Electronic Supplementary Material (ESI) for Nanoscale.

Figure S1: Schematic structures of the inverse bicontinuous cubic phase (Pn3m) and sponge phase. The grey surface represents the interface between the inner and outer leaflets of the bilayer. Bottom line: Unit cell. Top: Another view to highlight tetrahedral geometry of water channel.

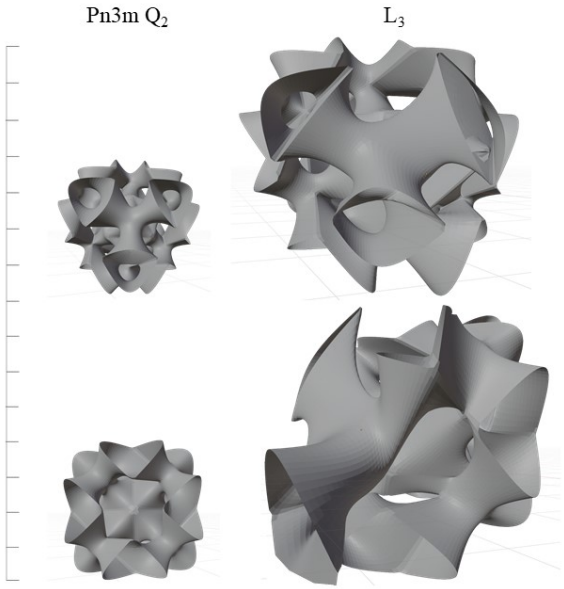


Figure S2: Cryo-TEM images of (left) L3NPs containing aspartic protease and (right) L3NPs containing β-galactosidase.

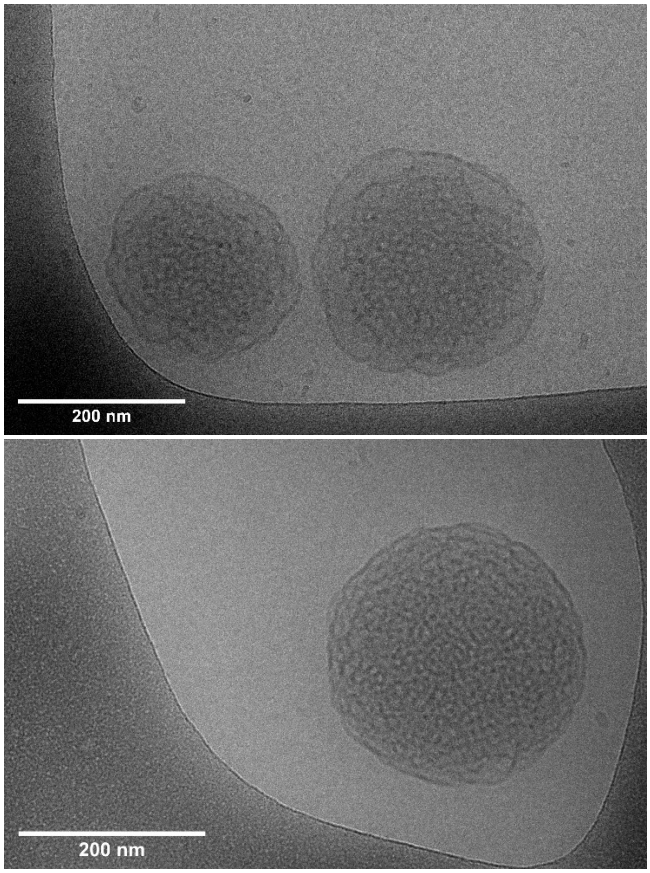


Figure S3: ISFs for L3NPs with (a) 15mg/mL aspartic protease, (b) 44mg/mL aspartic protease, (c) 15mg/mL β-galactosidase, (d) 44mg/mL β-galactosidase and (e) without enzyme. Fits including diffusion (equation (2)) are plotted as solid black lines and fits excluding diffusion (equation (1)) are plotted as dashed black lines.

