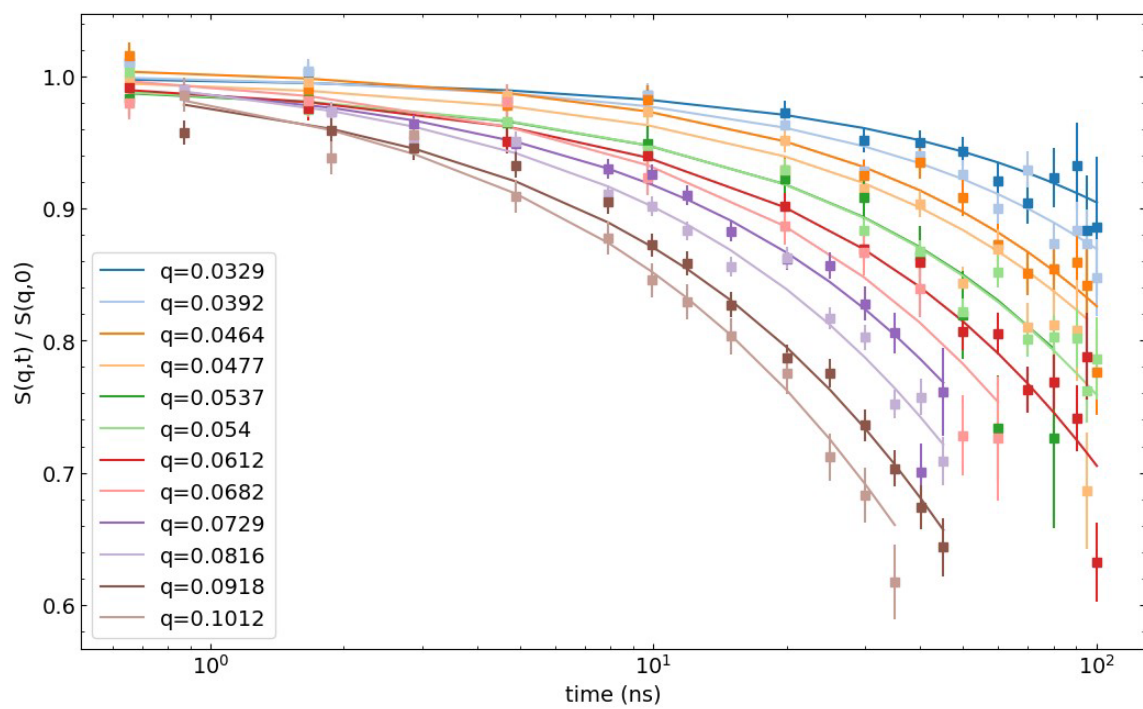| parameter or goodness of fit	lower limit	upper limit	median	-err	+err
η_m	0.0	10.5	1.2	-0.8	0.7
K_C	5.0	150.0	9.0	-3.0	14.0
χ^2	n/a	n/a	1.4	-0.1	0.1
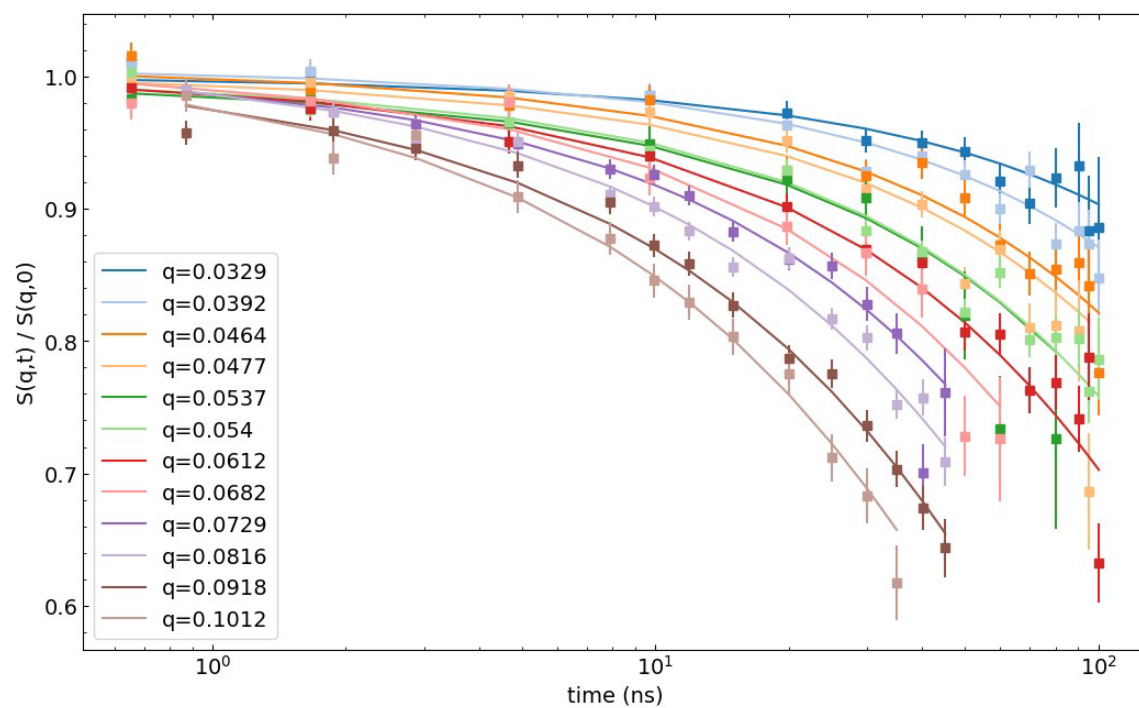
The units of q values are inverse Angstroms.

Note that some times were missing for q = 0.0477, 0.0537, 0.0682 and 0.1012 so these q values were not used for the averages in Fig. 2 in the main text. All data were used in the fits.
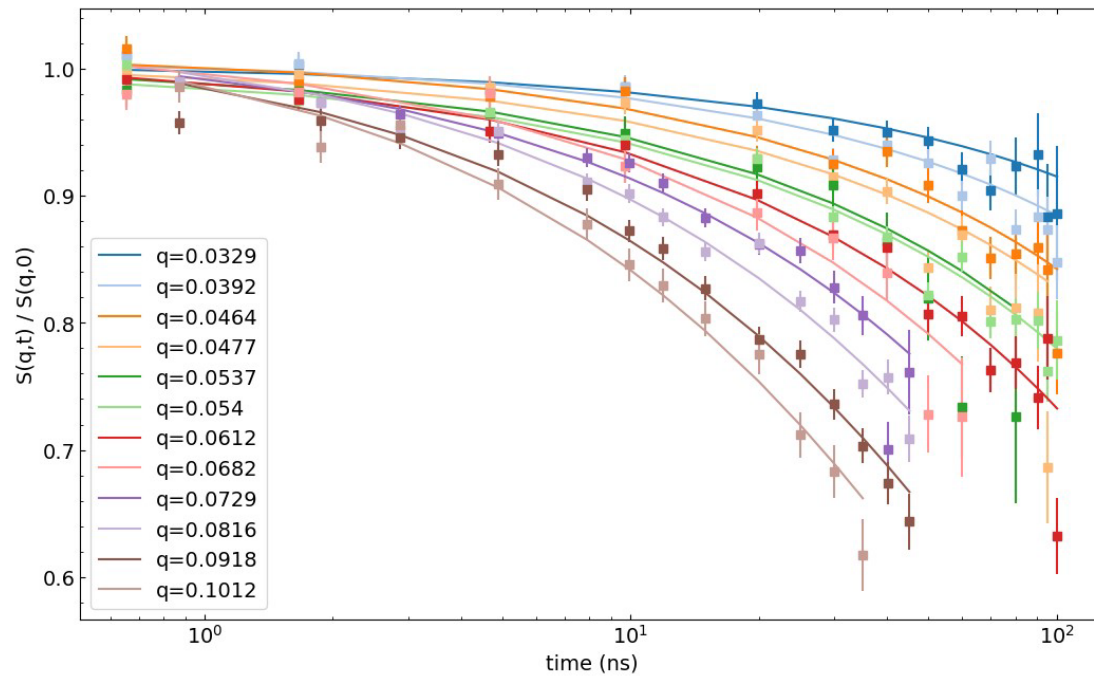
One-parameter fit of η_m

