# Direct measurement of the Transbilayer Movement of Phospholipids by Sum-frequency vibrational spectroscopy

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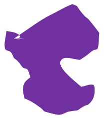
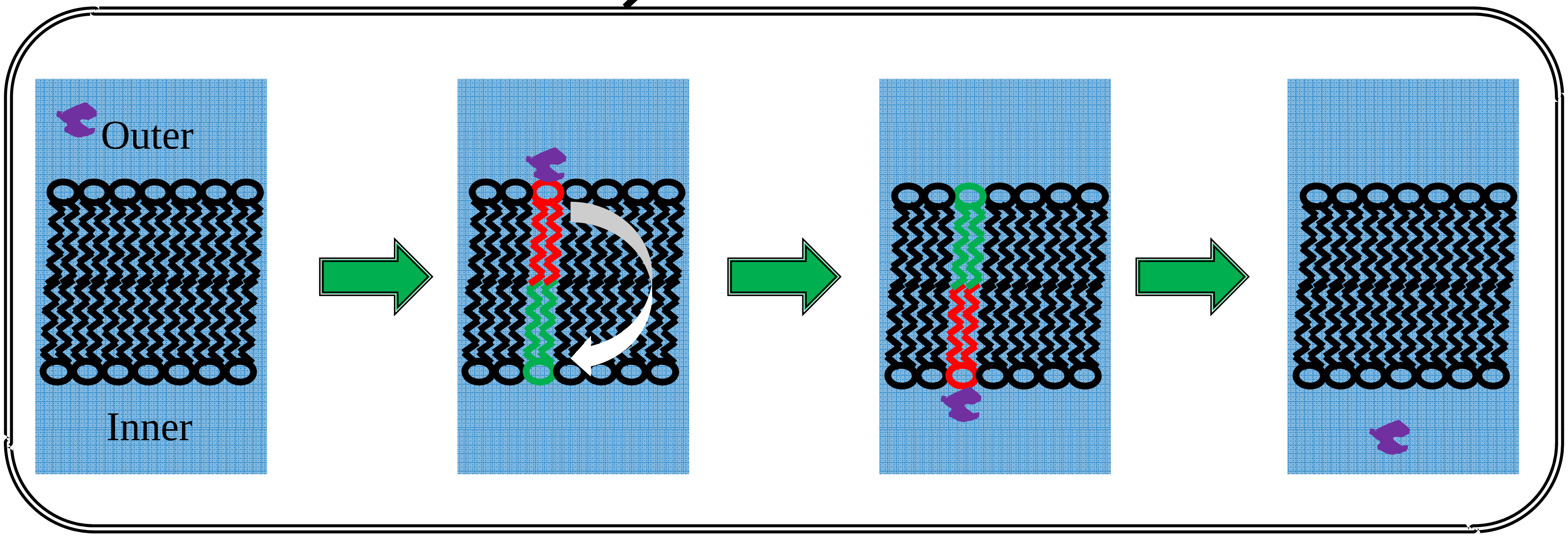
# Motivation – Translocation of lipid : Important in biological processes.



**Bio molecules : ex) proteins**

**Lipid : Amphiphilic**

**Bio molecule can get into the inner space by translocation between two lipids**



Translocation processes – Spontaneous, not affected by adsorption of molecule

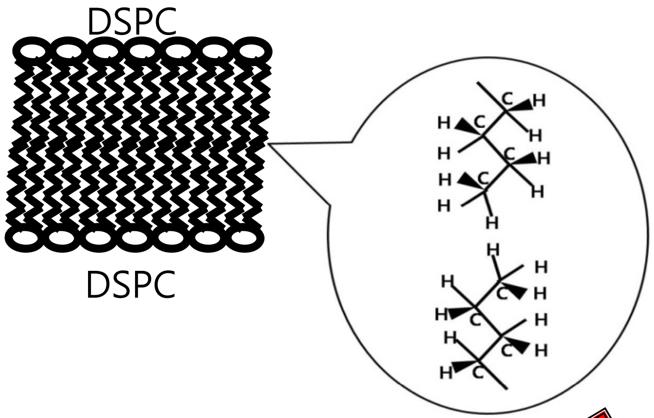
**Translocation by lipid membrane itself was studied by NMR, fluorescence and capacitance measurement.**

