

**Supporting Information For:**

**TRANSVERSE LIPID ORGANIZATION DICTATES BENDING FLUCTUATIONS IN MODEL  
PLASMA MEMBRANES**

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**Table S1: aLUV leaflet compositions prepared from POPE acceptor and ESM donor, determined from  $^{31}\text{P}$ -NMR and  $^1\text{H}$ -NMR. The overall mole fraction ( $\chi$ ), the mole fraction of the outer ( $\chi^{\text{out}}$ ) and inner ( $\chi^{\text{in}}$ ) leaflets and the fraction of total ESM in the outer leaflet ( $f^{\text{out}}$ ).**

| Component | $\chi$ | $f^{\text{out}}$ | $\chi^{\text{out}}$ | $\chi^{\text{in}}$ |
|-----------|--------|------------------|---------------------|--------------------|
| ESM       | 0.25   | 0.89             | 0.44                | 0.06               |
| POPE      | 0.75   | --               | 0.56                | 0.94               |
| Total     | 1      |                  | 1                   | 1                  |

**Table S2 Structural refinement of asymmetric ESM/POPE vesicles. Data were modeled with an symmetric five slab scattering length density profile. Parameter uncertainties are estimated to be < 5%. Modelled parameters indicate: area per lipid ( $A_L$ ;  $\text{\AA}^2$ ), bilayer thickness ( $D_B$ ;  $\text{\AA}$ ) and the hydrocarbon thickness ( $2DC$ ;  $\text{\AA}$ ).**

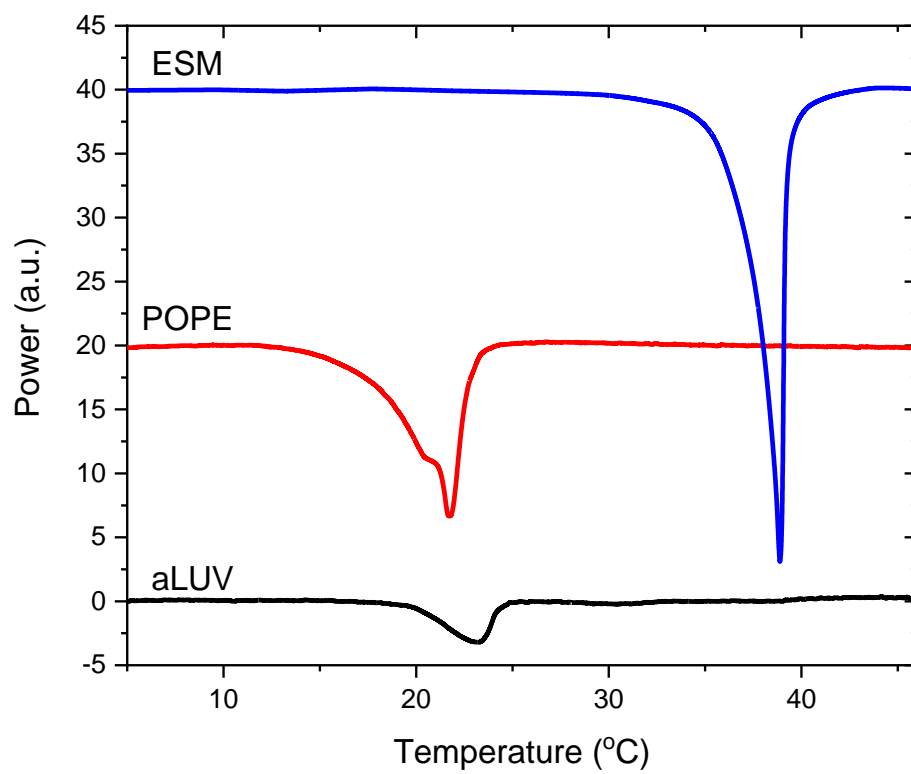
|       | aLUV <sup>a</sup> |             |             |
|-------|-------------------|-------------|-------------|
|       | <u>15°C</u>       | <u>30°C</u> | <u>45°C</u> |
| $A_L$ | 45.7              | 54.2        | 59.4        |
| $D_B$ | 48.6              | 42.5        | 39.6        |
| $2DC$ | 37.6              | 33.2        | 31.1        |

<sup>a</sup>Due to lack of leaflet contrast in the aLUV, data were treated with a symmetric bilayer model, thus parameters are reported rather than a per leaflet basis.