Parameter or goodness of fit	lower limit	upper limit	median	-err	+err
η_m	0.0	10.5	0.48	-0.03	0.04
χ^2	n/a	n/a	1.4	-0.1	0.1

One-parameter fit of K_C

Parameter or goodness of fit	lower limit	upper limit	median	-err	+err
K_C	5.0	350.0	73.0	-3.0	3.0
χ^2	n/a	n/a	1.8	-0.1	0.1

One-parameter fit of α using the diffusional softening model

Parameter or goodness of fit	lower limit	upper limit	median	-err	+err
α	0.0	1.0	0.02	-0.01	0.02
	n/a	n/a	1.4	-0.1	0.1
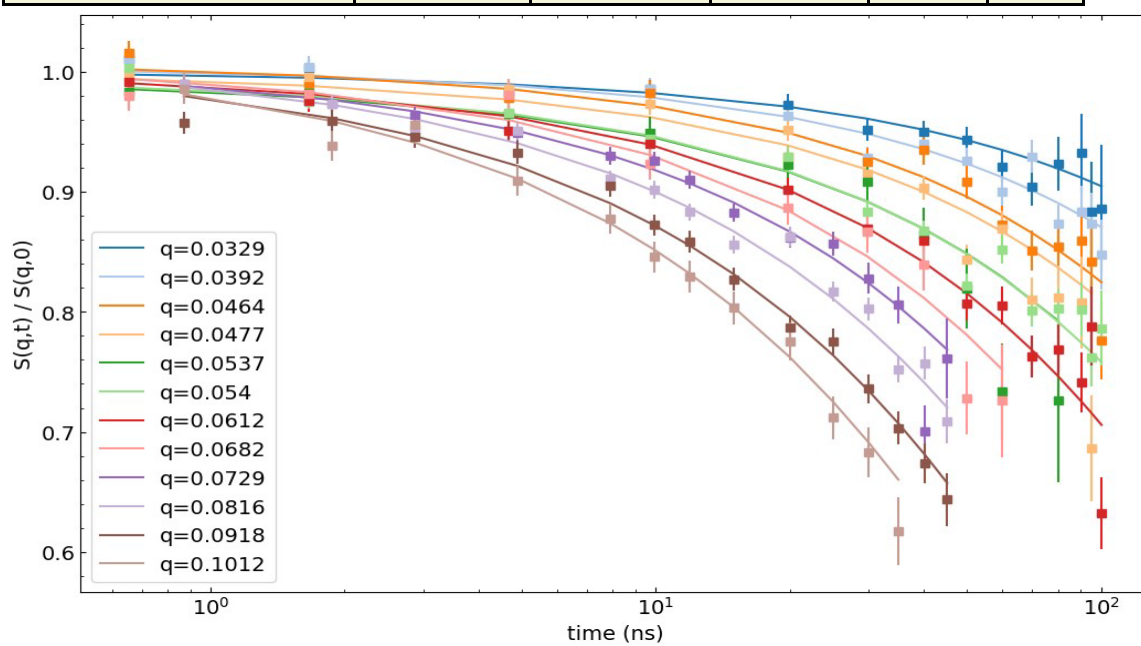
20% cholesterol

Two-parameter fit of K_C and η_m

Parameter or goodness of fit	lower limit	upper limit	median	-err	+err
η_m	0.0	10.5	2.0	-1.0	1.0
K_C	5.0	150.0	11.0	-5.0	18.0
χ^2	n/a	n/a	1.7	-0.1	0.1
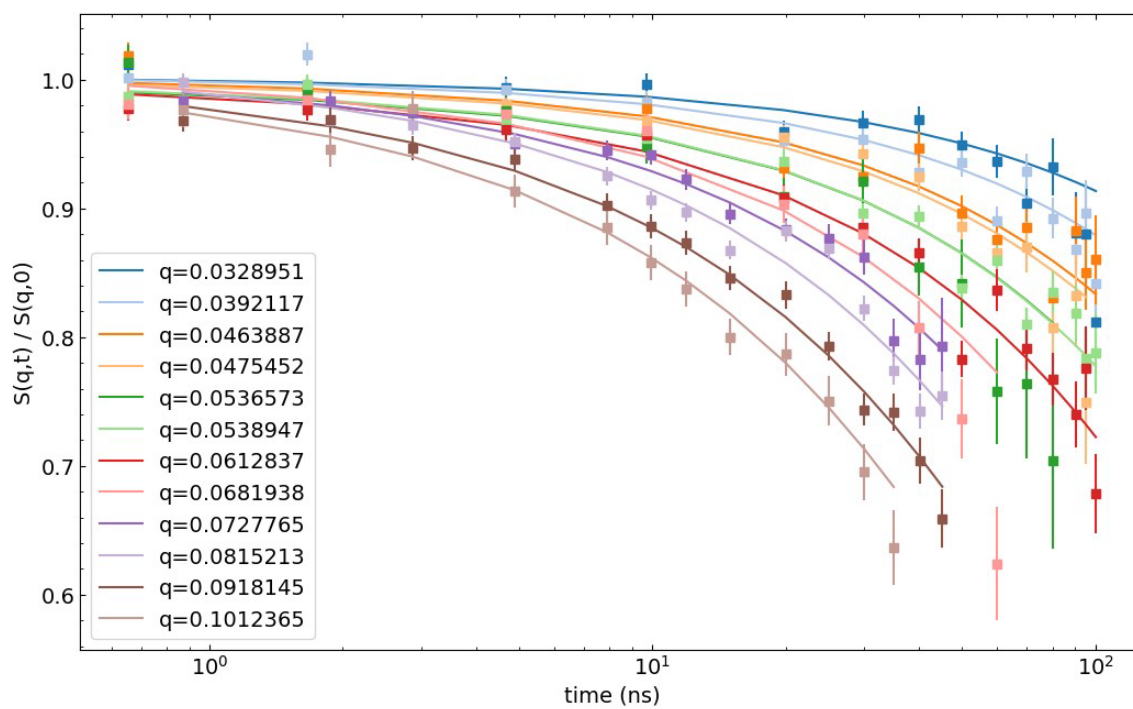
One-parameter fit of η_m

Parameter or goodness of fit	lower limit	upper limit	median	-err	+err
η_m	0.0	10.5	0.8	-0.06	0.07
χ^2	n/a	n/a	1.7	-0.1	0.1
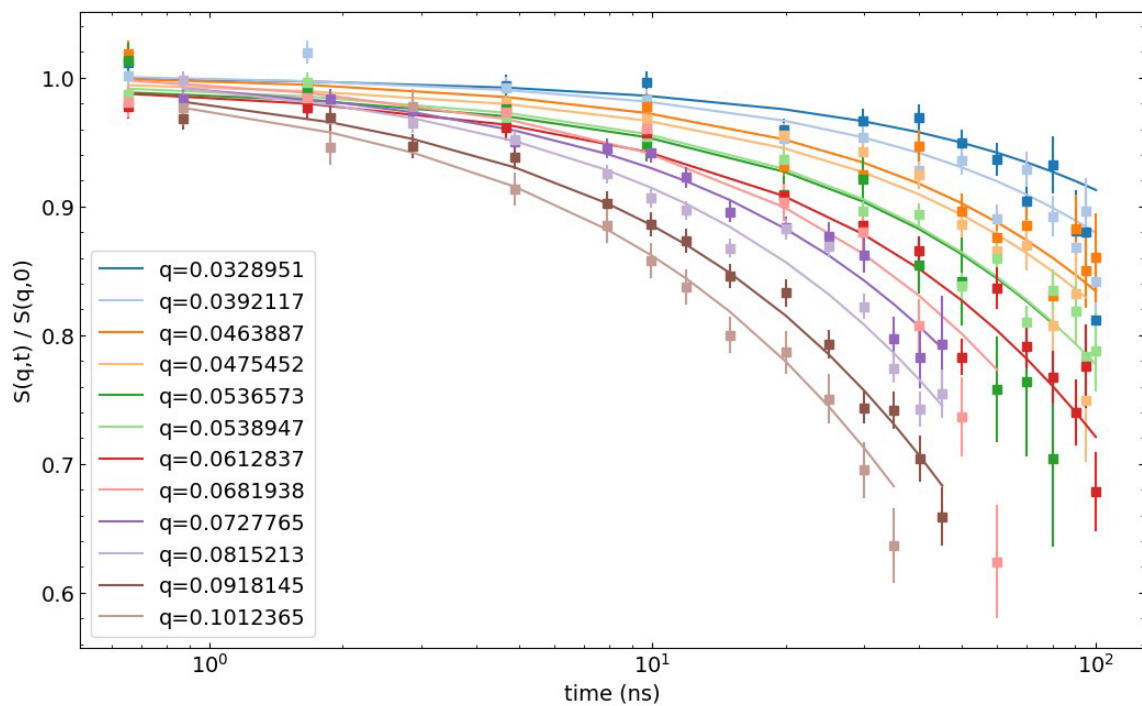
One-parameter fit of K_C