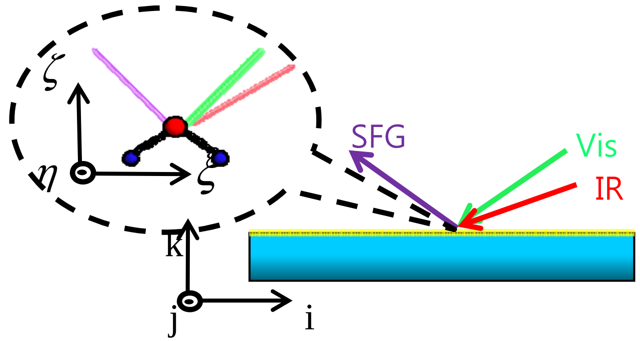
**Not surface specified , possibility of environment change by chemical treatment……**

**Vibrational Sum-frequency generation spectroscopy will be good candidate to observe the lipid flip-flop by translocation…..**

But how to ???

# SFG – Using SFG signal is forbidden in inversion symmetry

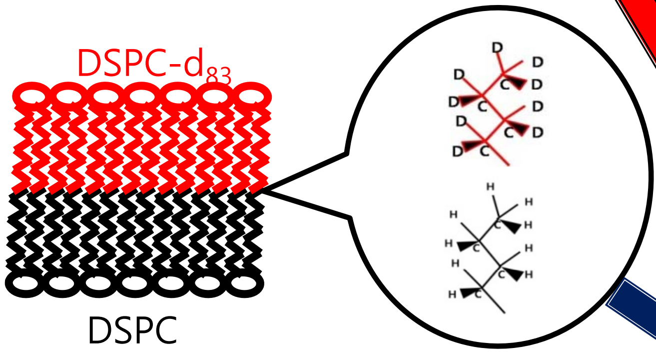



Summation of directional cosine average

$$\chi^{(2)}_{ijk} = N_s \sum_{\xi,\eta,\zeta} \alpha^{(2)}_{\xi\eta\zeta} < (\hat{\xi}\cdot\hat{i})(\hat{\eta}\cdot\hat{j})(\hat{\zeta}\cdot\hat{k}) >$$

$$, \alpha^{(2)}_{\xi\eta\zeta} = \alpha^{(2)}_{NR} + \sum_q \frac{\alpha_q}{\omega_{IR} - \omega_q + i\Gamma_q}$$

