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# Raman spectroscopic measurements on DEPC liposome: phase transition observation under Xe-gas pressure

**Tsutomu Uchida[1], Amadeu K. Sum[2]**

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Raman spectroscopic analysis was applied to observe the phase transition of dielaidoylphosphatidylcholine (DEPC) liposome under 0.5 MPa Xe pressurization conditions. After the band assignments on DEPC molecule, detailed measurements of the methylene C-H stretching mode spectra were performed. The data revealed that all three peaks associated with DEPC, including the C-H stretching mode, shifted to lower wavenumbers at lower temperatures and that the relative intensities of Raman spectra changed simultaneously. These changes on Raman spectra occurred at approximately 280 K, suggesting a gel to liquid crystalline phase transition. This temperature is lower than that under atmospheric pressure, and it is consistent with DEPC liposomes exposed to Xe measured with differential scanning calorimetric under similar conditions.

## 1. Introduction

Xenon (Xe) is the noble gas known to be a potent general anesthetic gas. General anesthesia is exhibited through the depression of nerve function, but the specific molecular mechanism by which anesthetic agents induce an anesthetic state remain poorly understood. One of the molecular mechanisms of general anesthesia which has been studied for over 80 years is known through the Mayer-Overton correlation. This model suggested that the anesthetic gases dissolve in the lipid bilayer of neurons to reduce the ion-channel activity (Mayer, 1899; Overton, 1901). Later, another molecular mechanism of general anesthetic was proposed by Pauling (1961) and Miller (1961). The theory proposed by Pauling and Miller suggested that anesthetic gases could form clathrate hydrates, so the gas would block the diffusion of signal-transmitting molecules at the synaptic junction by forming a clathrate structure. More recently, some researchers have focused on a mechanism involving direct interactions between hydrophobic pockets or clefts in proteins, especially integral membrane proteins such as gated-ion channels, and anesthetic gases (Franks and Lieb, 1984). In order to study the effect of Xe on the neuronal activities, Uchida et al. (2012) measured the firing of neuronal networks under Xe-gas pressure. They observed that the synchronized bursts in the neuronal network were inhibited when exposed to Xe-gas pressure while the single firing of neuron survived. While insightful, these measurements have still not been able to provide a more detailed molecular mechanism of general anesthetics.

Booker and Sum (in press) investigated the interaction of Xe with model phospholipid membranes using molecular dynamics (MD) simulations of pure dioleoylphoshatidyl choline (DOPC) bilayers in two initial configurations with Xe at a range of concentrations and pressures and over timescales of 150～300 ns. High-pressure differential scanning calorimetry (HP-DSC) measurements were also presented to characterize the effect of Xe on lipid (dielaidoyl- phosphatidylcholine: DEPC) membrane phase transition temperatures and fluidity. MD simulations revealed that Xe atoms exerted broad biophysical changes on the membrane fluidity, lateral pressure profiles near the lipid head groups, and bilayer structure by substantially increasing the lipid head groups spacing and bilayer thickness. The HP-DSC measurements revealed that the gel to liquid crystalline phase transition occurred at temperatures of 285.0, 282.5, and 278.4

1) Division of Applied Physics, Faculty of Engineering, Hokkaido University
2) Department of Chemical and Biological Engineering, Colorado School of Mines

K under 0, 0.3, and 1.0 MPa of Xe gas, respectively. These results differ from the pressure effect on the phase-transition temperature of lipid bilayer (Booker and Sum, in press).

Following their studies, we performed Raman spectroscopic measurements on DEPC liposomes to observe the change of molecular vibration across the membrane phase transition temperature under Xe pressure.

## 2. Experimental Procedures

The preparation procedures of liposome solution were similar to that of Booker and Sum (in press). Here we briefly present the procedure. As the model lipid, 18:1 (Δ9-Trans) phosphocholine (DEPC) in powder form, obtained from Avanti Polar Lipids, was used for the liposome (large multilamellar vesicles) solution by dissolving 20 mg/mL of powdered DEPC lipids into deionized water. Lipids were mixed vigorously for one hour and the liposomes were then allowed to age overnight at room temperature, approximately 300 K, which was above the DEPC phase transition temperature, $T_m$ = 285 K (Silvius, 1982).