Parameter or goodness of fit	lower limit	upper limit	median	-err	+err
K_C	5.0	350.0	97.0	-5.0	4.0
χ^2	n/a	n/a	2.1	-0.1	0.1
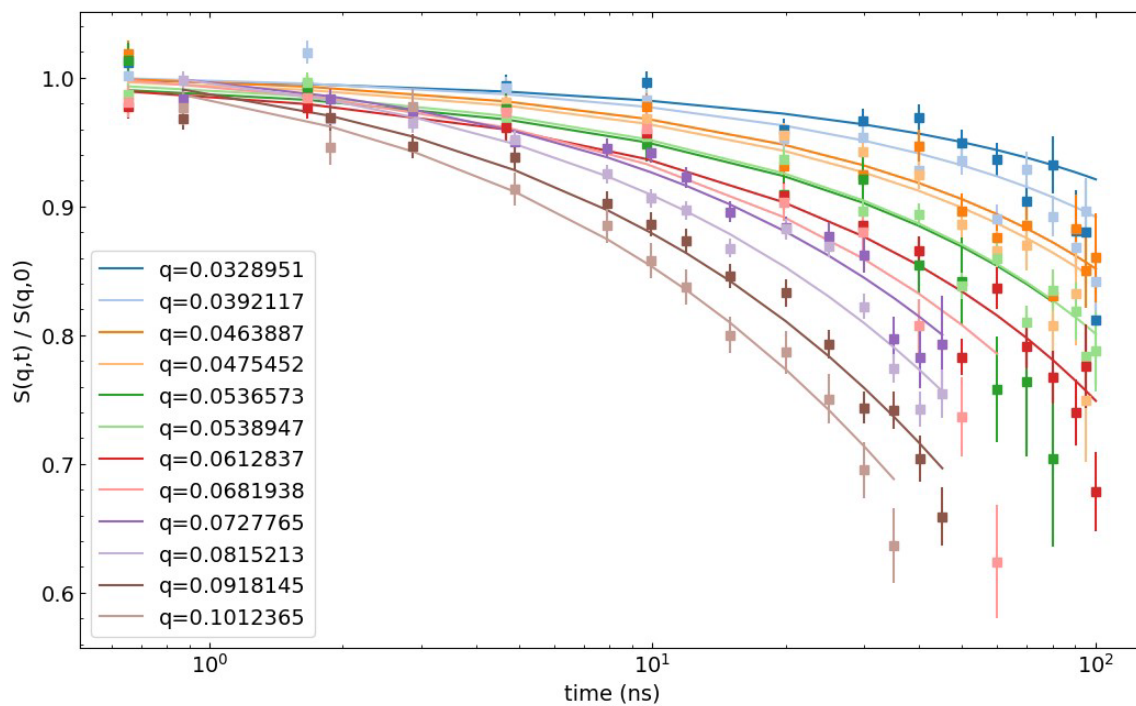
One-parameter fit of α using the diffusional softening model

Parameter or goodness of fit	lower limit	upper limit	median	-err	+err
α	0.0	1.0	0.2	-0.03	0.03
χ^2	n/a	n/a	1.7	-0.1	0.1
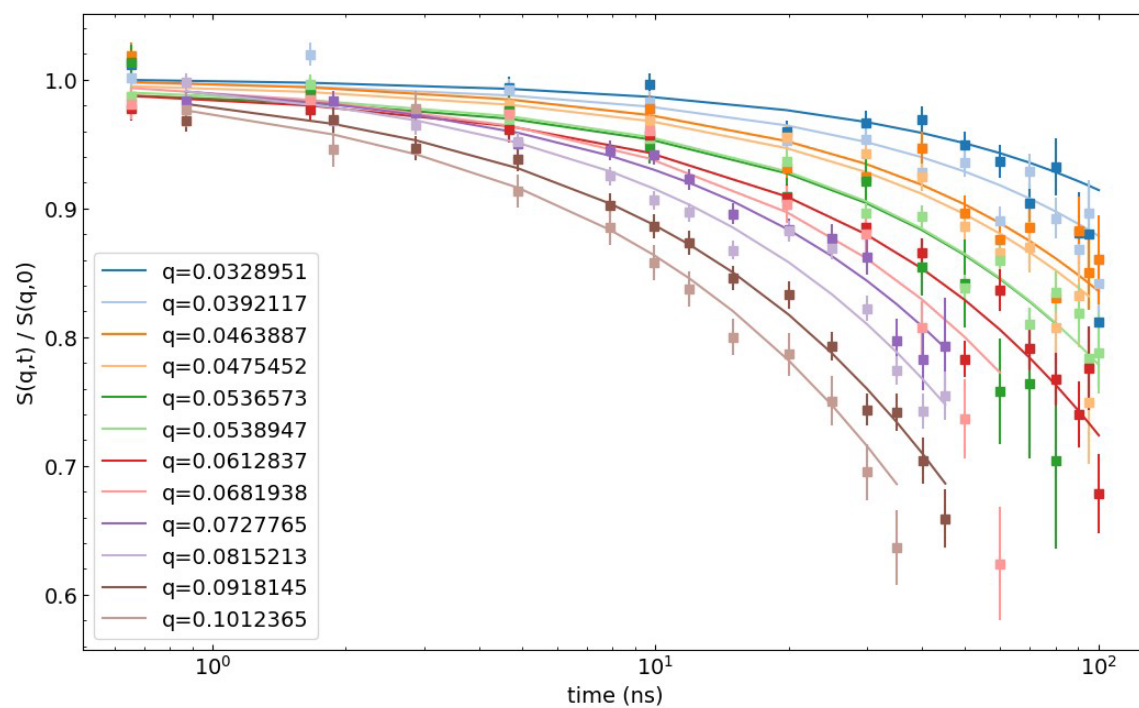
30% cholesterol