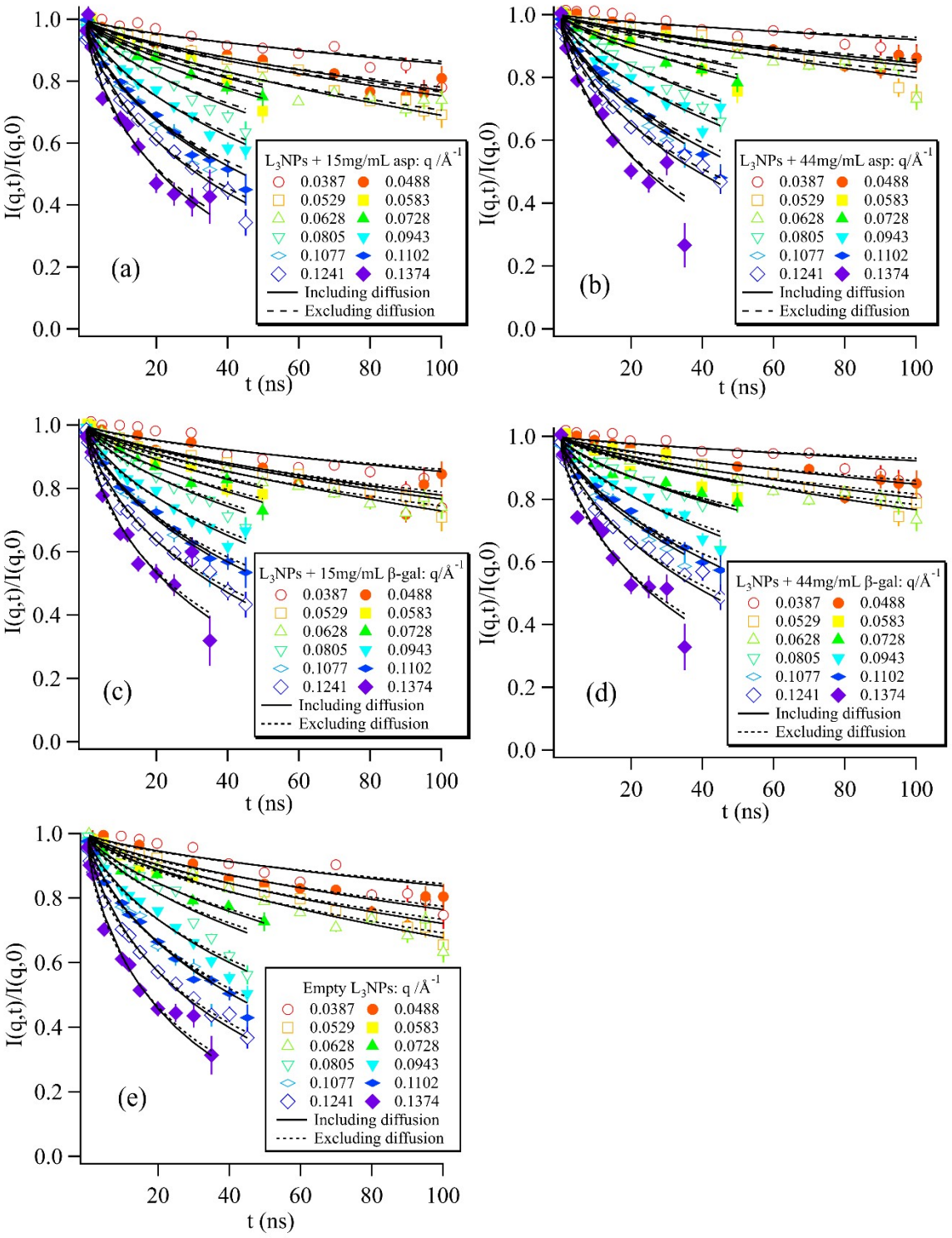
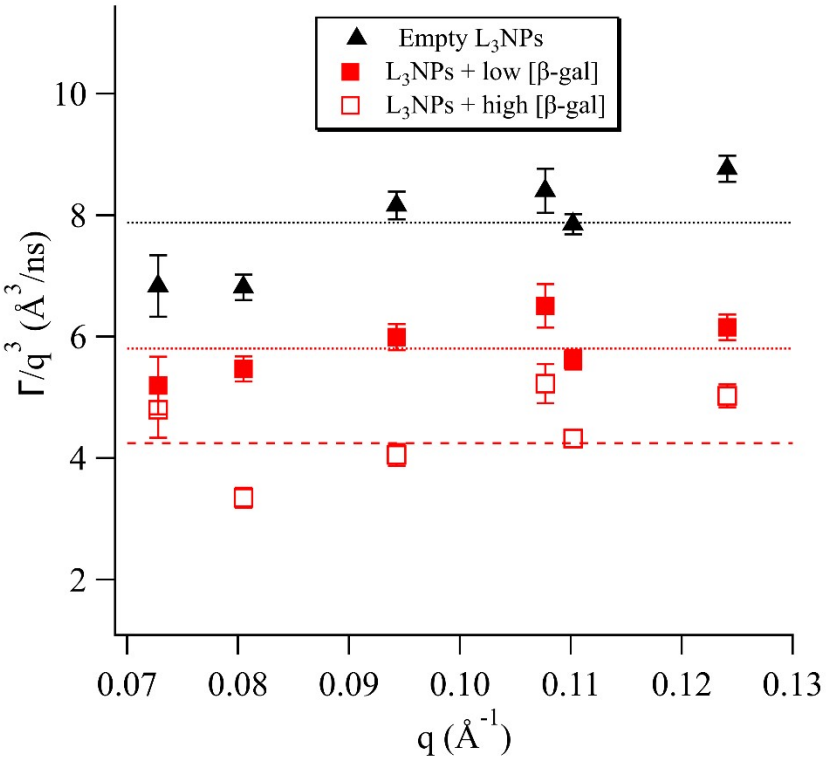