Prior to the Raman spectroscopic measurements, the macroscopic features of the prepared liposome sample were observed by a phase-contrast microscope (Olympus, CKX41). The detailed feature of each large multilamellar vesicle was observed by preparing the freeze-fractured replica sample of the solution. The detailed procedures of the replica preparation are described elsewhere (Uchida et al., 2011). A small amount of the liposome solution (approximately 15 $mm^3$) was rapidly frozen by immersing it into a liquid nitrogen bath. The frozen droplet was then fractured under vacuum ($10^{-4}$ to $10^{-5}$ Pa) and low temperature (approximately 100 K), and the replica film of the fractured surface was prepared by evaporating platinum and carbon (JEOL Ltd., JFD-9010). We used a field-emission gun-type transmission electron microscope (FEG-TEM; JEOL Ltd., JEM-2010) to observe the replica film at a 200 kV acceleration voltage.

Raman spectra were measured by SPEX Raman 750 spectrometer with 50 mW $Ar^+$ laser (514.5 nm) through the microscope equipped with the long-working distance objective lens (LMPlanFLN x50). The scattered radiation was collected through a slit with 180° geometry at 200 μm. The diameter of the incident laser beam focused on the specimen was maintained at approximately 10 μm. Each Raman spectrum was obtained by three consecutive 60 s accumulation. For the spatial averaging, Raman spectra at more than three positions for each condition was collected and averaged. The spectrum of the neon-emission was used for the correction of the wavenumber measurement. Each Raman spectrum was then analyzed by peak deconvolution with the peaks fitted to Voigt curves to estimate the intensity (area of the fitting curve) and the peak position (peak wavenumber) with commercially supplied software (Originlab Co., OriginPro 9.0).

Approximately 100 μL of the DEPC liposome solution was introduced to the high-pressure vessel (Taiatsu Techno Co.) equipped with the sapphire glass windows. The sample temperature was controlled by a refrigeration unit (Neslab RTE-111), which was monitored with thermocouple (T-type) to ±0.2 K. The high-pressure vessel was connected to a Xe cylinder (99.995% purity, Air-Water Inc.) and the pressure was monitored by a pressure transducer (Taiatsu Techno Co., SE700T). In the present study, Xe gas was pressurized at 0.5±0.02 MPa after flushing the cell a few times initially. Temperature and pressure readings were recorded with a data logger (Graphtech, GL220).

## 3. Results and Discussion

### 3.1 Observation of DEPC liposomes

Typical features of DEPC liposomes (large multilamellar vesicle) are shown in Fig. 1. The optical microscopic image (Fig. 1a) indicates that the vesicles (1～10 μm diameter) are formed in the solution although they are partly aggregated. In order to obtain good signal-to-noise ratio Raman scattering, we focused the laser on these aggregated vesicles. The FEG-TEM image of the vesicle (Fig. 1b) shows that the typical size of the vesicle ranged from submicrons to several microns in diameter. This is consistent with the optical microscopic observations. The surface of the vesicle is mainly smooth, but we sometimes observed the typical striped pattern (known as a bonded structure, shown by a thin arrow) and the island structures (shown by a dashed circle) on parts of the surface. The interval of the stripes is approximately 15 nm. When part of the vesicles was fractured, we were able to observe the cross section of the vesicle as shown by the thick arrow in Fig. 1b.

