Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Supporting Information for

Phase Transition Behaviours of the Supported DPPC Bilayer Investigated by Sum Frequency Generation (SFG) and Atomic Force Microscopy (AFM)

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Tilt angle of the DPPC molecules in the asymmetric bilayer during heating stage I

Although the changes in the SFG intensities are very small in the temperature range, it is interesting to note that the SFG intensity for the asymmetric stretching mode of CH_3 (CD_3) increases greater in comparison to that of the symmetric stretching mode using *ppp*-polarization (Figs. S1a and 1b). As described in our previous study,[1] the intensity ratio between the two modes in the *ppp*-polarized SFG spectra can be used to calculate the orientation angle (θ) of the terminal CH_3 (CD_3) group, thus the tilt angle (α) of the alkyl chain of the DPPC molecules. Detailed analysis procedure, equations and simulated curves has been described in previous paper,[1] here only the calculated results are given. It was found that tilt angle of the alkyl chain increases with the temperate with the values of 0 (±3) degrees (10°C to 12 (±3) degrees (30 °C) for both the top DPPC and bottom DPPC-d_{75} leaflets in the bilayer. Similar changes of the tilt angles of alkyl chains are also expected to occur in the symmetric DPPC bilayer. Therefore, the tilt angle of the alkyl chains of the DPPC molecules increases with the temperature before the phase transition. Similar changes in the tilt angles of the supported lipid bilayer have also been reported by Bain and his co-workers using *in situ* ATR-

Raman

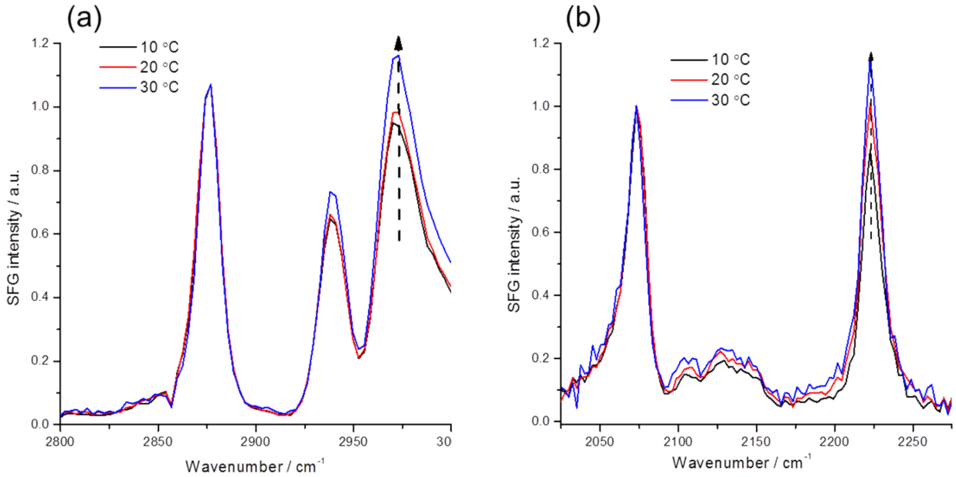

Figure S1. The *ppp*-polarized SFG spectra of DPPC/DPPC-d_{75} asymmetric bilayer deposited on a fused quartz surface in contact with water at the different temperatures of 10, 20 and 30°C observed in (a) C-H stretching region for DPPC top leaflet and (b) C-D stretching region for DPPC-d_{75}.

observations. In fact, when the temperature further increases, the flip-flop rate is significantly accelerated, thus the asymmetric features of the DPPC/DPPC-d_{75} bilayer quickly disappear. A similar analysis then becomes difficult.

SFG measurement for the cooling process of asymmetric DPPC bilayer

Figure S2(a) shows a series of *ssp*-polarized SFG spectra (2800–3000 cm^{-1}) for an asymmetric DPPC/DPPC-d_{75} bilayer on CaF_2 surface during the cooling process after heating to 50°C (Fig. 2). All the SFG peaks almost disappear at 50°C at which the phase transition is completed. This is reasonable since the asymmetric bilayer becomes fully symmetrical by the flip-fop movement and the phase-transition process. As the bilayer system is cooling, two SFG peaks appear at 2875 and 2939 cm^{-1} with the temperature decrease. These two peaks can be assigned to the CH_3,ss and $CH_{3,FR}$ of the DPPC molecules in the bilayer. Figure S2(b) shows the temperature dependence of the CH3,ss mode. A local maximum appears around 38°C although the maximum of the peak intensity is very weak in comparison to the first heating process (Fig. 2). The peak intensity then soon decreases to