Two-parameter fit of K_C and η_m

Parameter or goodness of fit	lower limit	upper limit	median	-err	+err
η_m	0.0	10.5	2.0	-1.0	2.0
K_C	5.0	150.0	10.0	-4.0	19.0
χ^2	n/a	n/a	1.8	-0.1	0.1
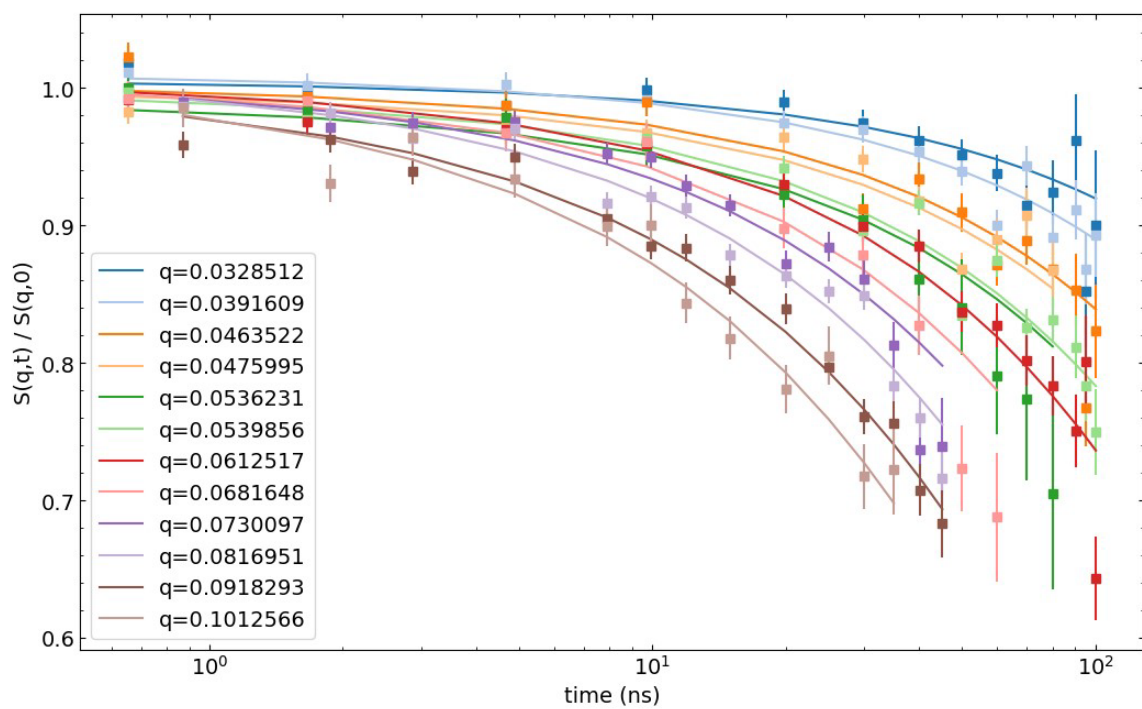
One-parameter fit of η_m

Parameter or goodness of fit	lower limit	upper limit	median	-err	+err
η_m	0.0	10.5	1.08	-0.09	0.11
χ^2	n/a	n/a	1.8	-0.1	0.1
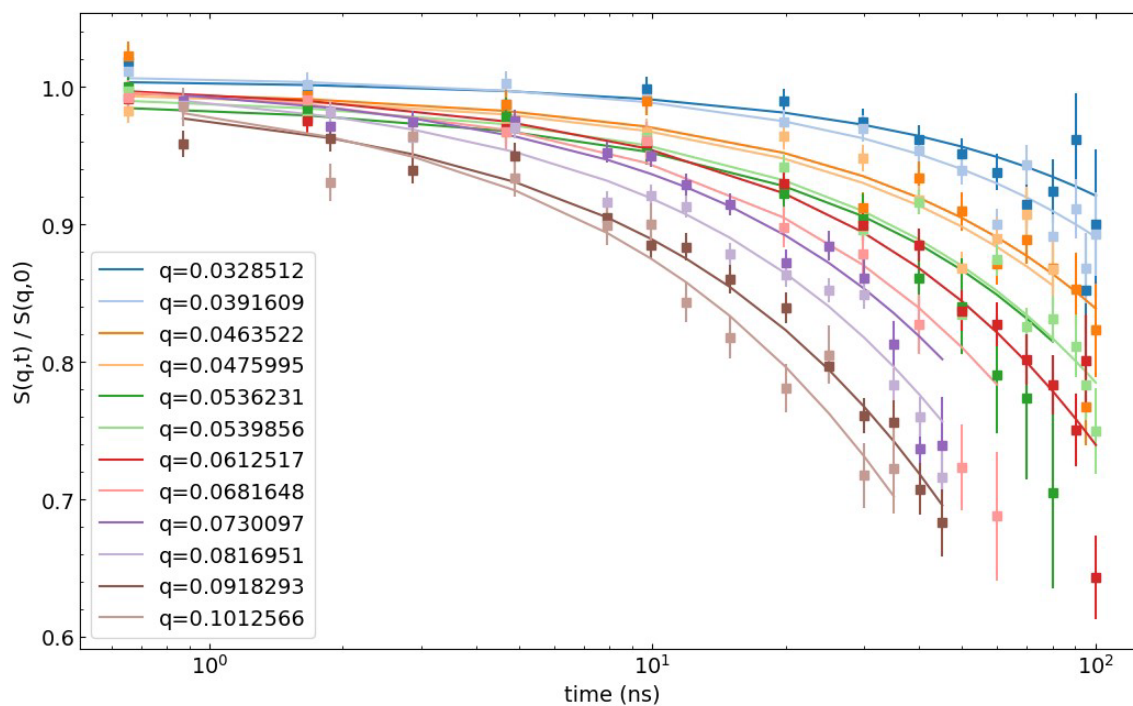
One-parameter fit of K_C

Parameter or goodness of fit	lower limit	upper limit	median	-err	+err
K_C	5.0	350.0	115.0	-6.0	6.0
χ^2	n/a	n/a	2.3	-0.1	0.1
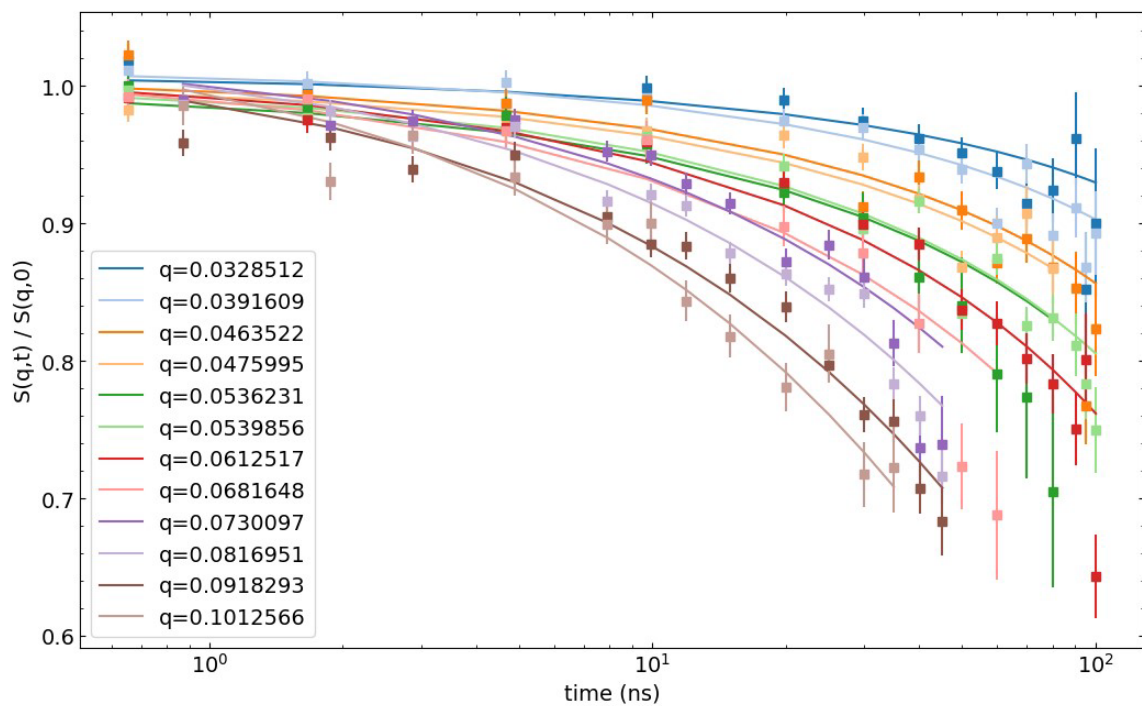
One-parameter fit of α using the diffusional softening model