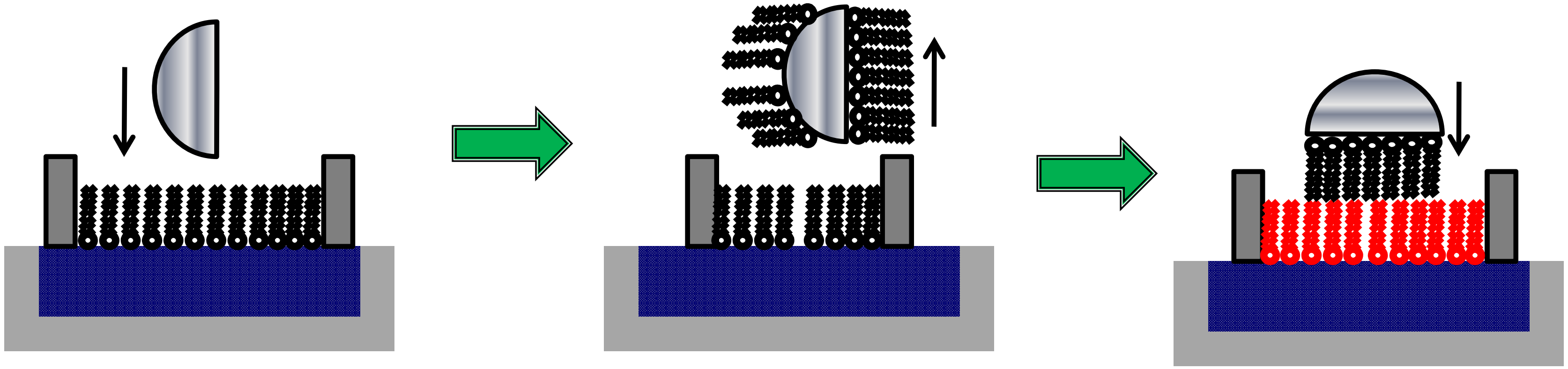


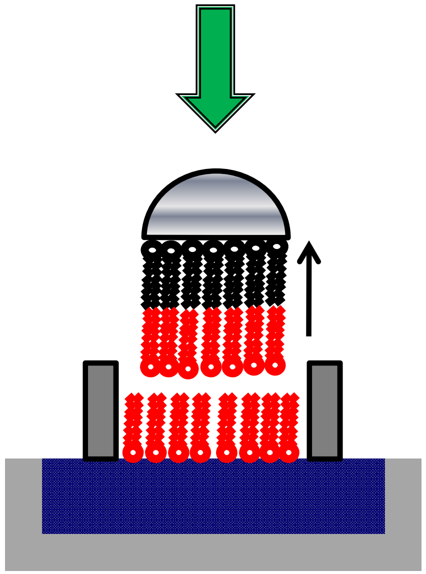

$$I_{SFG} \propto \overleftrightarrow{\chi}^{(2)} : \vec{E}\vec{E} = 0 \quad , \quad \because \overrightarrow{\chi}^{(2)} = 0$$

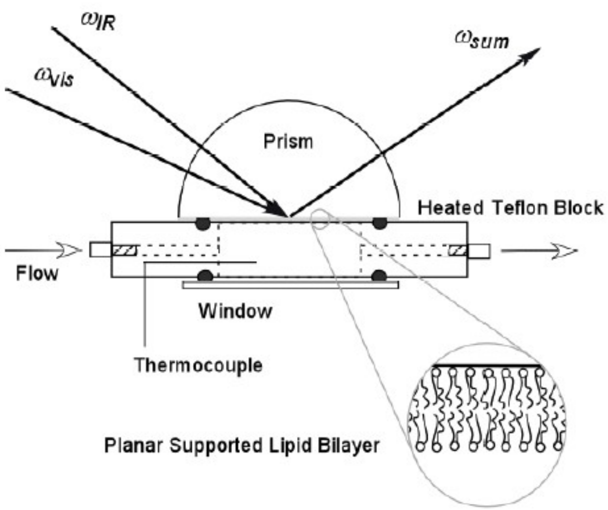
$$I_{SFG} \propto \overleftrightarrow{\chi}^{(2)} : \vec{E}\vec{E} \neq 0 \quad , \quad \because \overrightarrow{\chi}^{(2)} \neq 0$$

Translocation of lipid in asymmetric bilayer makes $I_{CH3ss}$ lower

# Experimental setup & sample preparation





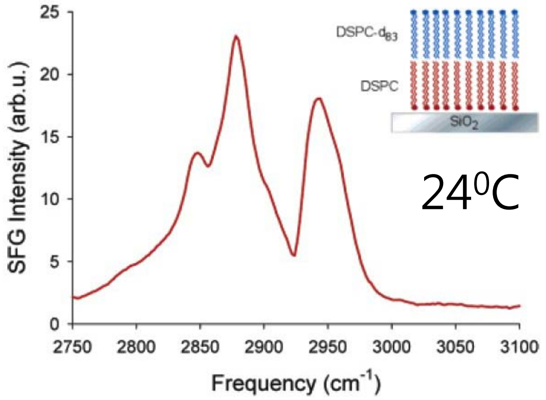



Deposite bilayer on IR grade Quartz

Heated by water jacket

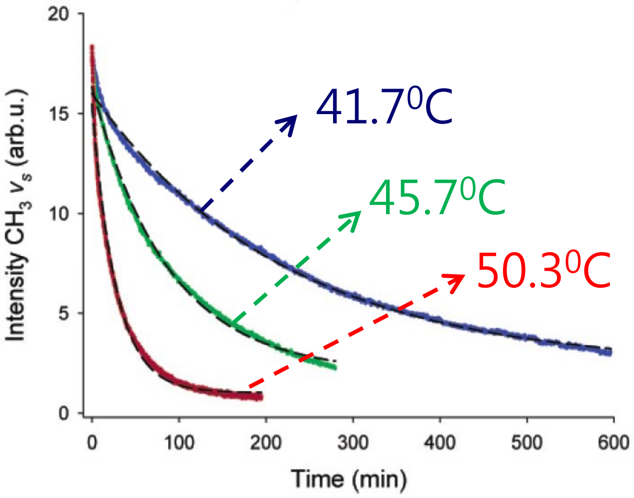
Incident angle of IR ,Vis
: critical angle of internal reflection

# Result - SSP SFG spectra of bilayer




Initially asymmetric bilayer : $CH_x$ spectra is clear and same as monolayer case.