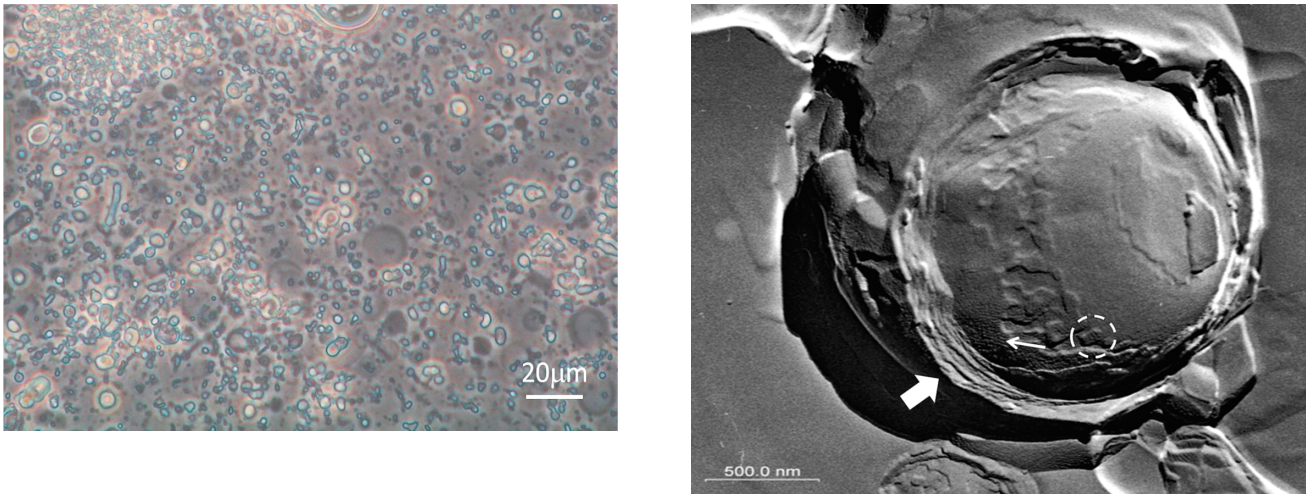


Fig. 1 : A typical feature of the DEPC liposome (large multilamellar vesicle): (a) optical microscopic image (scale bar 20 $\mu$m), and (b) FEG-TEM image of the freeze-fractured replica of the vesicle (scale bar 500 nm). A part of this vesicle has been removed with freeze-fracture process, and its cross section (thick arrow) shows the multiple DEPC layers (at least four layers). The typical stripped pattern (thin arrow) and the island structures (dashed circle) are observed on parts of the surface.

This figure shows that the vesicle is multilamellar (at least four layers), and each layer has a thickness of approximately 20 nm. Since each lipid bilayer is approximately 4 nm (Booker and Sum, in press), this thickness includes the water between the bilayers.

## 3.2 Raman spectra of DEPC under various conditions

Typical Raman spectra of the DEPC powder were recorded as the reference in the present study (Fig. 2a). Based on the band assignment for other lipids

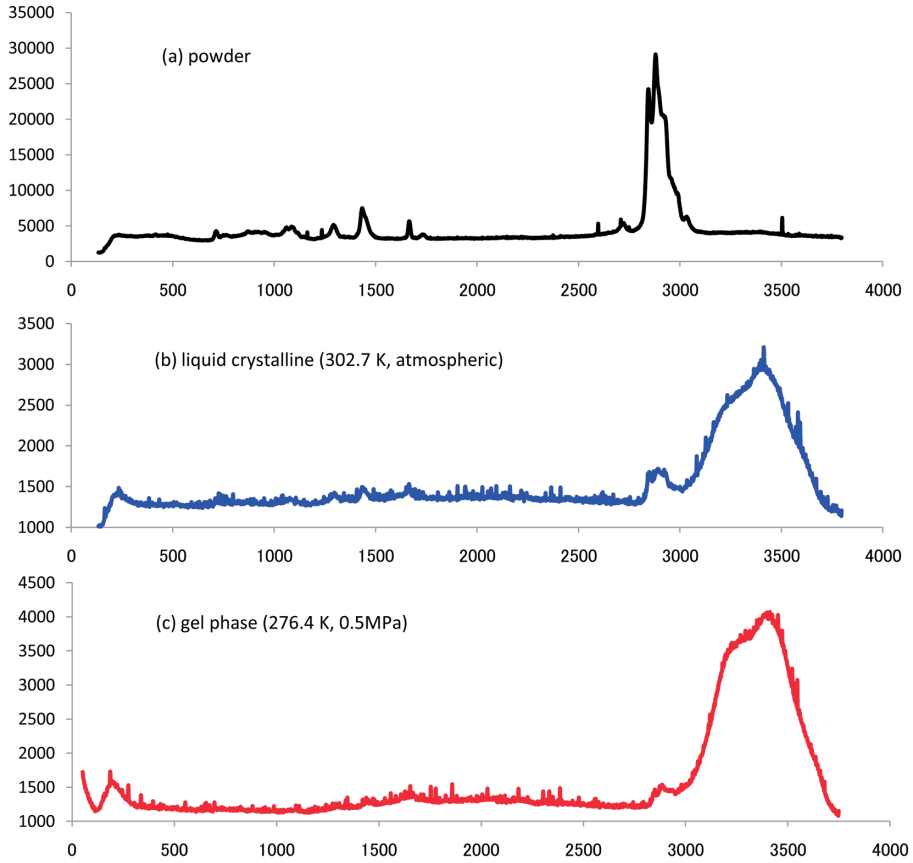


Fig. 2 : Typical Raman spectra of (a) DEPC powder and (b), (c) DEPC liposome solutions. Each measurement condition is (a) room temperature (~300 K), (b) 302.7 K at atmospheric pressure, and (c) 276.4 K at 0.5 MPa Xe pressure.