Figure S4: The normalised relaxation rate Γ/q3 vs q was plotted for sponge phase nanoparticles with different concentrations of encapsulated enzymes in the q range where the model is valid. The horizontal lines show the fit of the Zilman and Granek model for the relaxation rate Γ/q3 for bending fluctuations (equation 2).



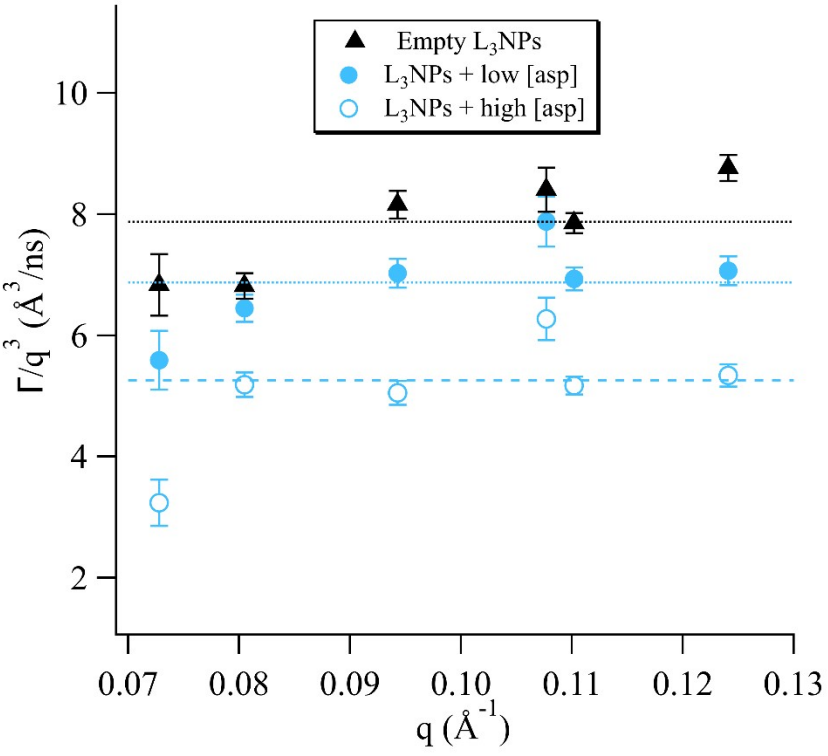


Figure S5: The non-normalised membrane bending moduli κ, extracted from the linear fits of equation 2 shown in Figure 1, are shown plotted against the final enzyme concentration in the sponge phase nanoparticles. Dotted lines included only to indicate trend.