Parameter or goodness of fit	lower limit	upper limit	median	-err	+err
α	0.0	1.0	0.3	-0.03	0.03
χ^2	n/a	n/a	1.8	-0.1	0.1
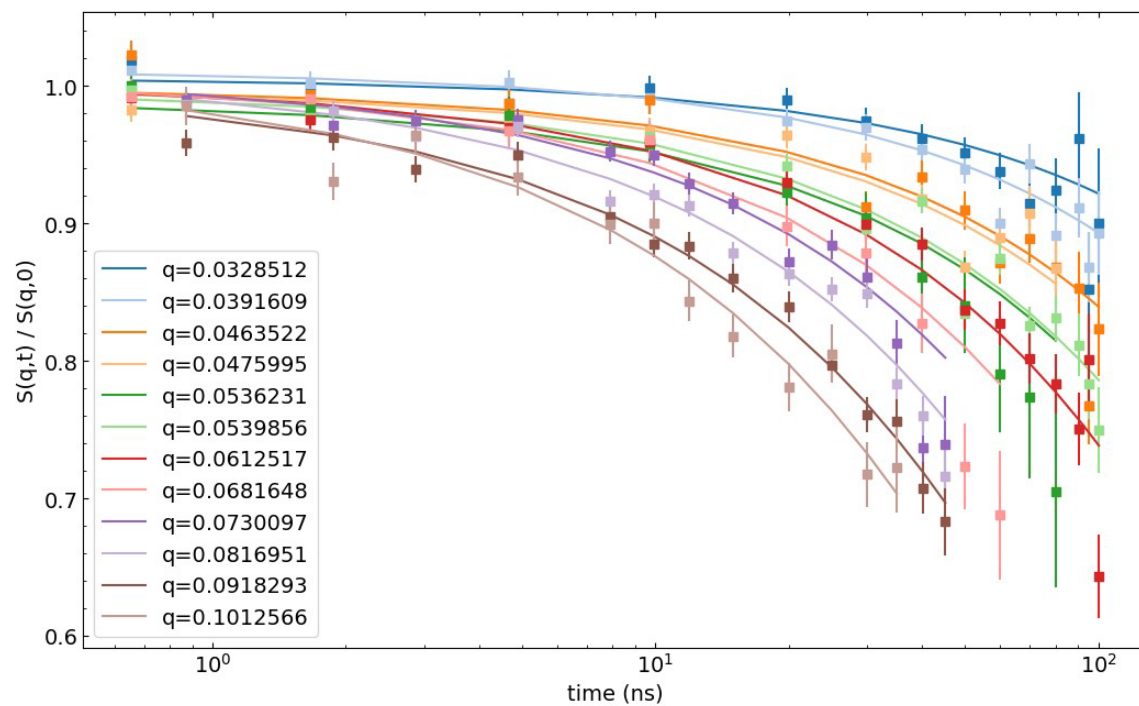
40% cholesterol

Two-parameter fit of K_C and η_m

Parameter or goodness of fit	lower limit	upper limit	median	-err	+err
η_m	0.0	10.5	4.0	-3.0	4.0
K_C	5.0	150.0	17.0	-8.0	39.0
χ^2	n/a	n/a	2.5	-0.1	0.1
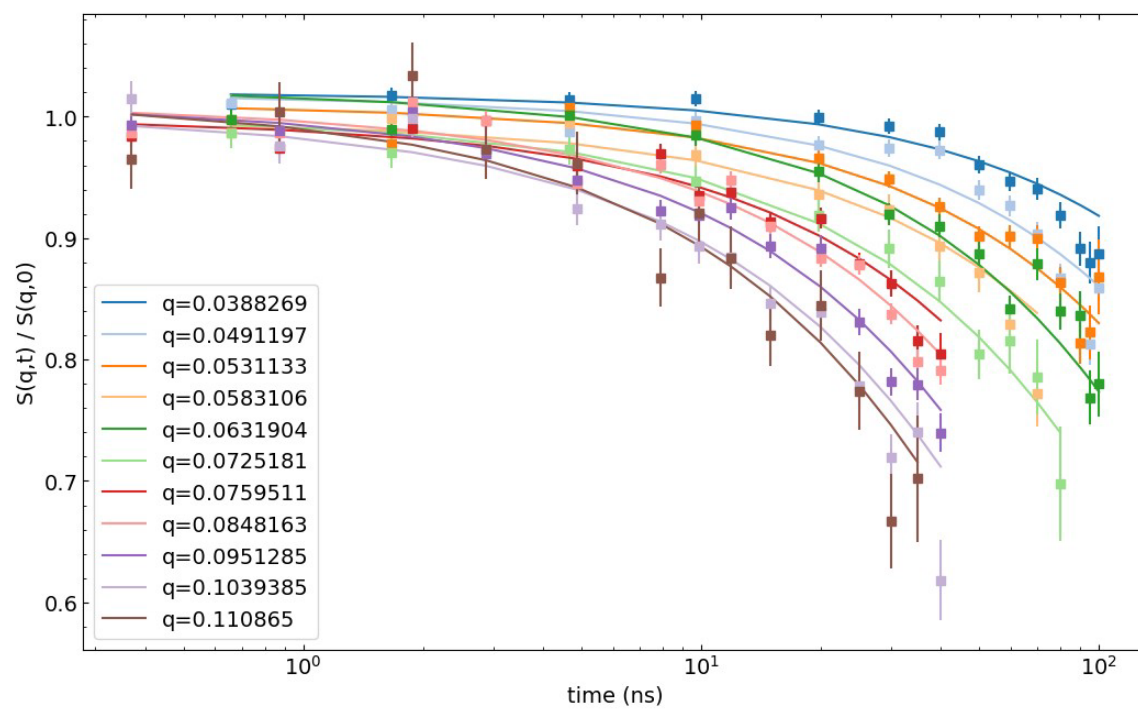
One-parameter fit of η_m

Parameter or goodness of fit	lower limit	upper limit	median	-err	+err
η_m	0.0	10.5	3.5	-0.4	0.4
χ^2	n/a	n/a	2.5	-0.1	0.1
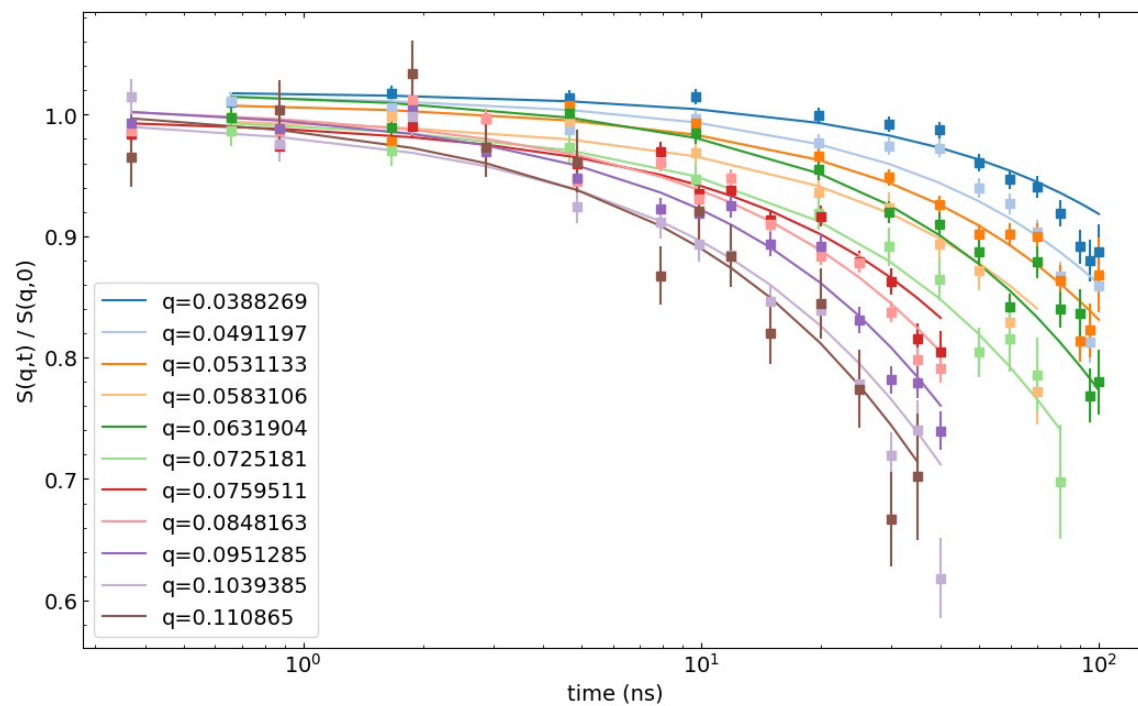
One-parameter fit of K_C