such as C(18):C(10)PC and C(18):C(10)TMPC (Batenjany, et al., 1994), several peaks are assigned as follows: C-N symmetric stretching mode (~750 $cm^{-1}$), methylene C-C stretching mode (triple peaks between 1030 and 1150 $cm^{-1}$), $CH_2$ twist mode and bending mode (at around 1300 and 1440 $cm^{-1}$, respectively), and methylene C-H stretching mode (triple peaks between 2800 and 3100 $cm^{-1}$).

The Raman spectrum for DEPC in solution is shown in Fig. 2b. It includes the peaks for the lipids and water (O-H stretching mode) between 3000 and 3800 $cm^{-1}$. Figure 2c shows the typical Raman spectrum of DEPC liposome solution at lower temperature under Xe gas pressure. As shown in Fig. 2, because the spectrum for the water O-H stretching mode is dominant, we focused our measurements in the methylene C-H stretching mode of DEPC, as it has the largest Raman scattering among the peaks for DEPC.

Figures 3a~b show the spectra for the C-H stretching mode for DEPC for the sample pressurized with 0.5 MPa Xe at 302.5 K. At those conditions, no significant changes on the Raman spectra were observed. Then the sample temperature was gradually decreased at approximately 2 K per hour. Since methylene C-H stretching mode is known to be composed of three peaks (Batenjany, et al., 1994), the C-H symmetric stretching mode (lowest wavenumber around 2840 $cm^{-1}$), the C-H anti-symmetric stretching mode (middle wavenumber around 2880 $cm^{-1}$) and the terminal $CH_3$ symmetric stretching mode (highest wavenumber around 2920 $cm^{-1}$), we deconvoluted each Raman spectra with three Voigt curves. The peak position of each deconvoluted peak is summarized as a function of temperature in Fig. 4. Since the variation in the peak position was smaller than the observed wavenumber range, the C-H symmetric stretching mode was selected to show the temperature dependence (see Fig. 4a, extended plot). These plots show that all three peaks are slightly shifted at around 280 K. The relative peak intensities of the lowest peak and the highest peak were calculated as the intensity ratios related to the intensity of the middle peak. The temperature dependence of the relative peak intensities (Fig. 5) also indicated changes at around 280 K. This coincidence in the change of the spectra suggests that the gel to liquid crystalline phase transition temperature of the DEPC liposome under 0.5 MPa Xe pressure.