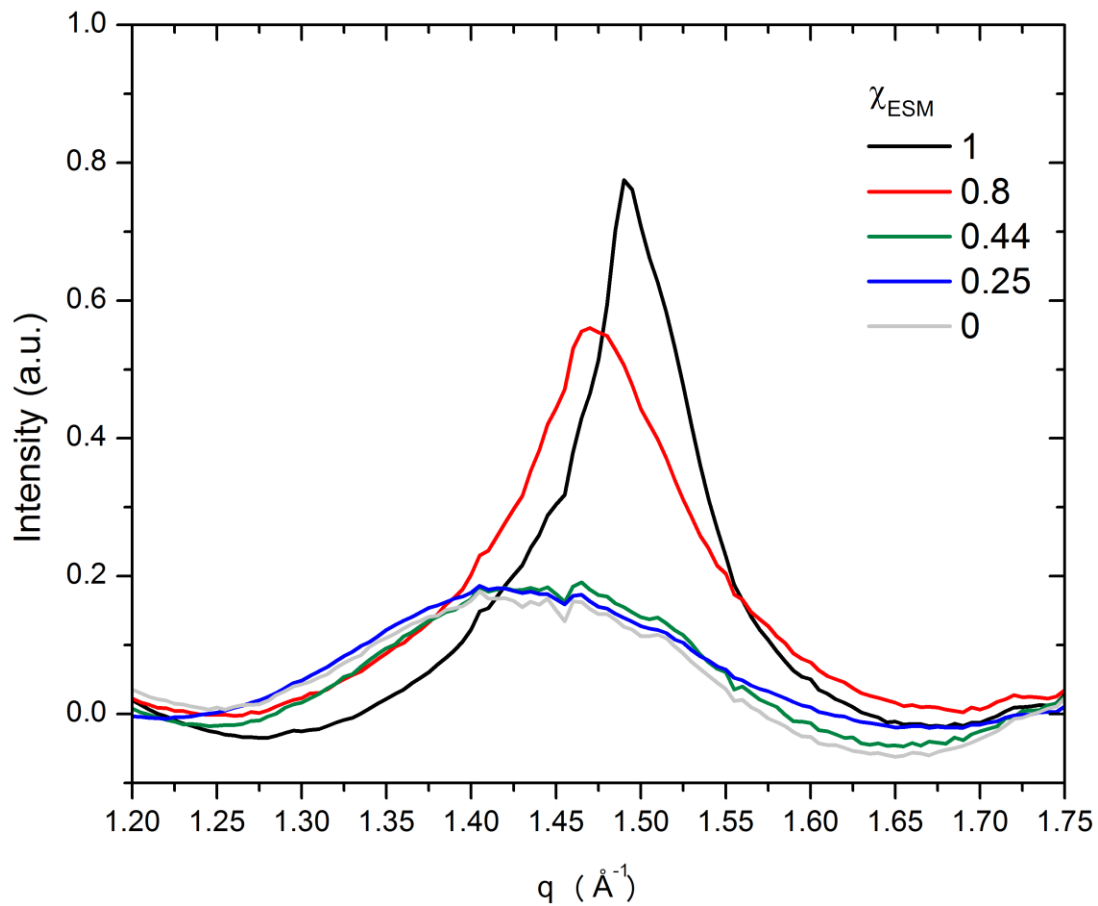
**Table S3 Structural refinement of symmetric vesicles. Data were modeled with an symmetric five slab scattering length density profile. Parameter uncertainties are estimated to be < 5%.**

| Temp (°C) | POPE      |           |           | ESM/POPE (1:3) <sup>a</sup> |           |           | ESM/POPE (4.4:5.6) |           |           | ESM/POPE (8:1) |           |           | ESM       |           |           |
|-----------|-----------|-----------|-----------|-----------------------------|-----------|-----------|--------------------|-----------|-----------|----------------|-----------|-----------|-----------|-----------|-----------|
|           | <u>15</u> | <u>30</u> | <u>45</u> | <u>15</u>                   | <u>30</u> | <u>45</u> | <u>15</u>          | <u>30</u> | <u>45</u> | <u>15</u>      | <u>30</u> | <u>45</u> | <u>15</u> | <u>30</u> | <u>45</u> |
| $A_L$     | 44.7      | 56.7      | 60.9      | -                           | 54.1      | 59        | 43.2               | 52.9      | 57.7      | -              | 50.5      | 55.9      | 43.8      | 49.7      | 55.5      |
| $D_B$     | 50.5      | 41.4      | 38.9      | -                           | 42.5      | 39.9      | 51                 | 42.9      | 40.6      | -              | 43.8      | 41.4      | 49.2      | 44.1      | 41.6      |
| $2DC$     | 39.5      | 32.6      | 30.8      | -                           | 33.2      | 31.3      | 39.1               | 33.2      | 31.6      | -              | 33.2      | 31.8      | 36.6      | 33        | 31.7      |