Parameter or goodness of fit	lower limit	upper limit	median	-err	+err
K_C	5.0	350.0	230.0	-10.0	10.0
χ^2	n/a	n/a	3.1	-0.1	0.1
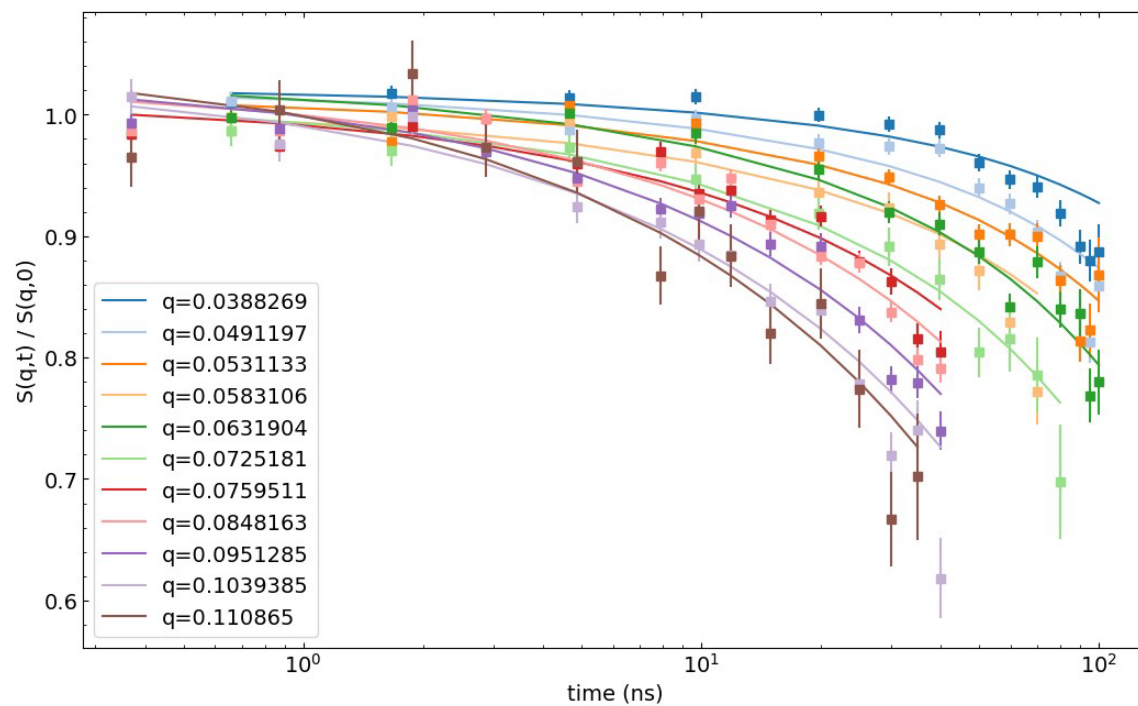
One-parameter fit of α using the diffusional softening model

Parameter or goodness of fit	lower limit	upper limit	median	-err	+err
α	0.0	1.0	0.6	-0.02	0.02
χ^2	n/a	n/a	2.4	-0.1	0.1
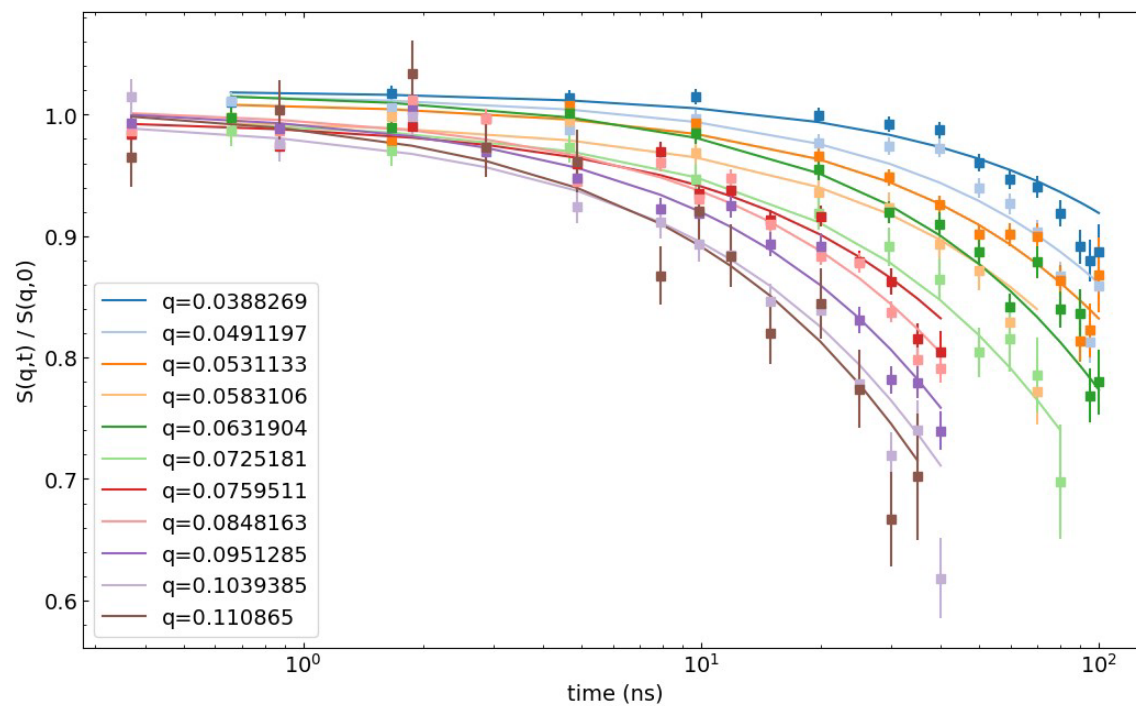
50% cholesterol

