# Lateral Pressure Dependence of the Phospholipid Transmembrane Diffusion Rate in Planar-Supported Lipid Bilayers

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# Introduction

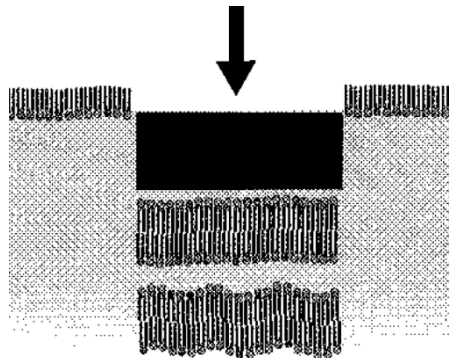Transbilayer migration of lipids in membranes.

- Investigation of the lateral pressure-dependent kinetics and
  thermodynamics of native lipid flip-flop
  using sum-frequency vibrational spectroscopy (SFVS)

# Langmuir-Blodgett / Langmuir Schaeffer Depositionc

Langmuir-Blodgett method → monolayer
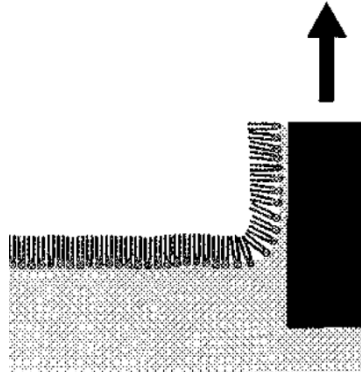
Langmuir-Schaeer method → bilayer

Planar supported lipid bilayer(PSLB) membranes

- controlling the lateral surface pressure

- packing density of the lipid film
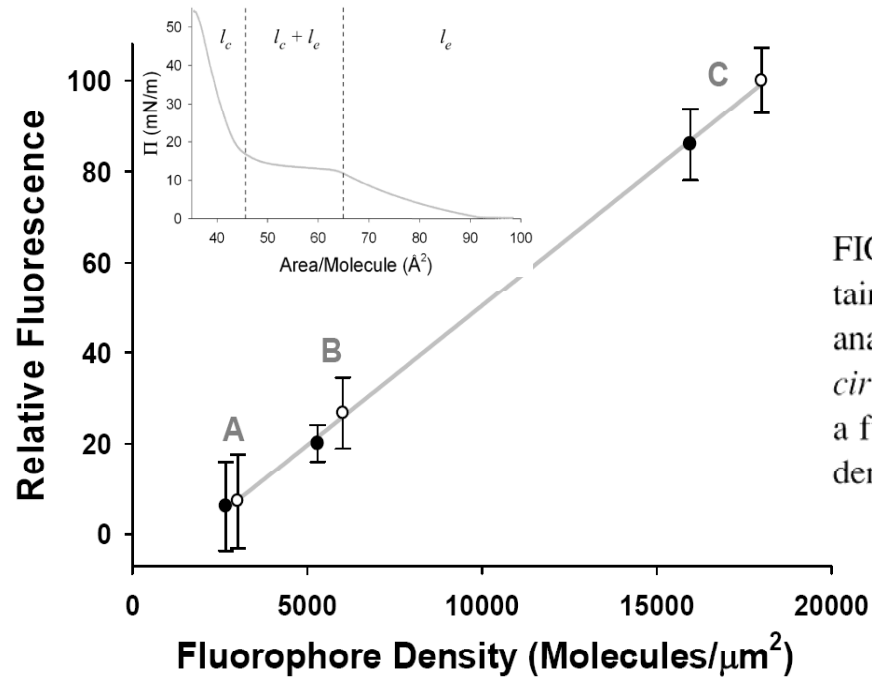
- forming asymmetric bilayers

FIGURE 1  Relative fluorescence intensity for DPPC monolayers containing either 0.12 (*A*), 0.23 (*B*), or 0.69 mol % (*C*) of an NBD labeled analog. For each concentration, samples were prepared at 20 mN/m (*solid circles*) and 40 mN/m (*open circles*), with the observed intensities shown as a function of calculated fluorophore surface density (see Fig. 3 for surface density/pressure data).