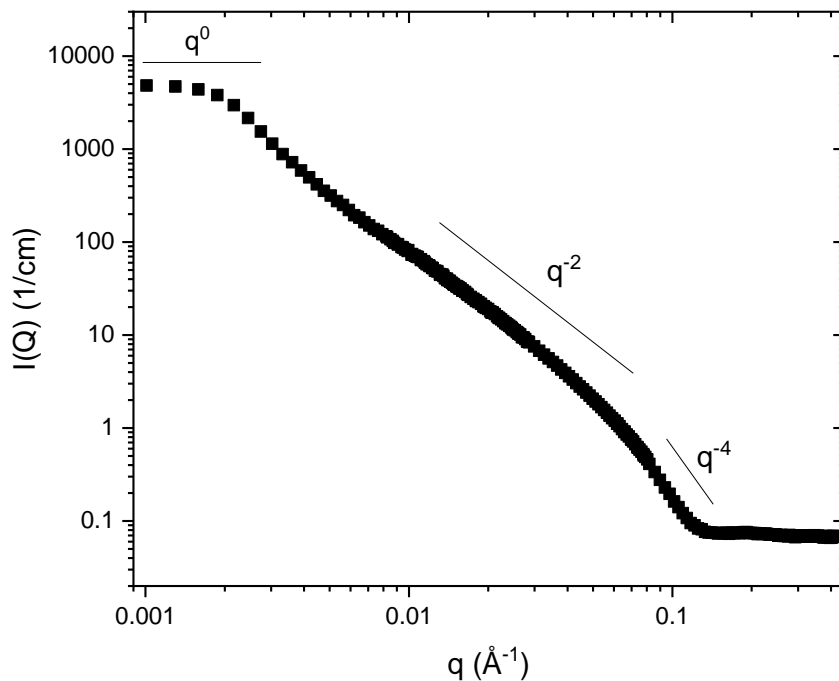
<sup>a</sup>SAXS only.



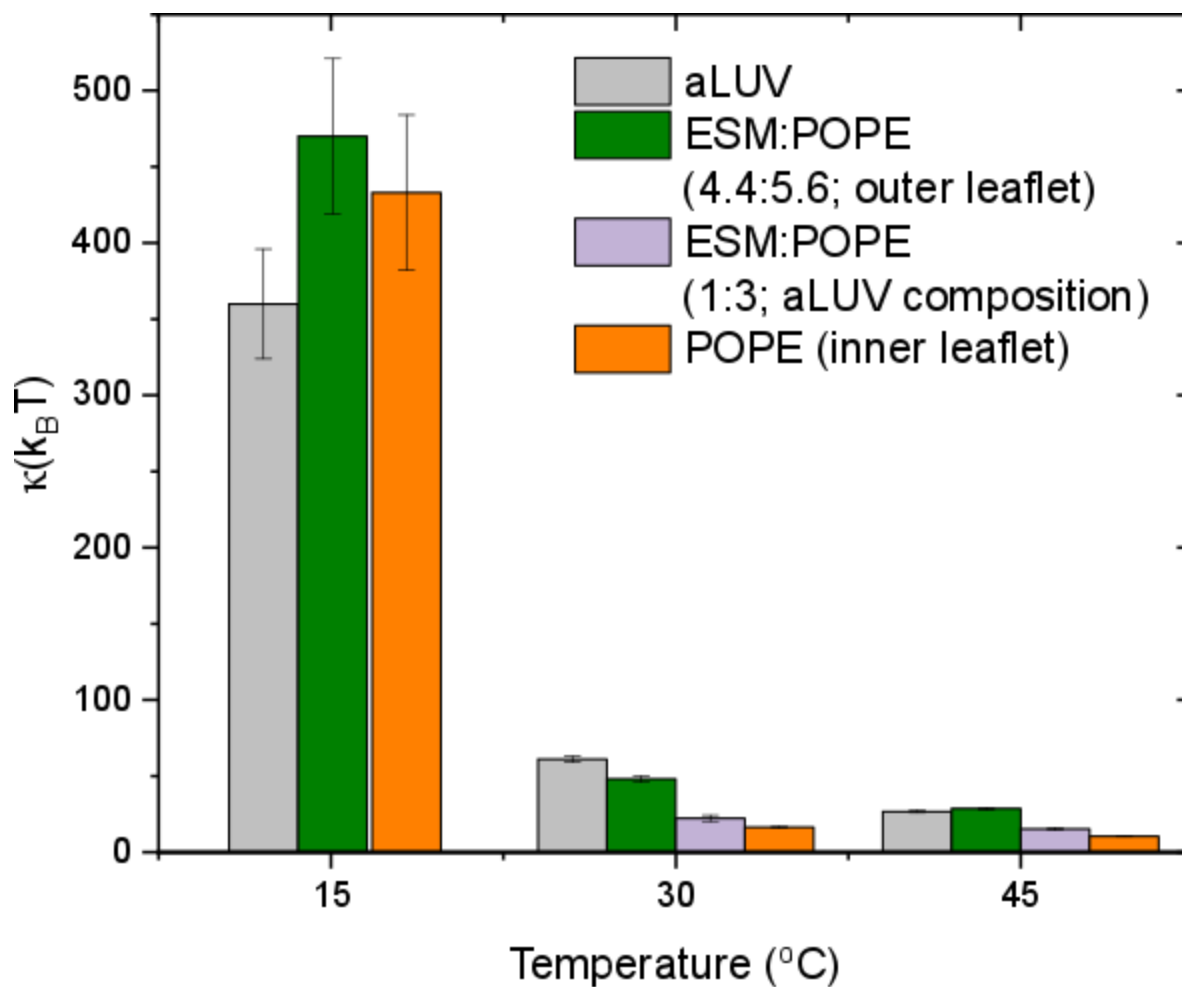
**Figure S1:** DSC thermograms of the aLUV (black) and its individual components ESM (blue) and POPE (red). These data are consistent with the notion that the aLUV is fluid at 30°C.