Two-parameter fit of K_C and η_m

Parameter or goodness of fit	lower limit	upper limit	median	-err	+err
η_m	0.0	10.5	2.0	-1.0	6.0
K_C	5.0	60.0	20.0	-20.0	20.0
χ^2	n/a	n/a	1.5	-0.1	0.1
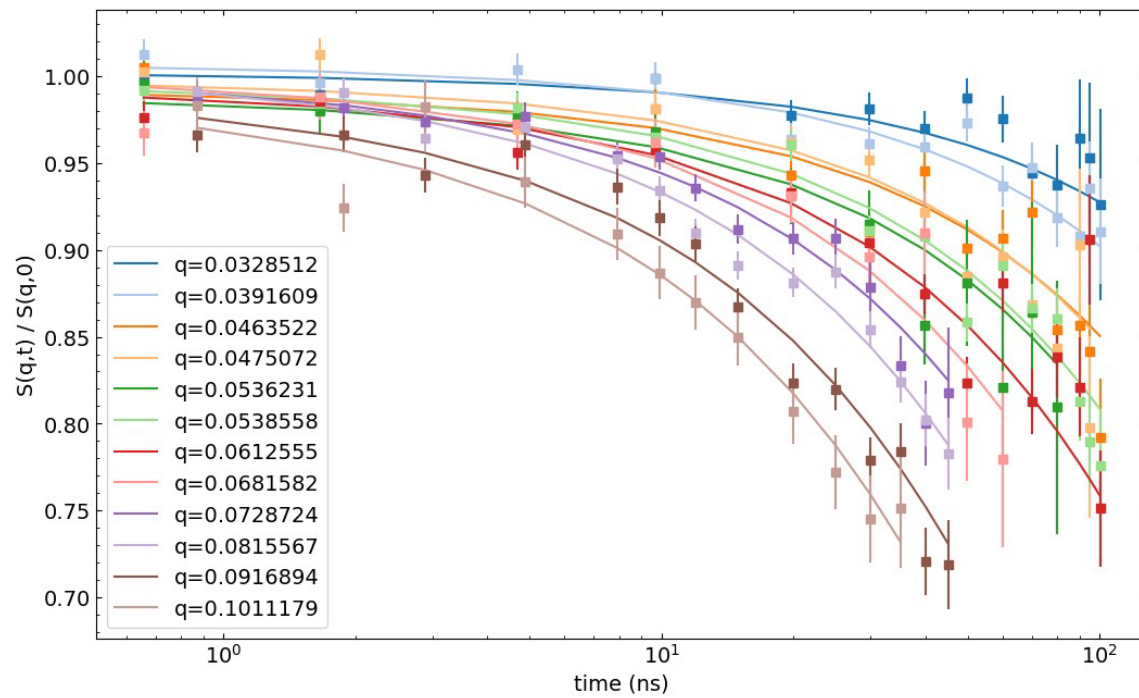
One-parameter fit of η_m

Parameter or goodness of fit	lower limit	upper limit	median	-err	+err
η_m	0.0	10.5	2.7	-0.4	0.4
χ^2	n/a	n/a	1.6	-0.1	0.1

One-parameter fit of K_c

Parameter or goodness of fit	lower limit	upper limit	median	-err	+err
η_m	5.0	350.0	180.0	-10.0	10.0
χ^2	n/a	n/a	1.7	-0.1	0.1
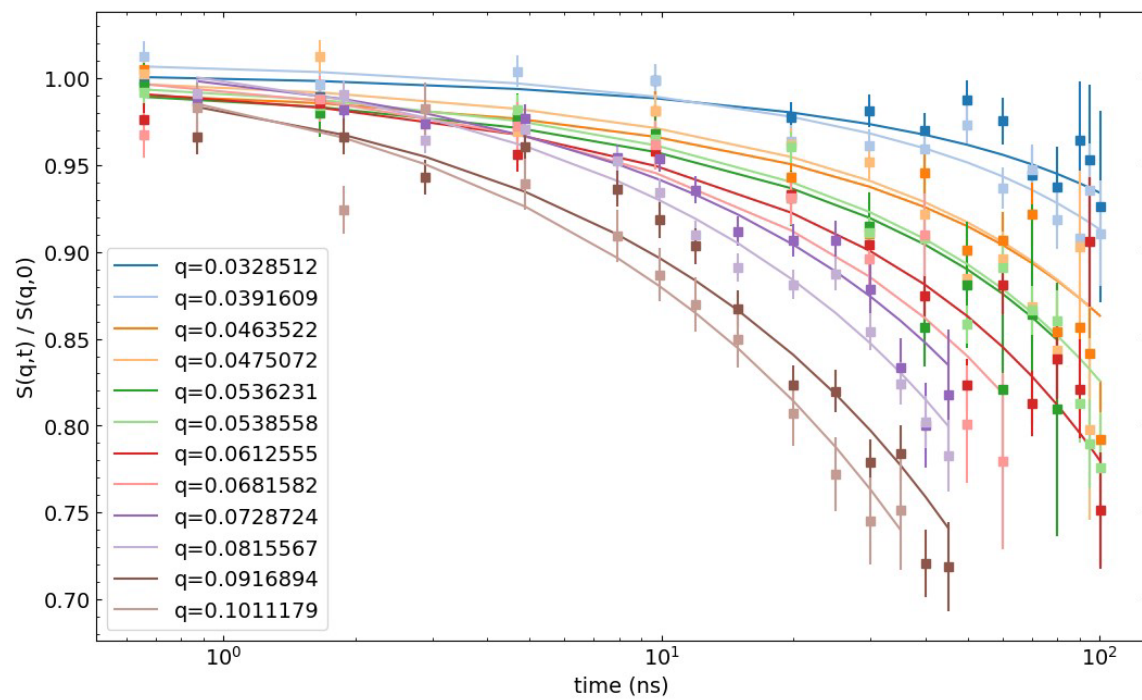
One-parameter fit of α using the diffusional softening model

Parameter or goodness of fit	lower limit	upper limit	median	-err	+err
α	0.0	1.0	0.54	-0.03	0.03
χ^2	n/a	n/a	1.5	-0.1	0.1

References

1. Chakraborty, S., M. Doktorova, T.R. Molugu, F.A. Heberle, H.L. Scott, B. Dzikovski, M. Nagao, L.-R. Stingaciu, R.F. Standaert, F.N. Barrera, J. Katsaras, G. Khelashvili, M.F. Brown, and R. Ashkar. 2020. How cholesterol stiffens unsaturated lipid membranes. *Proc. Natl. Acad. Sci.* 117:21896–21905.

2. Kirby, B.J., P.A. Kienzle, B.B. Maranville, N.F. Berk, J. Krycka, F. Heinrich, and C.F. Majkrzak. 2012. Phase-sensitive specular neutron reflectometry for imaging the nanometer scale composition depth profile of thin-film materials. *Current Opinion in Colloid & Interface Science*. 17:44–53.

S2. Results of using R = 40.3 nm instead of R = 30 nm

Fig. S2(6) in place of Fig. 6 in the text. Filled symbols show results of fitting either to K_{CD} (circles) with η_m fixed to 0 or to η_m (squares) with K_C/kT fixed to 20. Open symbols show the corresponding reduced χ^2.
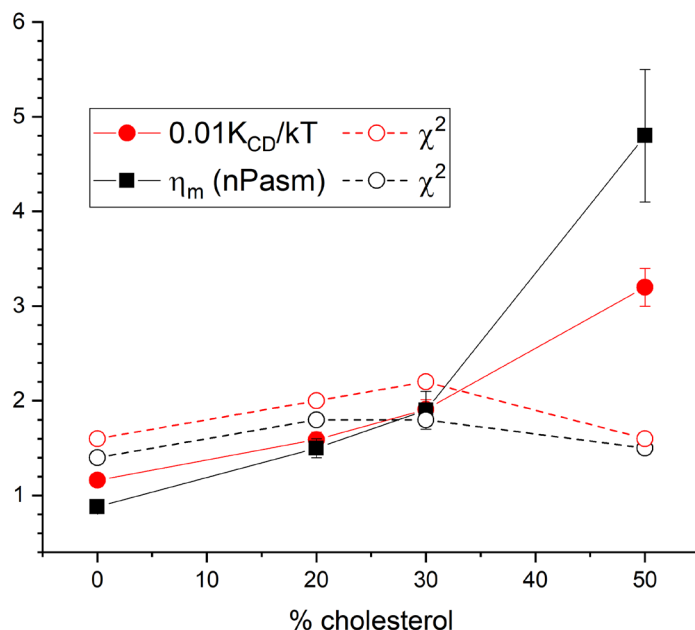