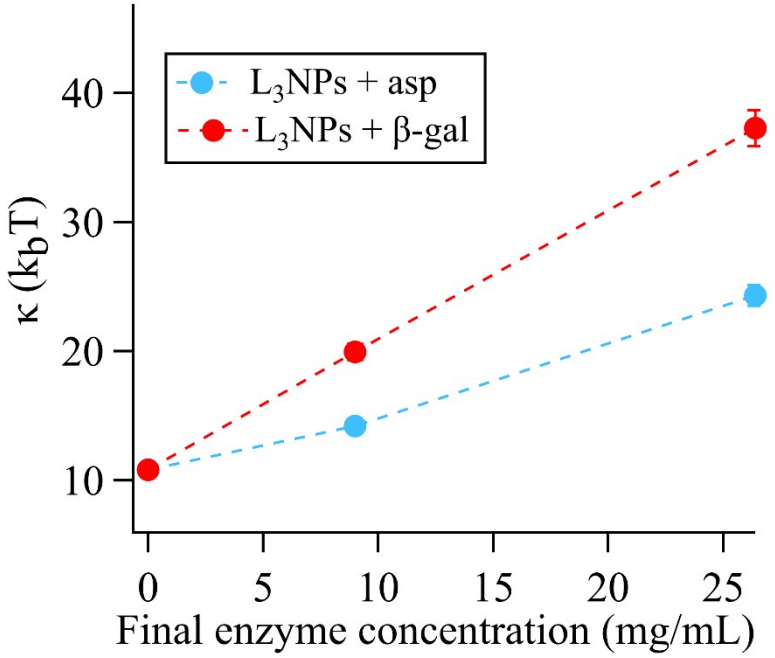


Figure S6: Comparison between fits for the bending rigidity including and excluding the contribution from diffusion for L3NPs with (a) 15mg/mL aspartic protease, (b) 44mg/mL aspartic protease, (c) 15mg/mL β-galactosidase, (d) 44mg/mL β-galactosidase and (e) without enzyme. Fits including diffusion are plotted as solid black lines and fits excluding diffusion are plotted as dashed black lines.

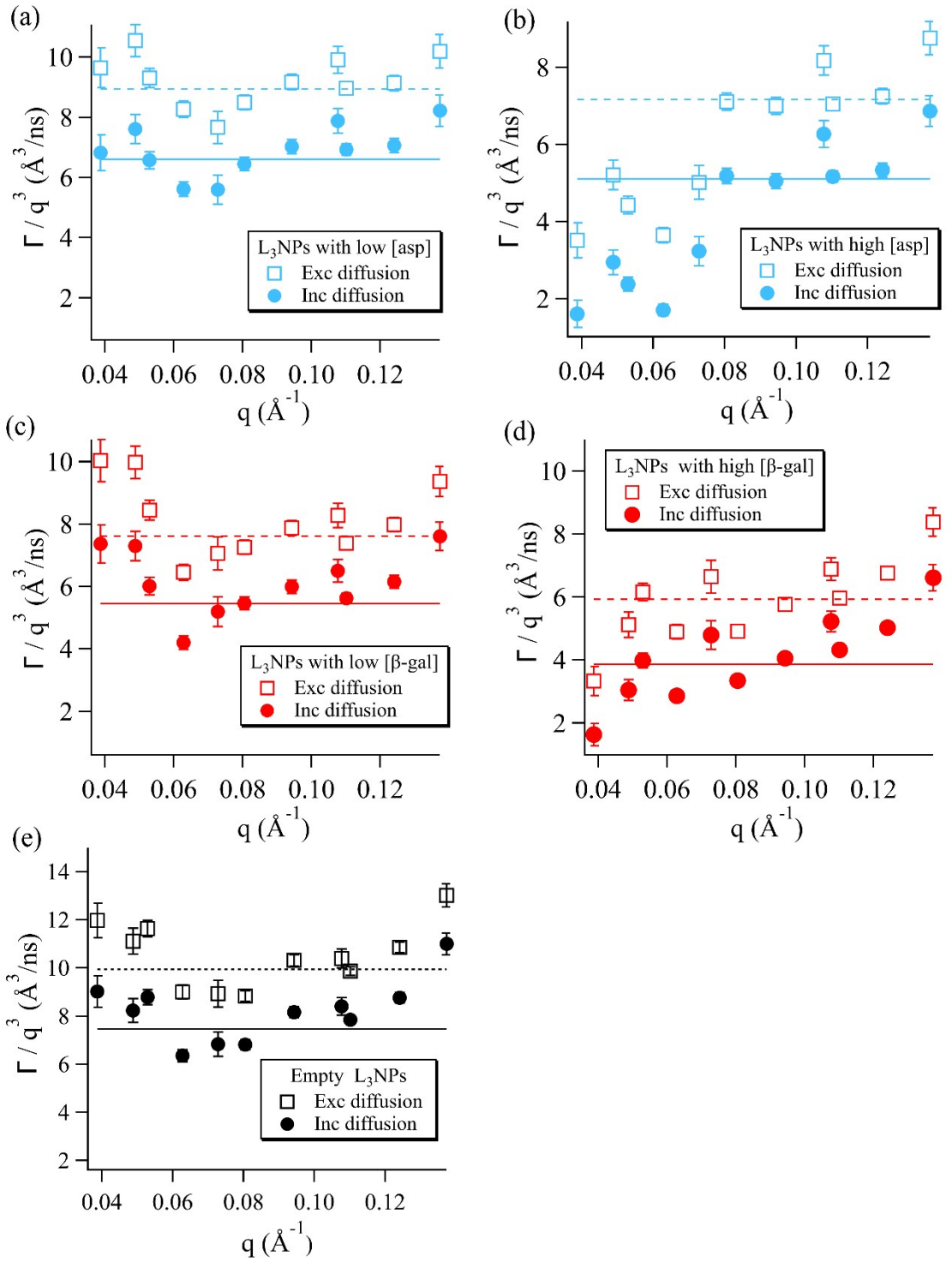


Figure S7: Structures of lipids used in molecular dynamics simulations.The models are available for download from the Zenodo repository.