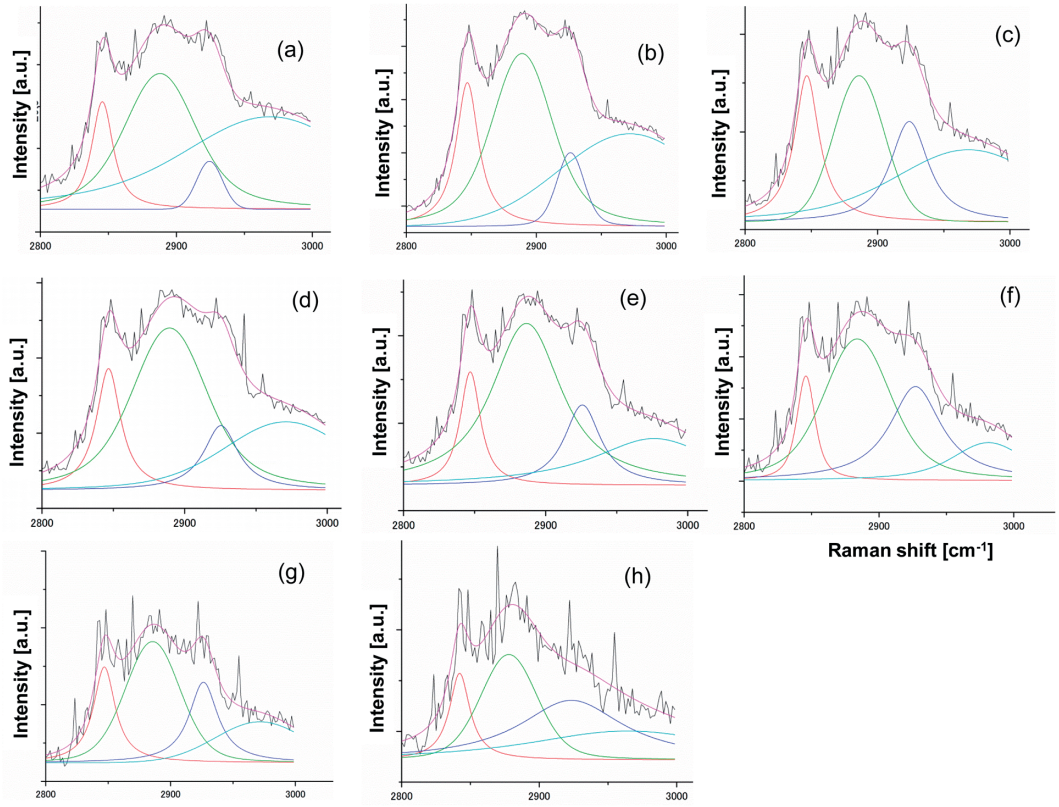


Fig. 3：Temperature dependence of Raman spectra of DEPC liposome on the methylene C-H stretching mode: (a) 302.7 K, atmospheric pressure, (b) 303.1 K, 0.5 MPa Xe, (c) 287.5 K, 0.5 MPa Xe, (d) 285.5 K, 0.5 MPa Xe, (e) 283.2 K, 0.5 MPa Xe, (f) 281.4 K, 0.5 MPa Xe, (g) 280.2 K, 0.5 MPa Xe, (h) 278.4 K, 0.5 MPa Xe.

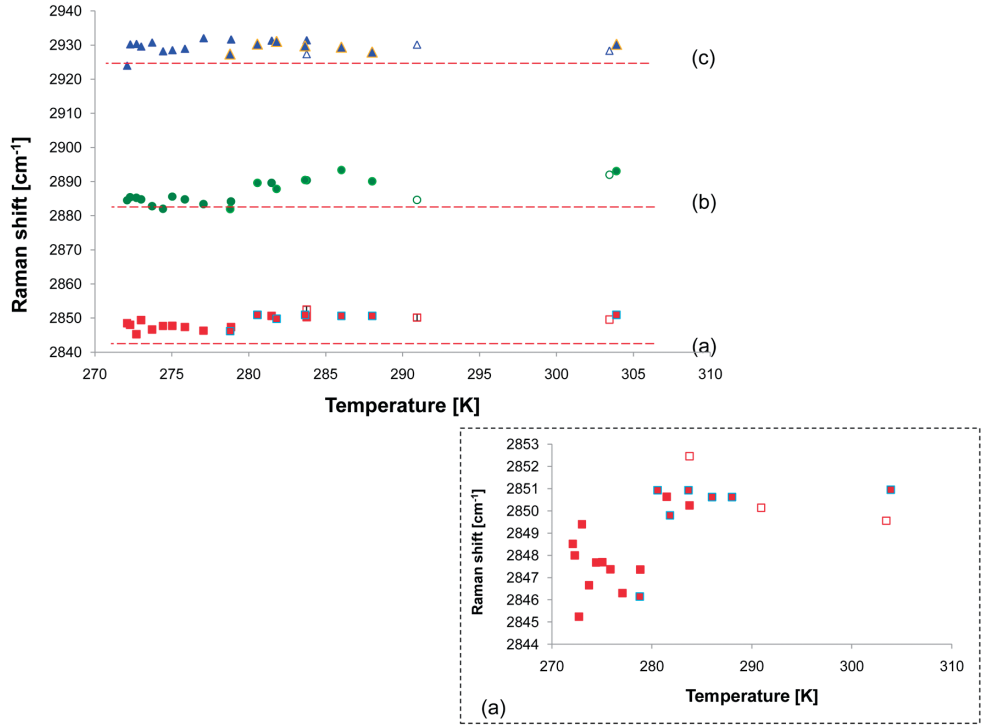


Fig. 4 : Temperature dependence of Raman peak wavenumbers of DEPC on the methylene C-H stretching mode: (a) C-H symmetric stretching mode (lowest wavenumber around 2840 $cm^{-1}$: squares), (b) C-H anti-symmetric stretching mode (middle wavenumber around 2880 $cm^{-1}$: circles), and (c) terminal $CH_3$ symmetric stretching mode (highest wavenumber around 2920 $cm^{-1}$: triangles). Open marks indicate the data under atmospheric pressure and solid marks are under 0.5 MPa Xe pressure. Each dashed line shows the peak position of powder DEPC. The extended figure is the enlargement of (a).