Fig. S2(8) in place of Fig. 8 in the text. Results that include dynamical diffusional softening theory in fits to the NSE data. The estimated values of α (times 10, upward triangles) were used to fit the data resulting in the membrane viscosity (circles) and the unsoftened κ in Eq. 10. The downward triangles show values of α (times five) that were obtained by assuming that the viscosity did not change with cholesterol.
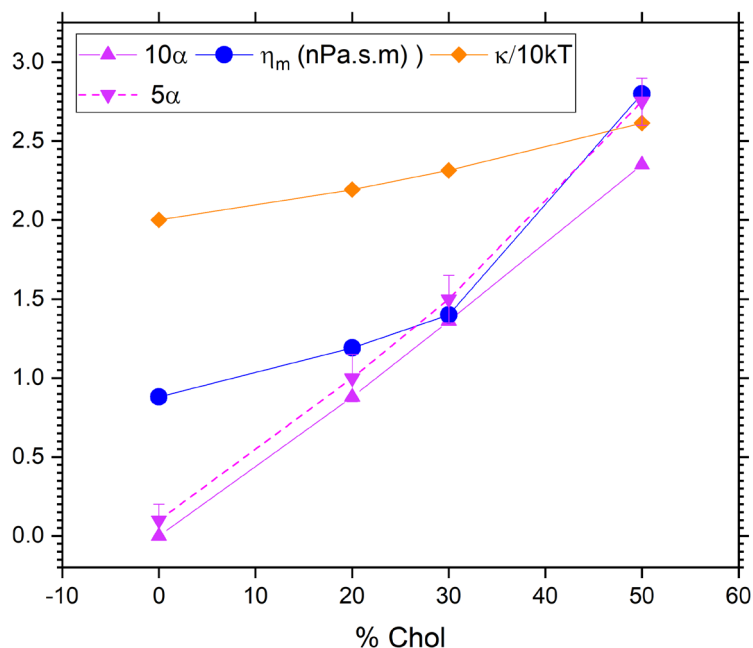
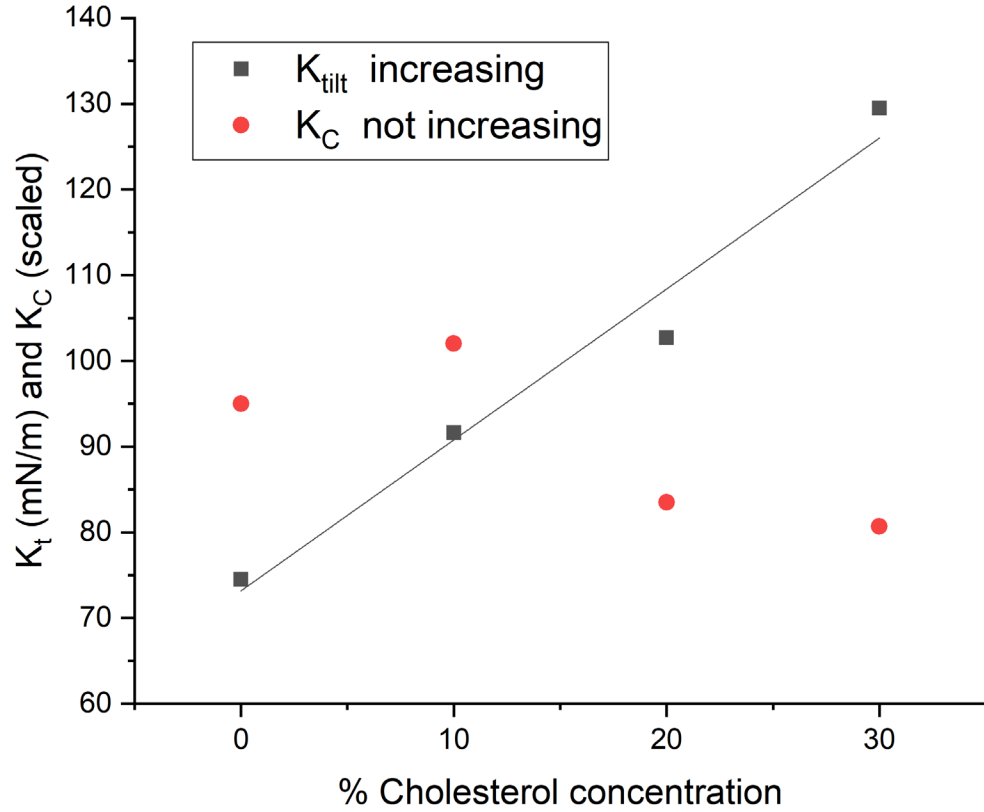## S3. Effect of cholesterol on the tilt modulus of DOPC

Fig. S3. Result of re-analysis of the original x-ray diffuse scattering data, Pan et al., Phys Rev E 80 (2009) 021931, that includes the tilt modulus K_t as well as the bending modulus K_C using the analysis method of Jablin et al., Phys. Rev. Lett. 113 (2014) 248102. The average K_C/kT was 20 for no cholesterol.

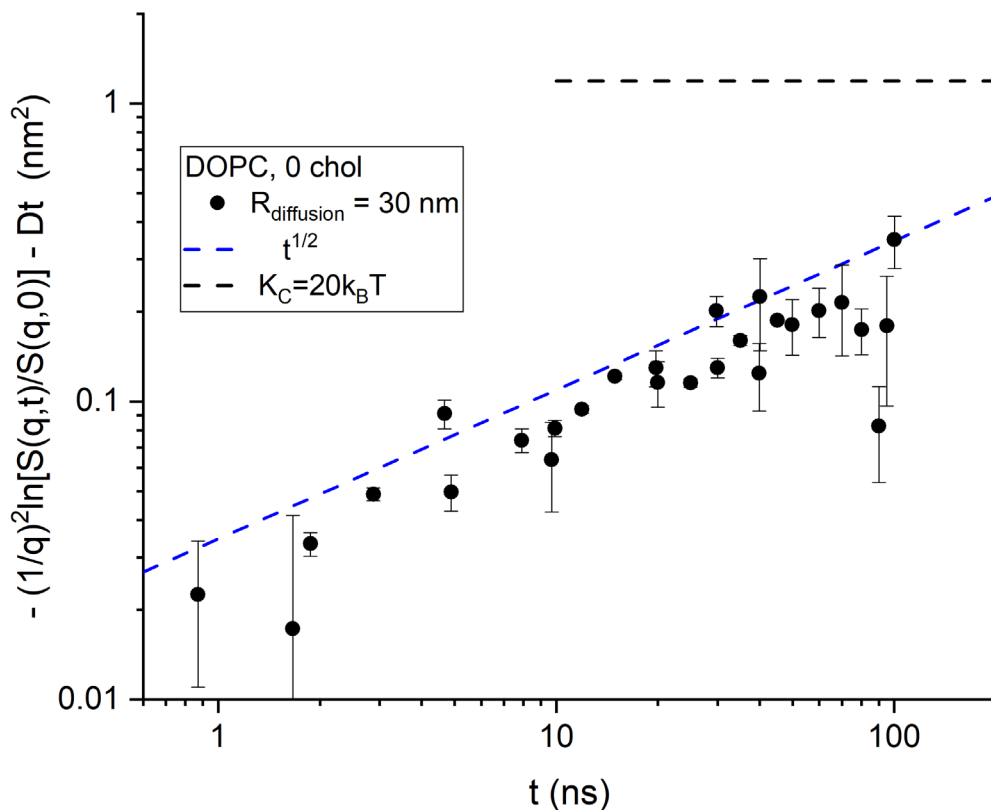## S4. Effect of smaller radius for diffusion on MSD

Fig. S4. Time dependence of the MSD assuming the radius for diffusion is 30 nm. The required monotonicity is doubtful, suggesting that diffusion has been over-corrected.

Supposing that at long time the MSD saturates near 0.2 would require an equilibrium K_C about 120 kT, far higher than any measurement. It may also be argued that these NSE data might saturate temporarily in this short time window and then the slow mode in the traditional theory of Watson et al.[26] and Seifert & Langer[27] kicks in during a later time, similar to the curves with non-zero α in Fig. 10 in the main text. Then, the saturation level would be consistent with a reasonable value of K_{CD} if we could use Eq. (11) for spheres with the theory for flat plaquettes. However, the latter theory predicts a 2/3 power law whereas Fig. S4 is more consistent with a ½ power law of the spherical theory with viscosity, so we prefer to interpret this figure as indicating the inevitable non-monotonicity from over-correcting vesicle diffusion, especially since a hydrodynamic radius should be larger than the structural radius and then the K_{CD} from Eq. (11) would become too large.