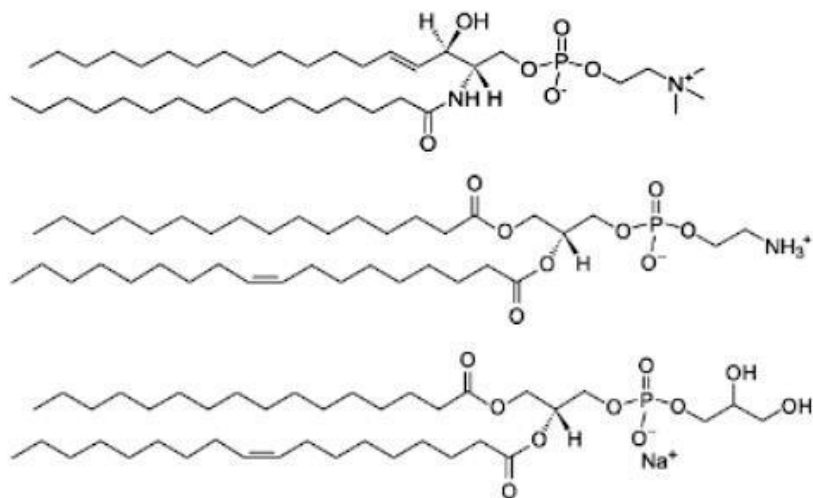
**Figure S2:** Wide angle X-ray scattering data multi-lamellar vesicles (MLV) composed of different mole fractions of ESM ( $\chi_{\text{ESM}}$ ) at 30°C. These data suggest that compositions up to  $\chi_{\text{ESM}}=0.8$  are fluid at 30°C as seen by the lack of a sharp WAXS peak for  $\chi_{\text{ESM}}=0, 0.25$  and  $0.44$ .



**Figure S3:** The SANS data for the aLUV. The data displays a  $q^0$  dependence at low- $q$ , a  $q^{-2}$  dependence at medium- $q$ , arising from the lamellar bilayer of the aLUV which transitions to a  $q^{-4}$  dependence indicative of objects with smooth surfaces and sharp interfaces. All of which are characteristics of structurally sound vesicles. aLUV solution was diluted by a factor of 10 from the sample used for structural analysis.