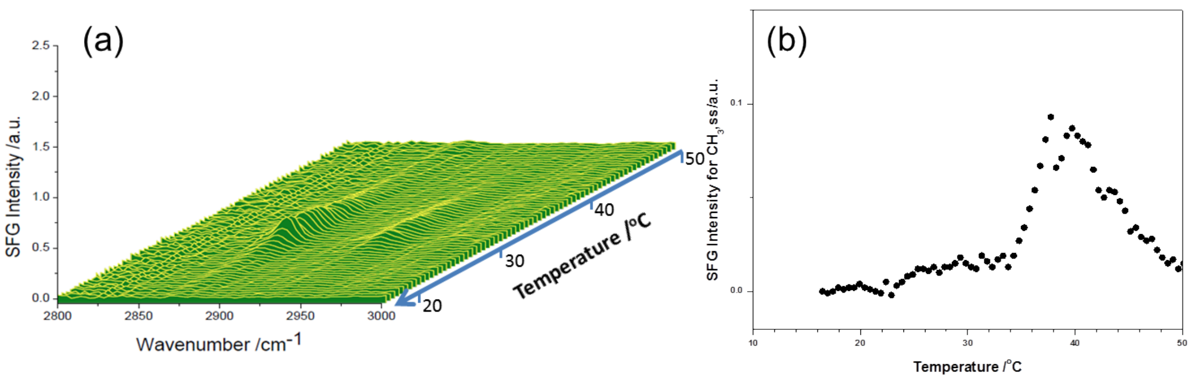

Figure S2. (a) The *ssp*-polarized SFG spectra of DPPC/DPPC-d_{75} asymmetric bilayer during the cooling process from 50°C after the measurement shown in Fig. 2; (b) Time profiles for SFG intensity of CH_3,ss. See text for details.

the background level.

Since the as-prepared asymmetric DPPC/DPPC-d_{75} bilayer becomes fully symmetrical after the phase transition, it is hard to attribute the peaks to the top or bottom leaflet, but to the structural difference or population difference between the top and bottom leaflets. As discussed for the symmetric bilayer in Fig. 3, the former one seems to be possible. The phase transition of the *liquid* to *gel* phase should occur around the T_m. As discussed in the main text, similar structural changes occur during the cooling process, i.e., the DPPC molecules in the bottom leaflet first freeze, then those in top leaflet change to a *gel* structure. A clear hysteresis was observed for the local maximum in the heating (Fig. 2b) and cooling processes (Fig. S2b). This implies that some irreversibility is present in the melting and freezing processes. Similar features were also observed in the symmetric bilayer.

On the other hand, it is interesting to note that no methylene peaks were observed in the cooling process although such peaks were observed in the heating process (Fig. 2). This indicates that the conformational disordering is fully released during the phase transition process of the *gel* to *liquid* phase and will not appear in the reversed phase transition process.