Fixing the IR wavenumber at 2875cm$^{-1}$ ($CH_{3ss}$), they checked the intensity decay in various temperature below $T_m$.




**Decaying rate increased with temperature.**

# Does the translocation contribute such an SFG intensity decaying???

1) Check the heating effect by IR absorption

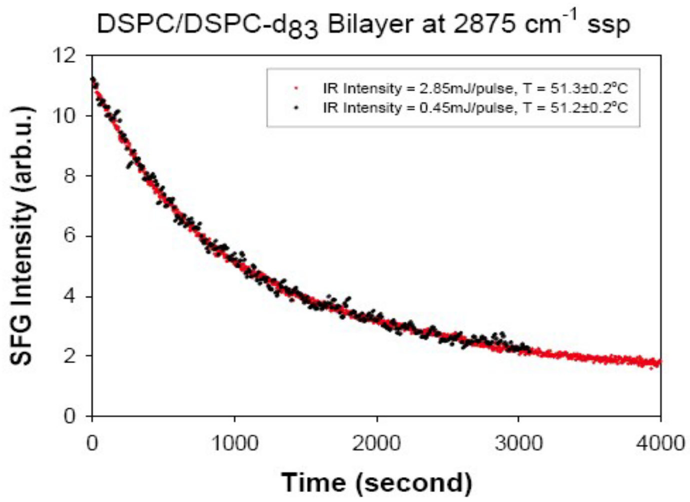



**Figure 2.** The effect of IR intensity on the measured $CH_3$ $\nu_s$ intensity decay, red points were recorded at an IR intensity of 2.85 mJ/pulse and black points at 0.45 mJ/pulse.

2) Check the structural change by temperature
& signal conversing to mixed state

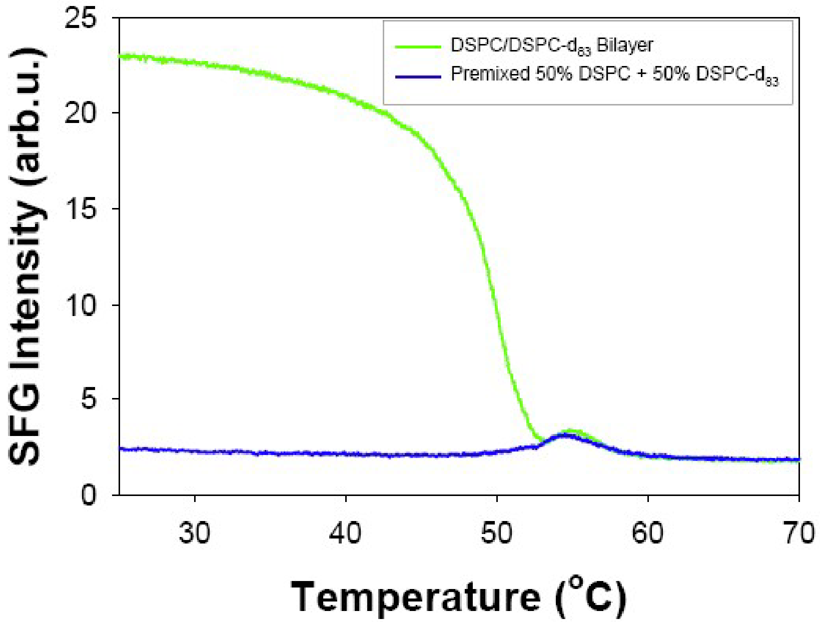
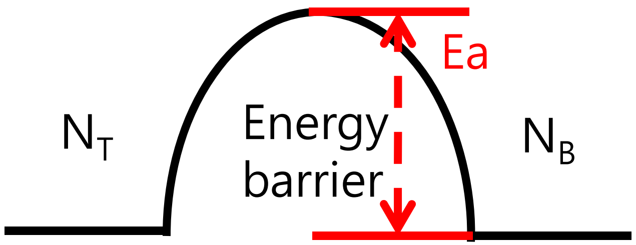



**Figure 3.** Temperature dependent response of the $CH_3$ $\nu_s$ intensity for an asymmetric bilayer composed of monolayer of DSPC-$d_{83}$ on top of a monolayer of DSPC on a fused silica support (green), and the response from a premixed 50% DSPC + 50% DSPC-$d_{83}$ bilayer. Both curves were recorded at a scan rate of 0.2°C/min.