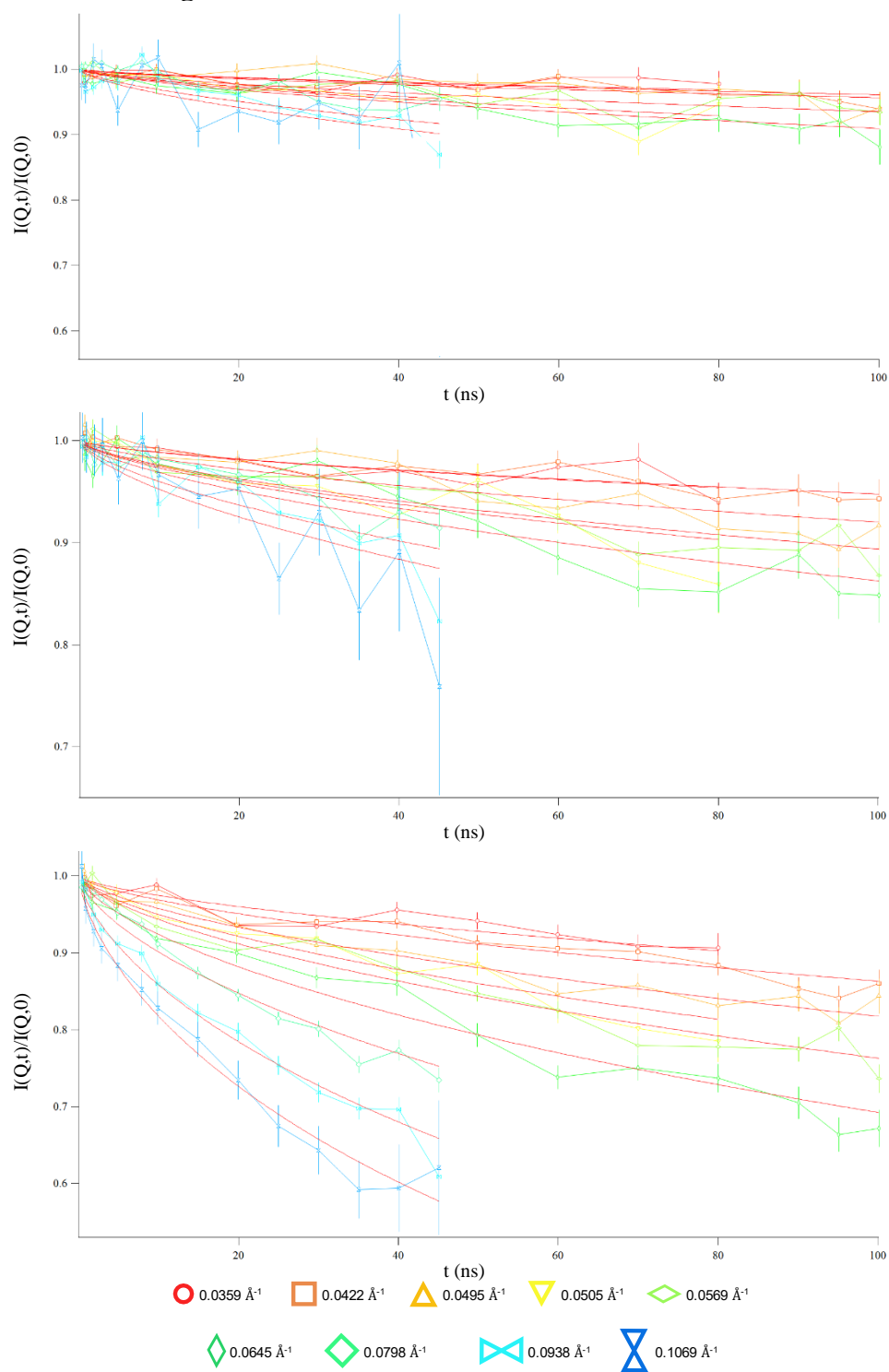


**Figure S4:** Bending moduli ( $\kappa$ ) for the aLUVs and symmetric vesicles with compositions corresponding to the inner leaflet, outer leaflet and overall aLUV composition measured with NSE at 15  $^{\circ}C$ , 30  $^{\circ}C$  and 45  $^{\circ}C$ .



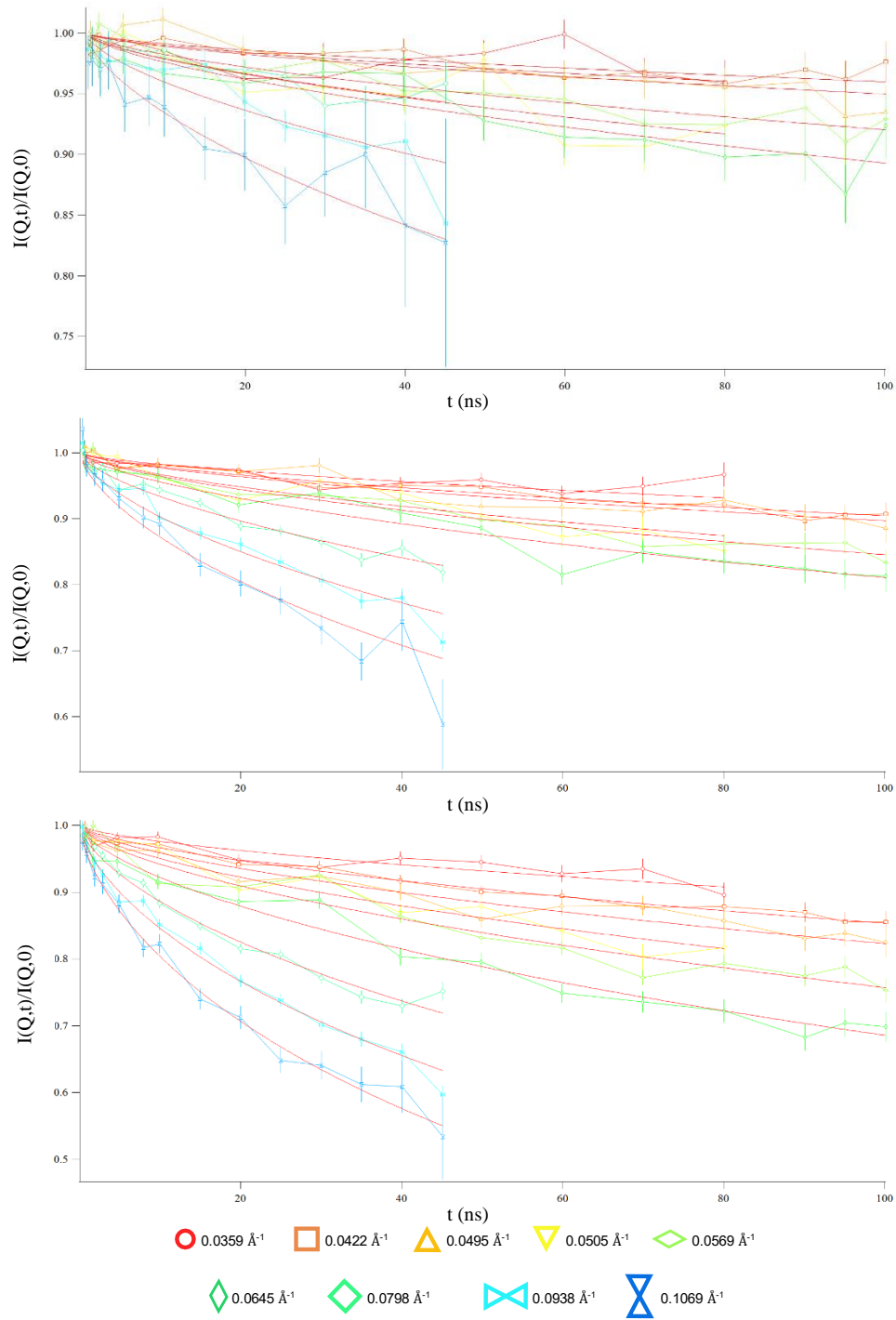
**Figure S5:** A) Most abundant sphingomyelin structure in ESM. B) Structure of POPE. C) Structure of POPG.