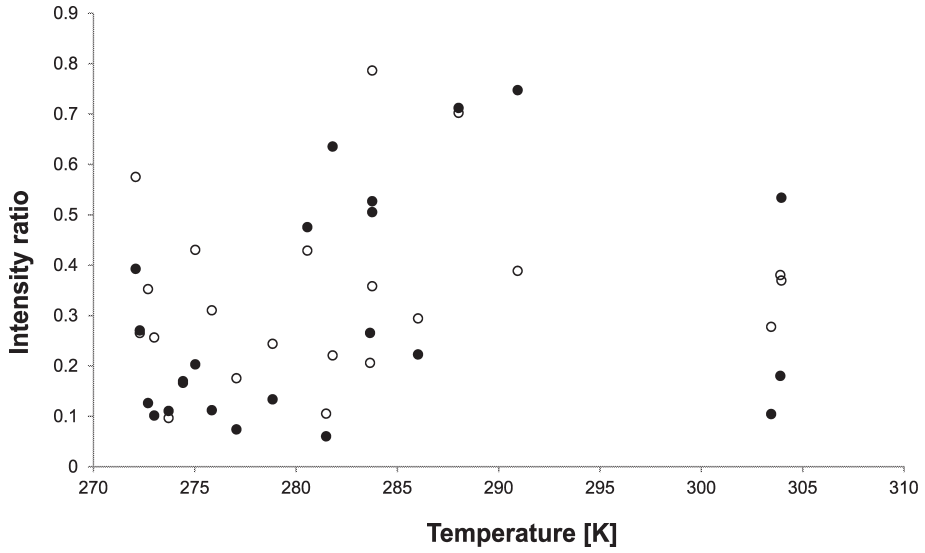


Fig. 5 : Temperature dependence of Raman peak intensities of DEPC on the methylene C-H stretching mode: (open circles) the C-H symmetric stretching mode ($I_{2840}$) / the C-H anti-symmetric stretching mode ($I_{2880}$), and (solid circles) the terminal $CH_3$ symmetric stretching mode ($I_{2920}$) / the C-H anti-symmetric stretching mode ($I_{2880}$).

The peak position of the three methylene C-H stretching modes shifted to lower wavenumbers and the peak intensity of the C-H anti-symmetric stretching mode became larger below the phase transition temperature. These spectra change suggested the mobility change of the lipid molecules in the liposome. These Raman spectra changes qualitatively coincided to other lipids such as 1,2-di- *O*-hexadecyl-*sn*-glycero-3-phosphocholine (DHPC) (Levin, et al., 1985), 1-stearoy-2-capryl-*sn*- glycero-3-phosphocoline (C(18): C(10)PC) (Batenjany, et al., 1994), 1-stearoy-2-capryl-*sn*-glycero-3- phospho-*N*-trimethylpropanolamin

(C(18):C(10) TMPC) (Batenjany, et al., 1994), and 1,2-dipalmitoyl-*sn*-glycero-3-phosphocoline (DPPC) (Fox, et al., 2007). This mobility change was maintained to lower temperatures than the normal liposome due to the existence of Xe molecules in the lipid bilayers.

Based on the HP-DSC measurements (Booker and Sum, in press), the phase transition temperature would be approximately 280 K. This temperature is in good agreement with that observed in the present study.

## 4. Conclusions

Raman spectroscopic analysis was applied to observe the phase transition of DEPC liposome under pressurized Xe conditions. The detailed measurements of the methylene C-H stretching mode spectra revealed that the phase transition temperature was shifted to lower temperature, approximately 280 K, under 0.5 MPa Xe pressure. This temperature is consistent with that estimated by another study with HP-DSC measurements.

This study showed several technical achievements, demonstrating the challenge in quantifying the biophysical changes to liposomes. In particular, we were able to obtain accurate peak analysis from low S/N spectra and accurate temperature and pressure controlling and monitoring on the sample. This experimental approach has the advantage of combining the microscopic scale of the molecular motions suggested by molecular dynamics simulations and the mesoscopic scale of lipid activity changes from molecular vibration.

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