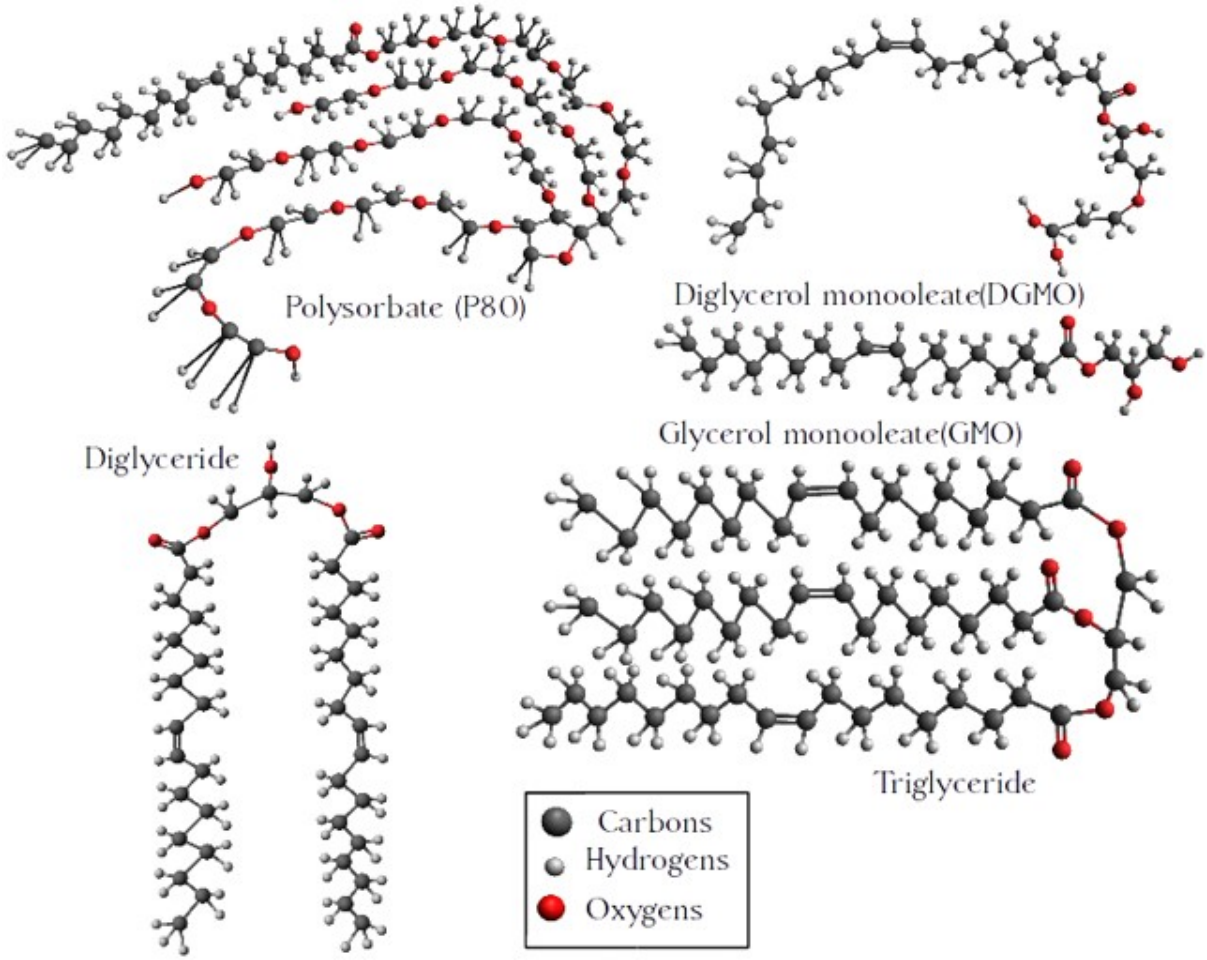


Figure S8: Secondary structure of aspartic protease during the last 100 ns of simulation. Codes for the secondary structures are the following: “T”- turn, “E” - extended conformation, “B” - isolated β-bridge, “H” - α-helix, “G” - 310 -helix, “I” - π-helix. The structures are named using VMD software [12,13].