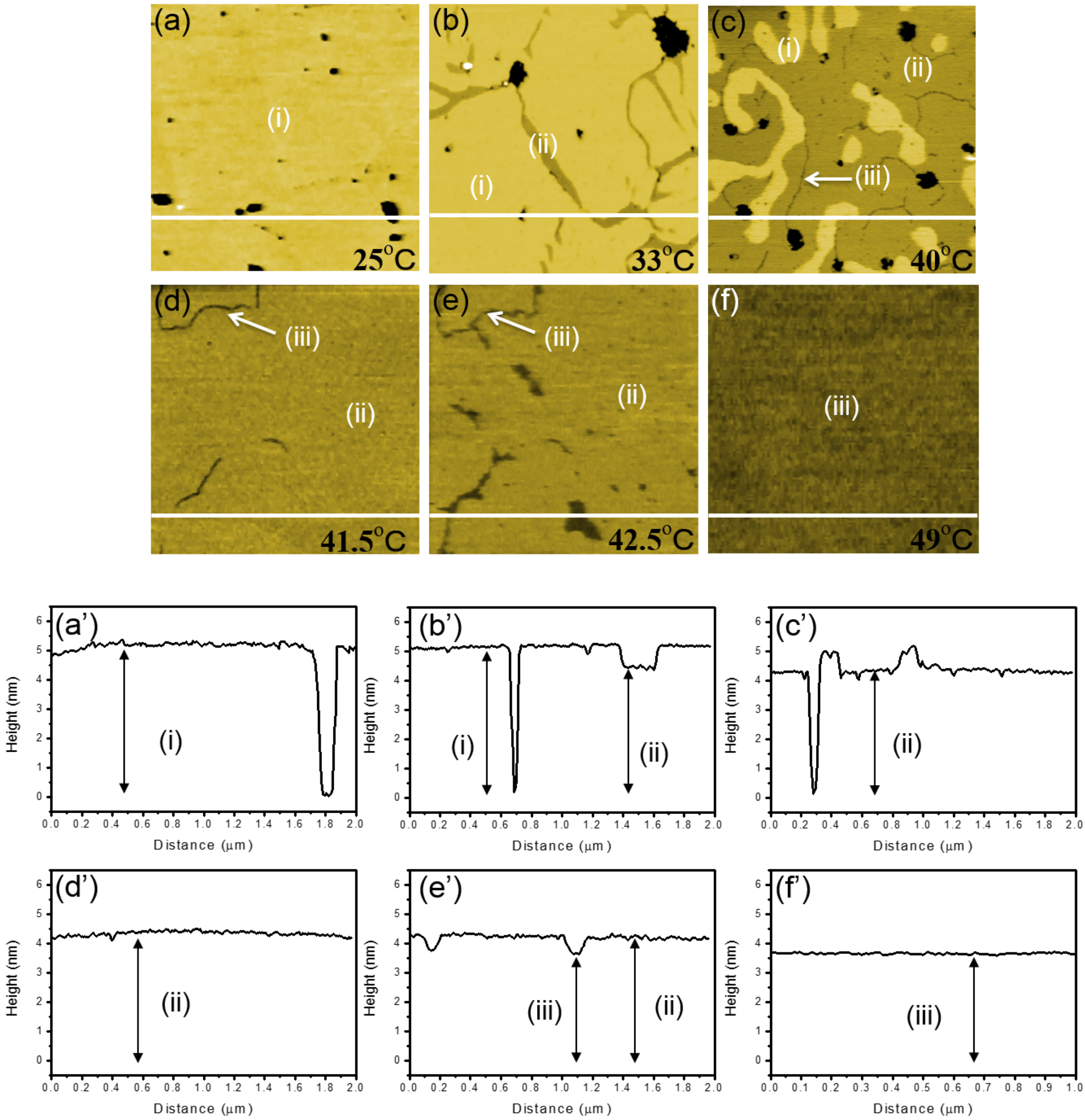

Figure S3 The cross section profiles of the DPPC bilayer (marked by white line). AFM images recorded at $^{+}$ (a) **25**^{o}C, (b) **33**^{o}C, (c) **40**^{o}C, (d) **41.5**^{o}C, (e) **42.5**^{o}C, and (f) **49**^{o}C in a tris-buffer solution containing 5mM Ca^{2} were shown in Fig.4