## S2: Intermediate Scattering curves.

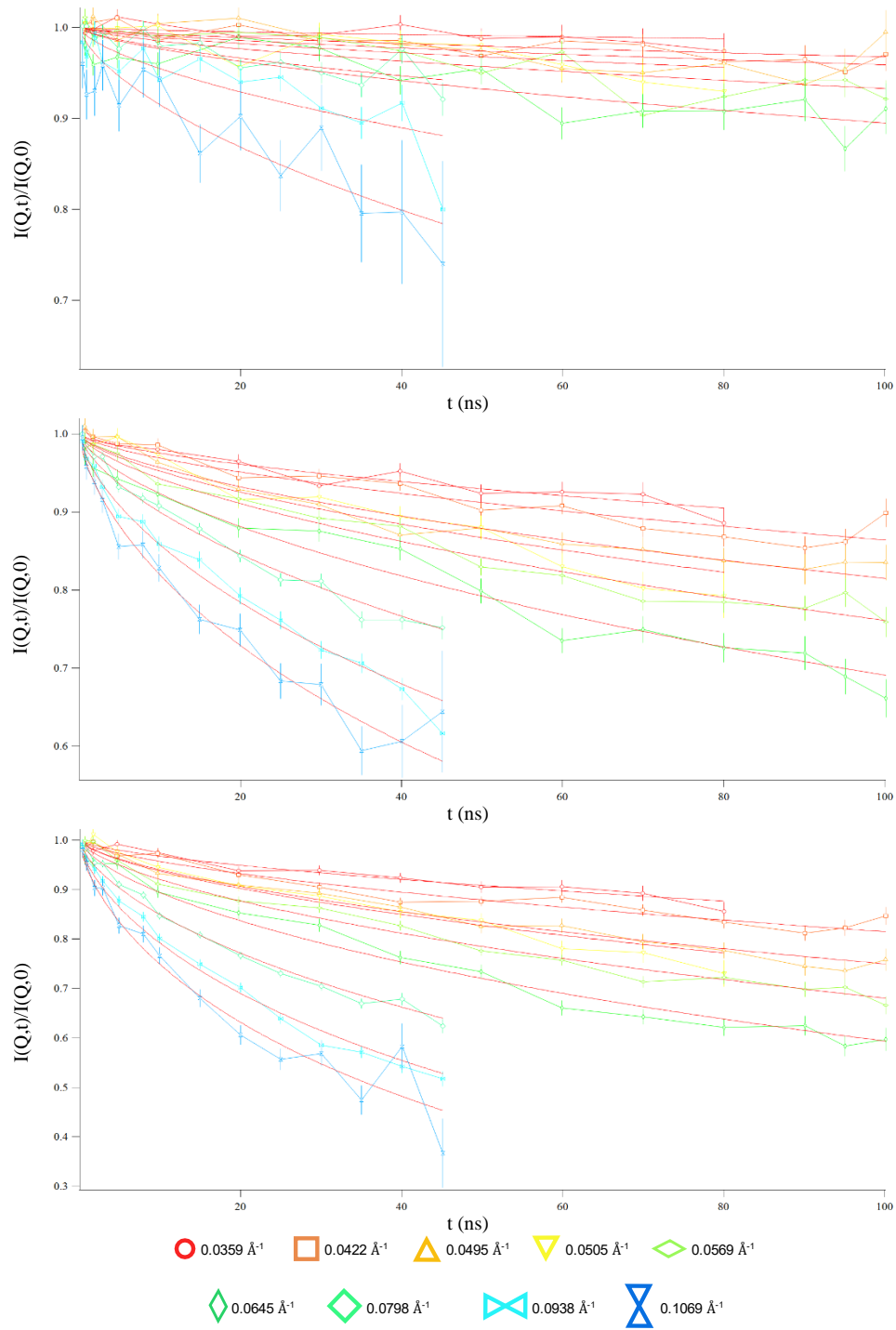


**Figure S2.1:** Intermediate scattering function measured by NSE for the ESM control at  $T = 288$  K (top), 303 K (middle), and 318 K (bottom). The legend below displays the various colors/patterns in the decay plot that correspond to the different wavenumber transfer values. Error bars represent one standard deviation.