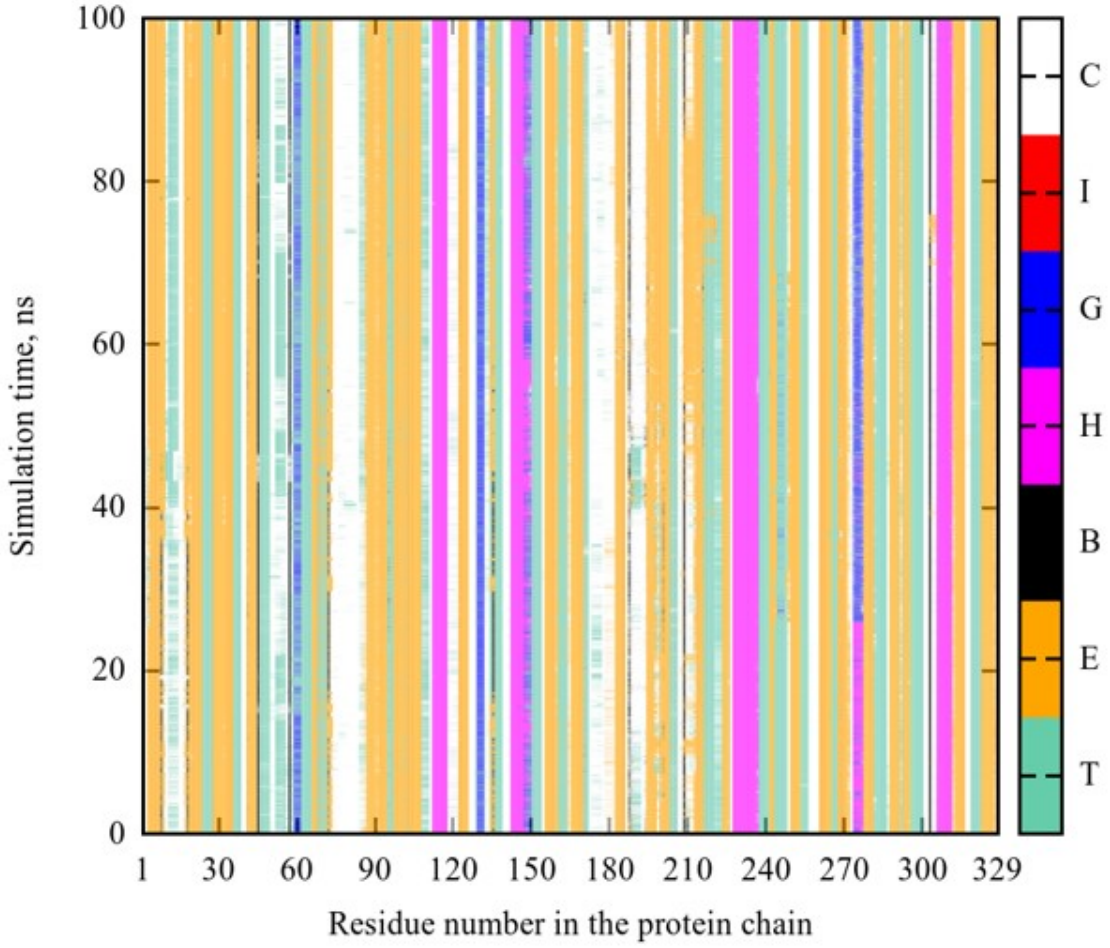


Figure S9: Self-intermediate scattering functions for 7 q-values for water and selected parts of lipid tails. Whole left panel shows results from simulations without the protein. The right panel shows results from simulations with protein.