# Model - Reaction model of translocation (Arrhenius behavior)

$N_T \underset{k_-}{\overset{k_+}{\rightleftharpoons}} N_B$ $N_T$ , $N_B$ : fractions of DSPC molecules locate at top or bottom

➡ Unimolecular processes : energy of two states are same. $K_+ = K_-$




➡ In Arrhenius behavior, reaction rate k increase with temperature, because high temperature give more chance to molecules to jump over the activation energy, Ea

$$k = Ae^{-Ea/RT}$$

Solving the dynamic equation of this processes with I.C. : $N_B = 1$

$$\frac{dN_B}{dt} = k_+ N_T - k_- N_B = -k(2N_B - 1) \longrightarrow (2N_B - 1) = e^{-2kt}$$

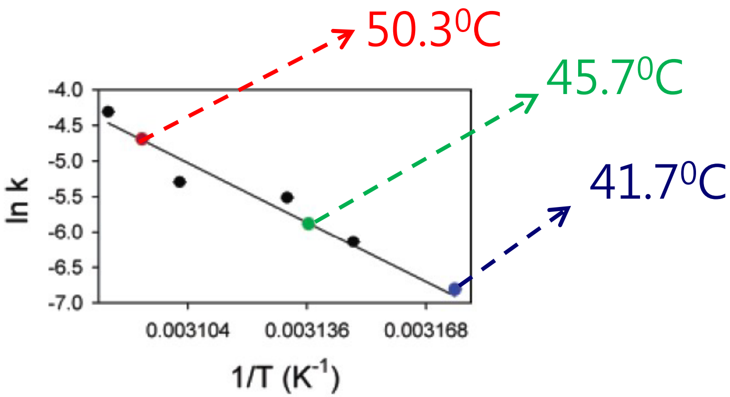
# Fitting with model

Relate to intensity of SFG,

$$I^{SFG}_{CH3_{ss}} \propto (N_T - N_B)^2 = (2N_B - 1)^2 = e^{-4kt}$$

$$I^{SFG}_{CH3_{ss}} = I_{max} e^{-4kt} + I_{nr}$$

$$t_{1/2} = \frac{\ln 2}{2k}$$ Half-life time of intensity




$$\ln k = \ln A - \frac{E_a}{R}\left(\frac{1}{T}\right) \Leftrightarrow k = Ae^{-\frac{E_a}{RT}}$$

***Table 1.*** Temperature-Dependent Rate Constants and $t_{1/2}$ for the Exchange for a DSPC Bilayer

| temp (°C) | rate $k$ ($\times 10^{-3}$ min$^{-1}$)[a] | $t_{1/2}$ (min) |
|---|---|---|
| $41.7 \pm 0.3$ | 1.11 | 312 |
| $44.5 \pm 0.3$ | 2.16 | 160 |
| $45.7 \pm 0.3$ | 2.80 | 124 |
| $46.3 \pm 0.4$ | 4.03 | 86.0 |
| $49.2 \pm 0.2$ | 5.01 | 69.2 |
| $50.3 \pm 0.1$ | 9.10 | 38.1 |
| $51.3 \pm 0.2$ | 13.4 | 25.9 |

[a] Error is estimated at less than 5%.

$E_a = 206 \pm 18 KJ/mol$ High energetic cost

Because hydrophilic head should move to hydrophobic core

## Conclusion & Summary

- By SFG, spontaneous translocation in lipid bilayer was monitored.

- Intensity decaying tendency followed simple Arrhenius model, and It is well fitted.

- From the fitting, calculated activation energy, $E_a$ is about 206kJ/mol And it can be expected that $t_{1/2}$ = 18days in room temperature

At 36.5C$^0$, $t_{1/2}$ = 18hours