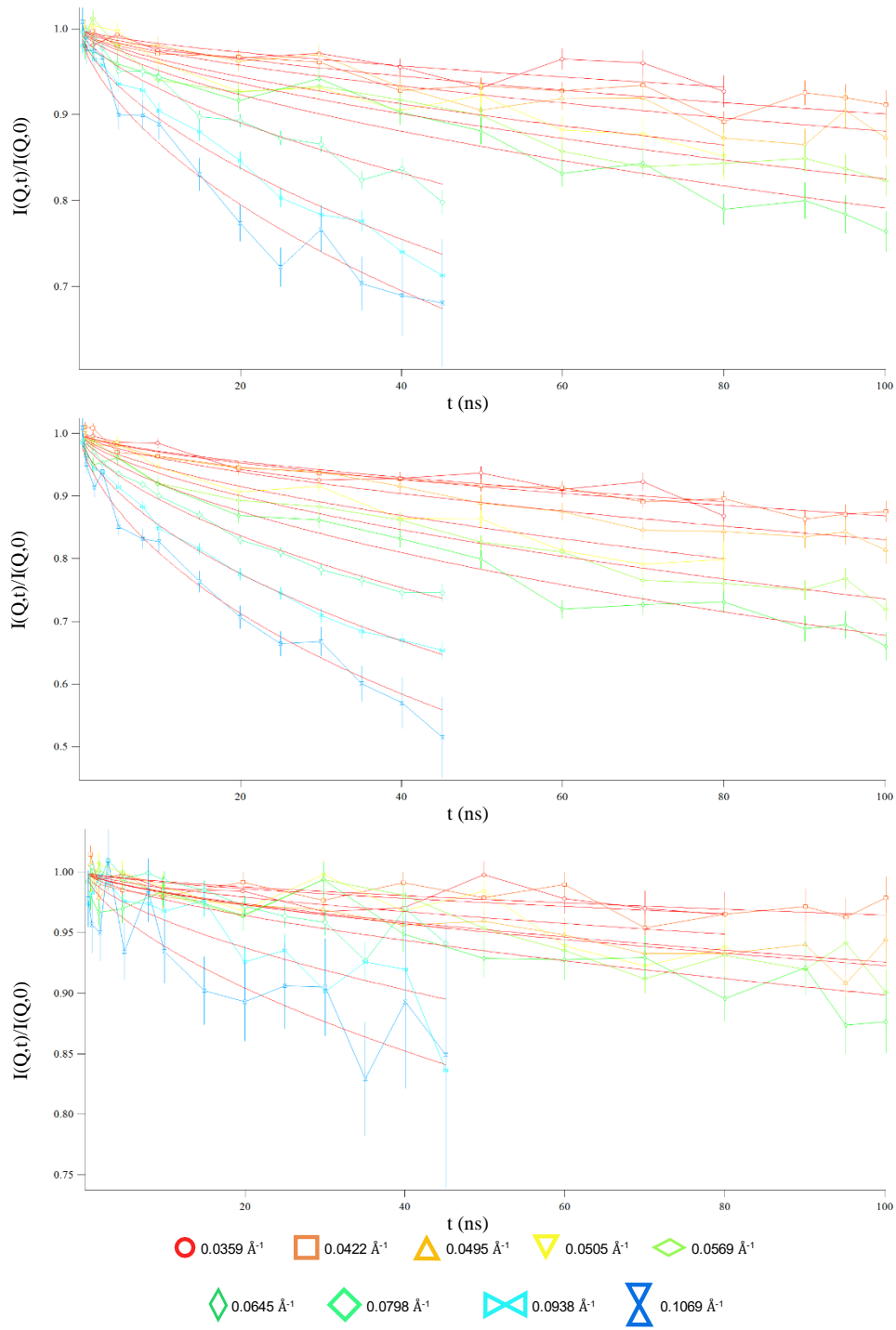




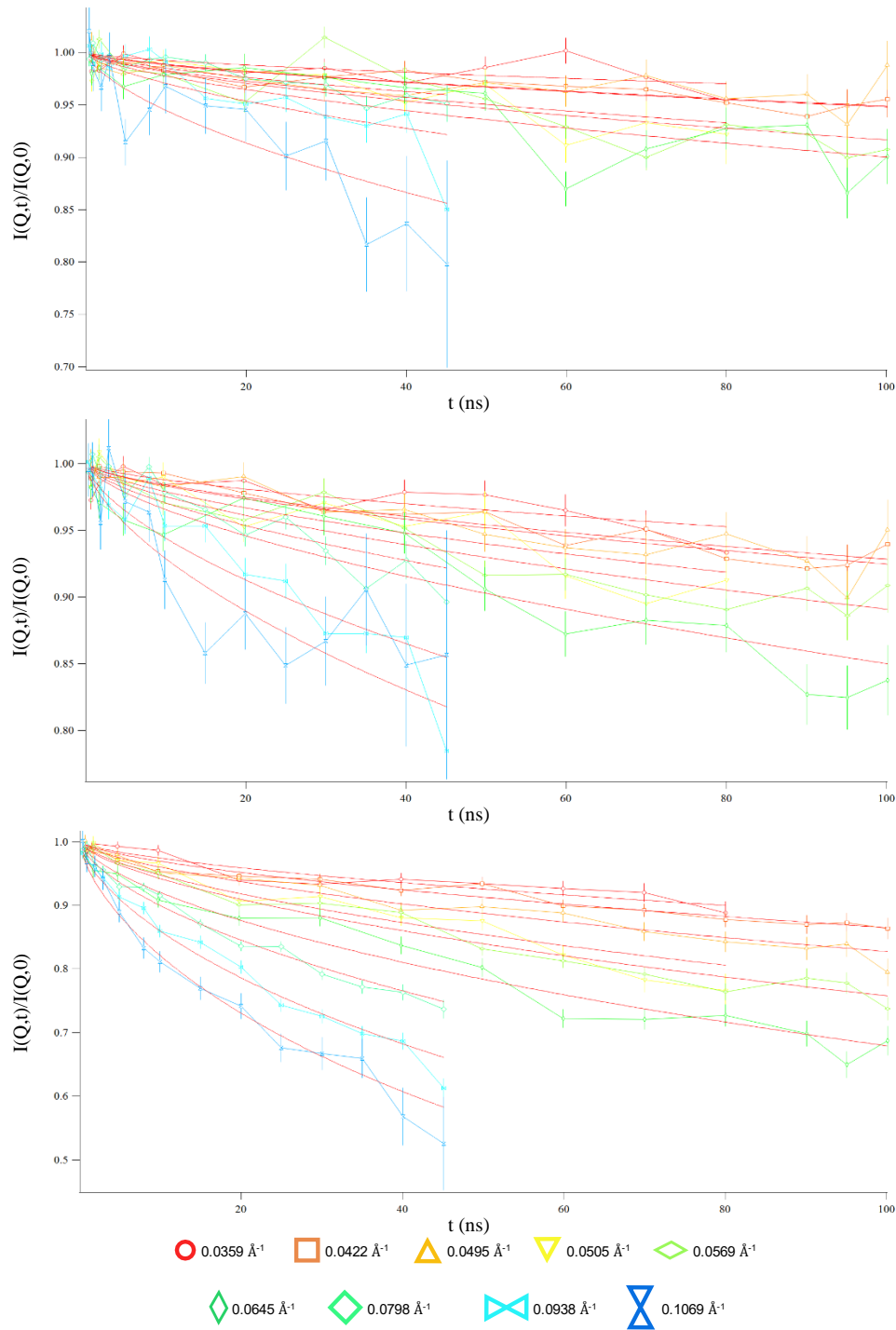
**Figure S2.2:** Intermediate scattering function measured by NSE for the asymmetric LUV at  $T = 288$  K (top),  $303$  K (middle), and  $318$  K (bottom). The legend below displays the various colors/patterns in the decay plot that correspond to the different wavenumber transfer values. Error bars represent one standard deviation.



**Figure S2.3:** Intermediate scattering function measured by NSE for the POPE control (with NaCl) at  $T = 288$  K (top), 303 K (middle), and 318 K (bottom). The legend below displays the various colors/patterns in the decay plot that correspond to the different wavenumber transfer values. Error bars represent one standard deviation.



**Figure S2.4:** Intermediate scattering function measured by NSE for the POPE/ESM (1:1) control at  $T = 288$  K (top), 303 K (middle), and 318 K (bottom). The legend below displays the various colors/patterns in the decay plot that correspond to the different wavenumber transfer values. Error bars represent one standard deviation.



**Figure S2.5:** Intermediate scattering function measured by NSE for the POPE/ESM (1:4) control at  $T = 288$  K (top), 303 K (middle), and 318 K (bottom). The legend below displays the various colors/patterns in the decay plot that correspond to the different wavenumber transfer values. Error bars represent one standard deviation.

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