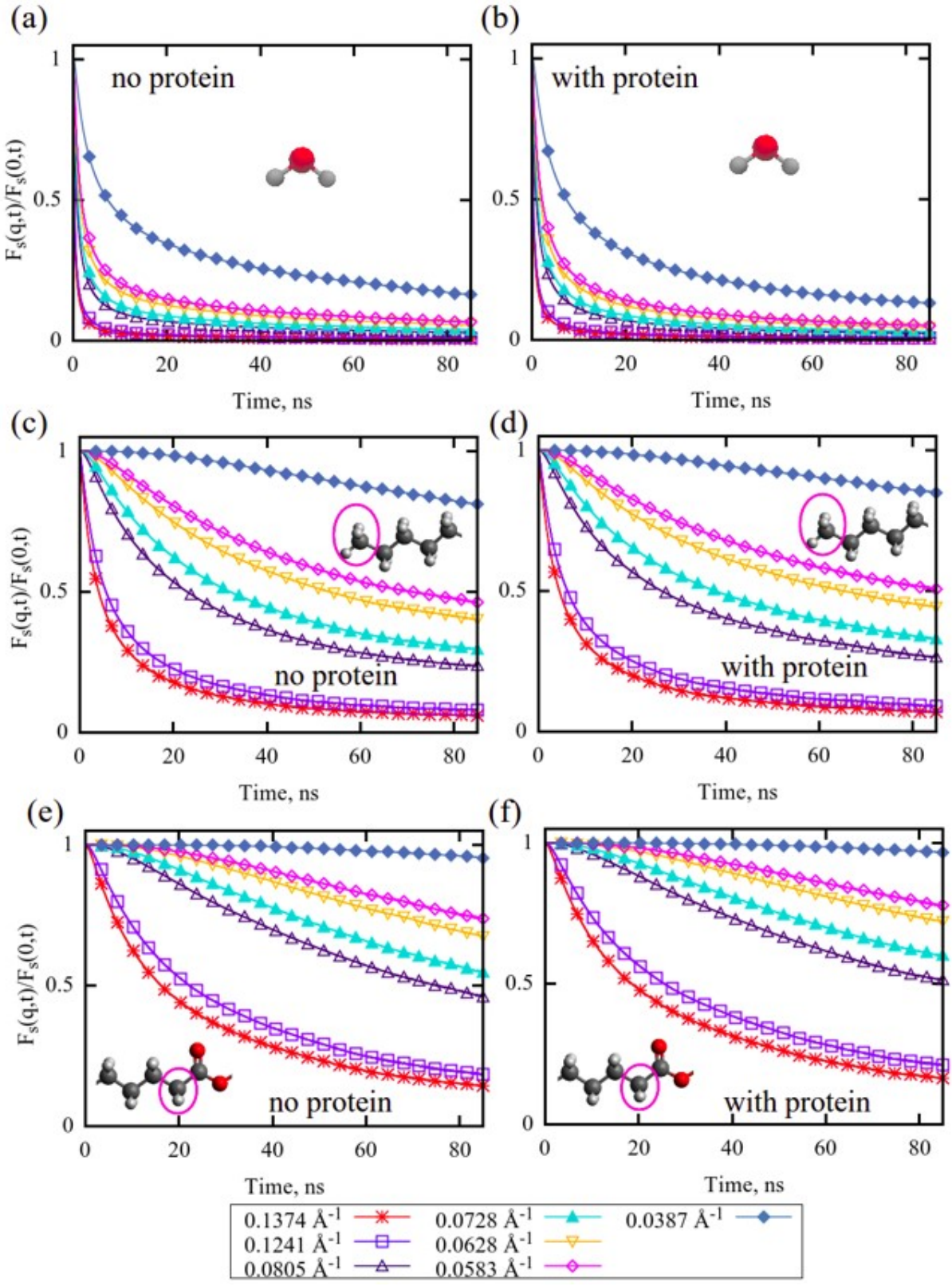


Figure S10: Snapshots from simulation containing aspartic protease, with the water molecules shown in blue. For clarity and to highlight the protein's position relative to the water channels, the lipids are not shown but reside in the space not occupied by the water molecules.