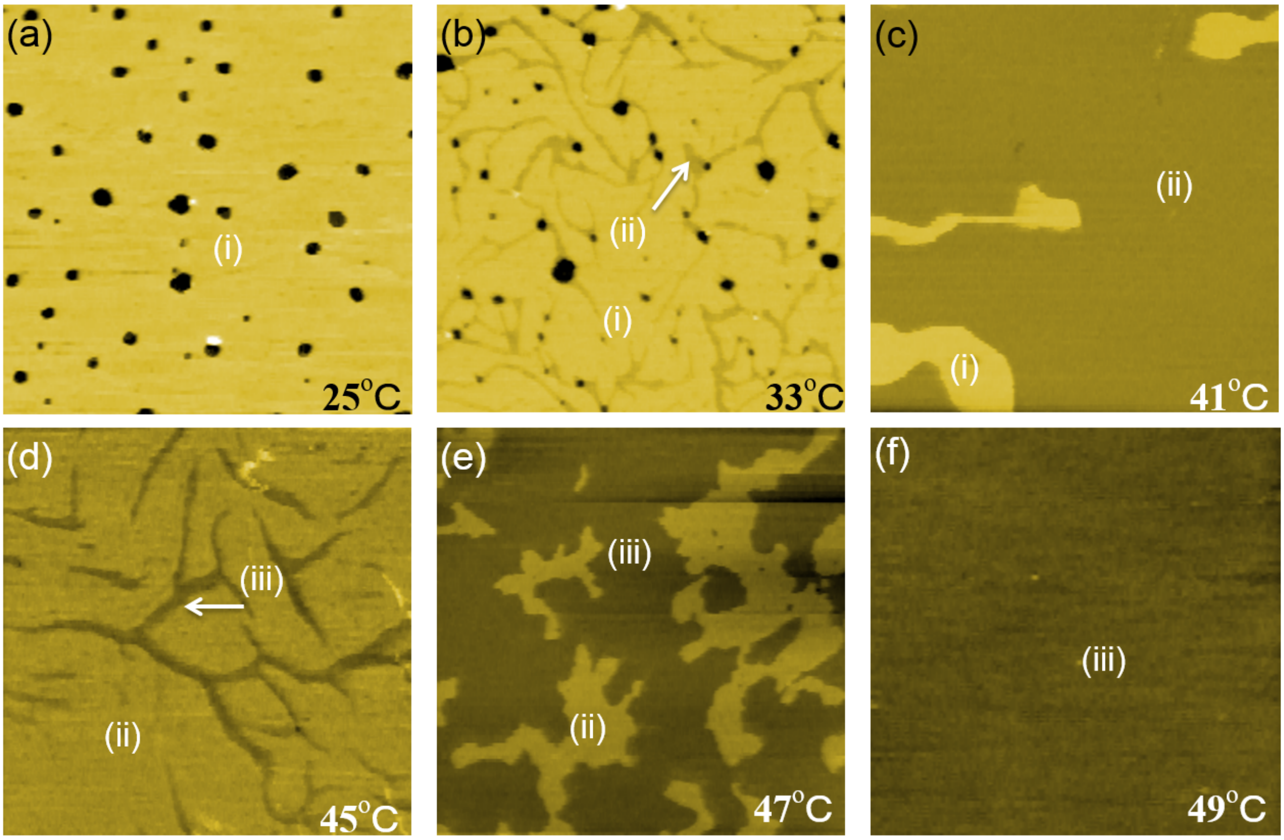

Figure S4. AFM images for a supported DPPC bilayer recorded at + (a) **25**°C, (b) **33**°C, (c) **41**°C, (d) **45**°C, (e) **47**°C, and (f) **49**°C in pure water. Size of AFM images is 2μm×2 μm.

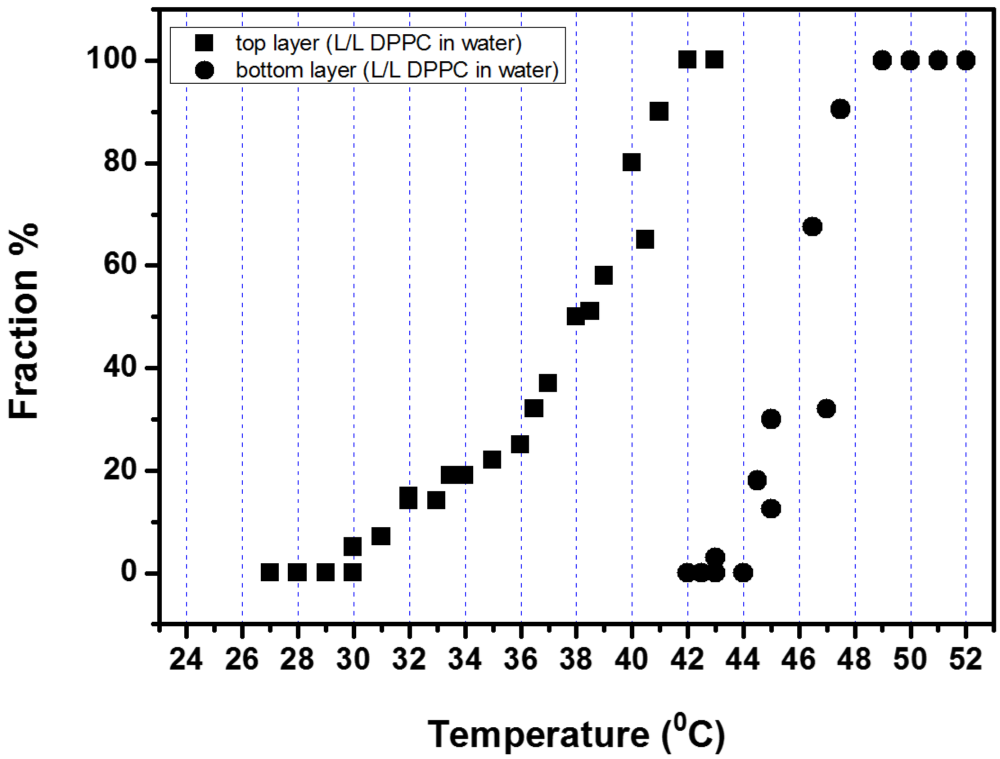

Figure 5. Fraction of domain (ii) (black squares) and (iii) (back circles) determined from many *in situ* AFM observations in pure water as a function of temperature.

Reference

1. Tong, Y.; Li, N.; Liu, H.; Ge, A.; Osawa, M.; Ye, S., Mechanistic studies by sum-frequency generation spectroscopy: Hydrolysis of a supported phospholipid bilayer by phospholipase A_2, *Angew. Chem. Int. Ed.* **2010,** *49*, 2319-2323.