model No. BX4 microscope (Olympus, Melville, NY) with a 10x objective using epi-illumination and collection

control of film packing density and lipid composition
→ the LB method.

the amount of material deposited on the substrate

$\|$ equal

the surface coverage at the air-water interface
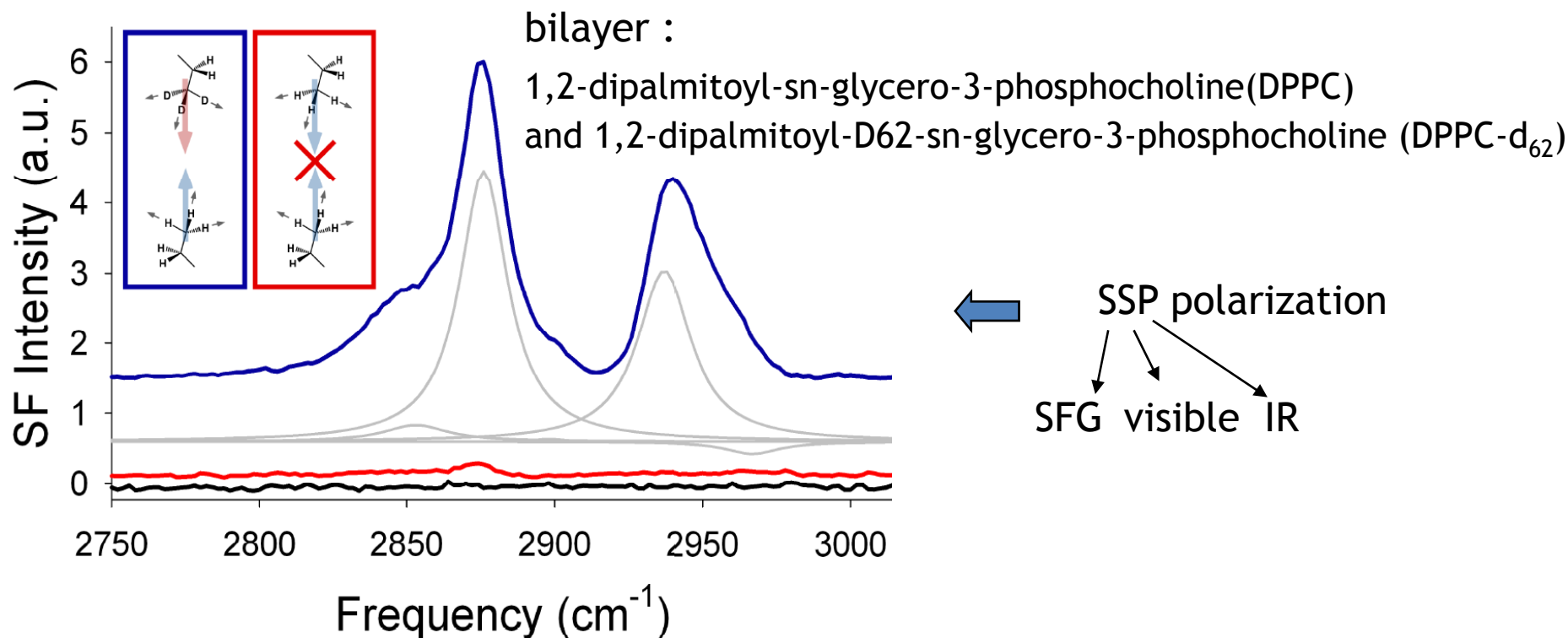
# Sum−frequency vibrational spectroscopy



bilayer :

1,2-dipalmitoyl-sn-glycero-3-phosphocholine(DPPC)

and 1,2-dipalmitoyl-D62-sn-glycero-3-phosphocholine (DPPC-$d_{62}$)