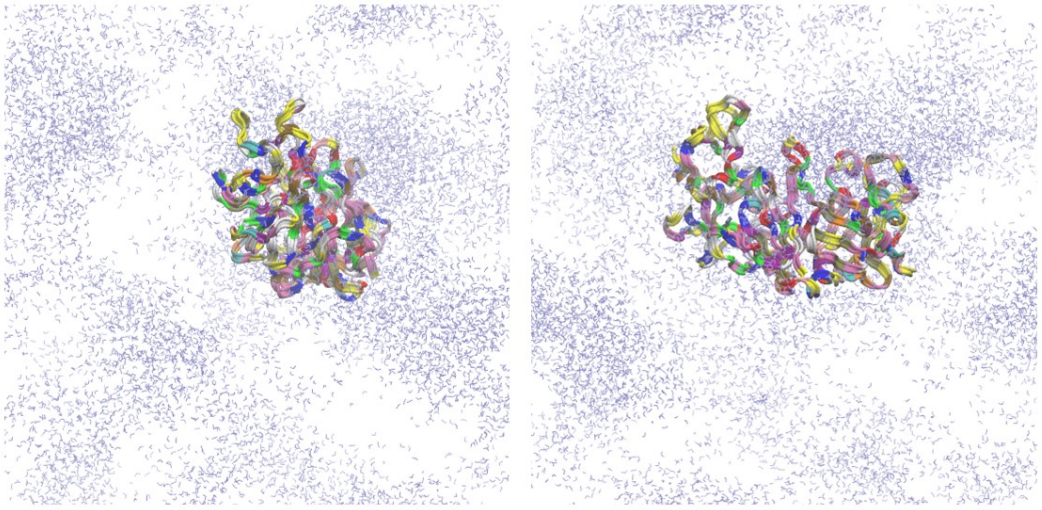
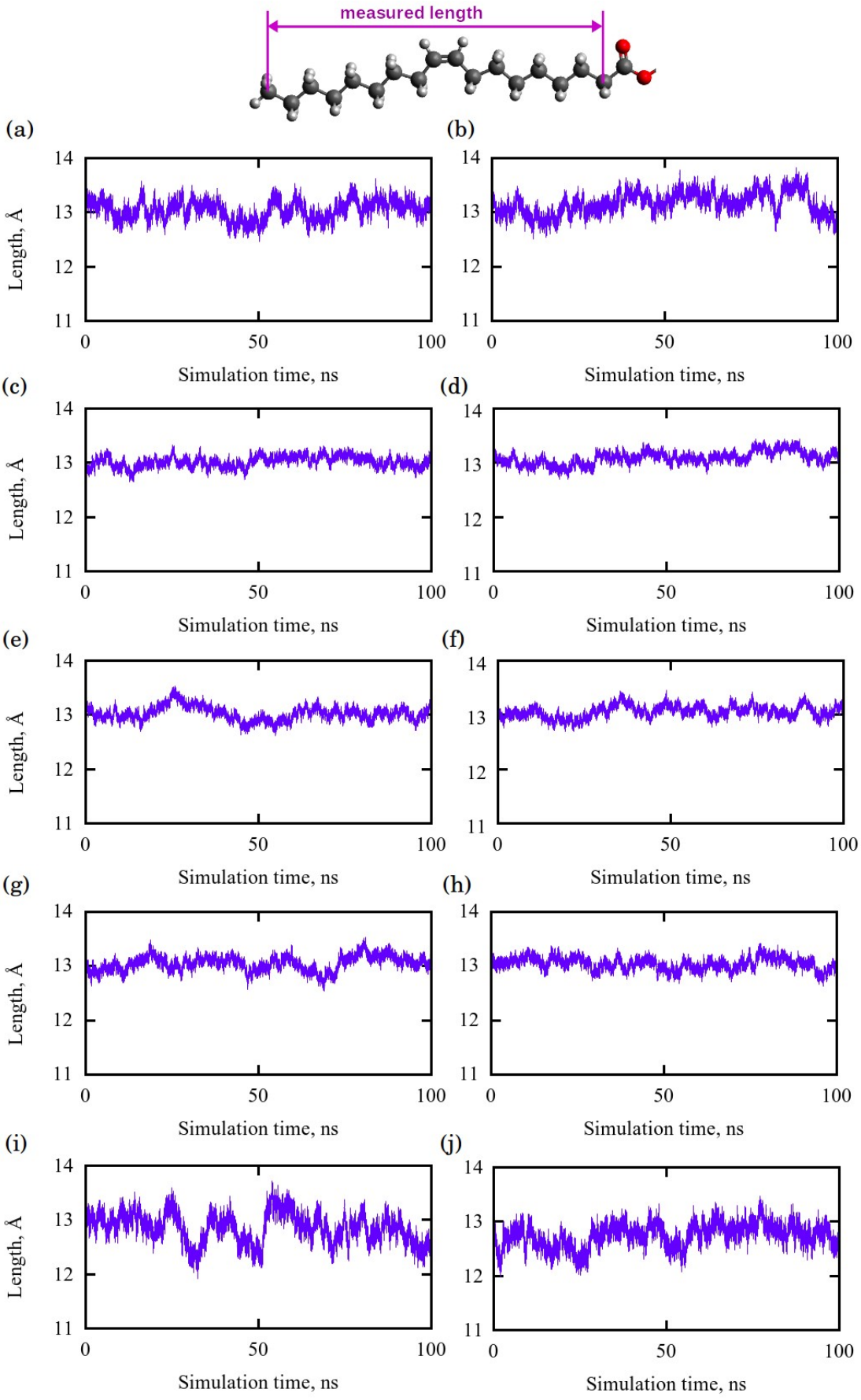


Figure S11:


measured length
(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
(i)
(j)
Length, Å
Simulation time, ns
14
13
12
11
0
50
100