SSP polarization

SFG  visible  IR

FIGURE 2  A SFVS spectrum of an asymmetric bilayer of DPPC (proximal layer) and DPPC-$d_{62}$ (distal layer) at 23°C is shown in blue. Individual peaks corresponding to the $CH_2$ $\nu_s$ (2850 cm$^{-1}$), $CH_3$ $\nu_s$ (2876 cm$^{-1}$), $CH_2$ Fermi resonance (2900 cm$^{-1}$), $CH_3$ Fermi resonance (2937 cm$^{-1}$), and $CH_3$ asymmetric stretch ($\nu_{as}$) (2967 cm$^{-1}$) are shown in gray, determined by fitting the spectrum to Eq. 4 (44). A SFVS spectrum of the same bilayer after heating to 50°C for several minutes and subsequently cooling to 23°C (red). For comparison, the SFVS spectrum of a symmetric bilayer consisting of 1:1 DPPC/DPPC-$d_{62}$ recorded at 23°C (black) is also shown. Spectra are offset for clarity. (Inset) Illustration of dipole cancellation for opposed methyl groups in a symmetric lipid bilayer and the absence of dipole cancellation for an asymmetric bilayer in which one leaflet is deuterated.

The susceptibility x$^{(2)}$ :

narrow distribution of orientations

$$\chi^{(2)} = \chi_R^{(2)} + \chi_{NR}^{(2)},$$

$$\chi_R^{(2)} = \sum_\nu \frac{N\langle A_i M_{jk}\rangle}{\omega_\nu - \omega_{IR} - i\Gamma_\nu}$$

$$\implies \chi^{(2)} = \frac{N_{distal}}{\varepsilon_0} < \beta^{CH_3\nu_s} > - \frac{N_{proximal}}{\varepsilon_0} < \beta^{CH_3\nu_s} >$$

number of molecules    hyperpolarizability

The interference of the CH$_3$ v$_s$ may be exploited to examine the time-dependent asymmetry of a lipid bilayer

The relationship between
the time-dependent asymmetry of the bilayer and the observed SFVS
intensity :

$$I_{CH_3}(t) \propto (N_{Distal} - N_{Proximal})^2$$

The kinetics of lipid translocation can be determined from the time evolution of the SFVS $CH_3$ $v_s$ intensity using

$$I_{CH_3}(t) = I_{Max}e^{(-4kt)} + I_o$$

Where 

$I_{max}$ : maximum $CH_3$ vs intensity,
k : rate constant for flip-flop,
t : time in seconds, $I_o$ : baseline offset

# MATERIALS AND METHODS

Materials：： 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC)

1,2-dipalmitoyl-D62-sn-glycero-3-phosphocholine (DPPC-d$_{62}$)

Dissolvent： CHCl$_3$ (1 mg/mL)

Substrate : Hemicylindrical fused silica prisms

Bilayer samples were prepared on a Minitrough (KSV Instruments, Helsinki, Finland)

SFVS experiments were conducted using a custom OPO/OPA system (LaserVision, Bellevue, WA) pumped by a 7-ns Surelite 1 NdYAG laser (Continuum, Santa Clara, CA) at 10 Hz repetition rate.
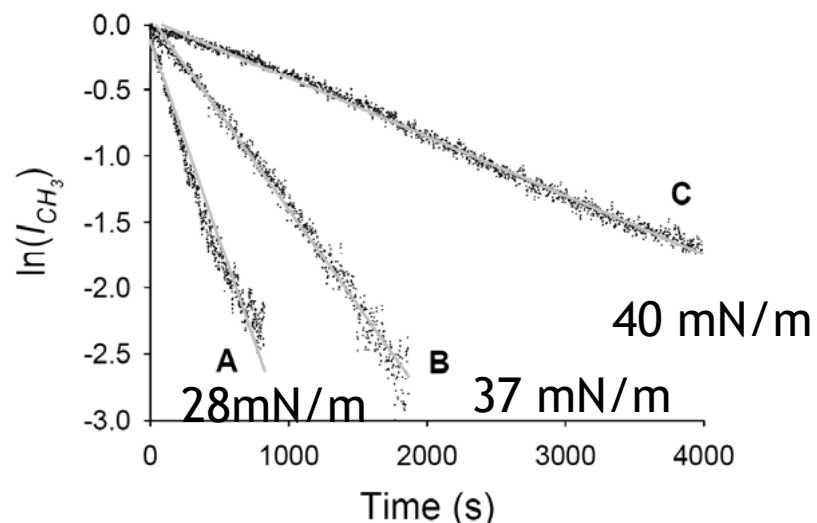
A surface pressure/area isotherm for DPPC at 23°C.
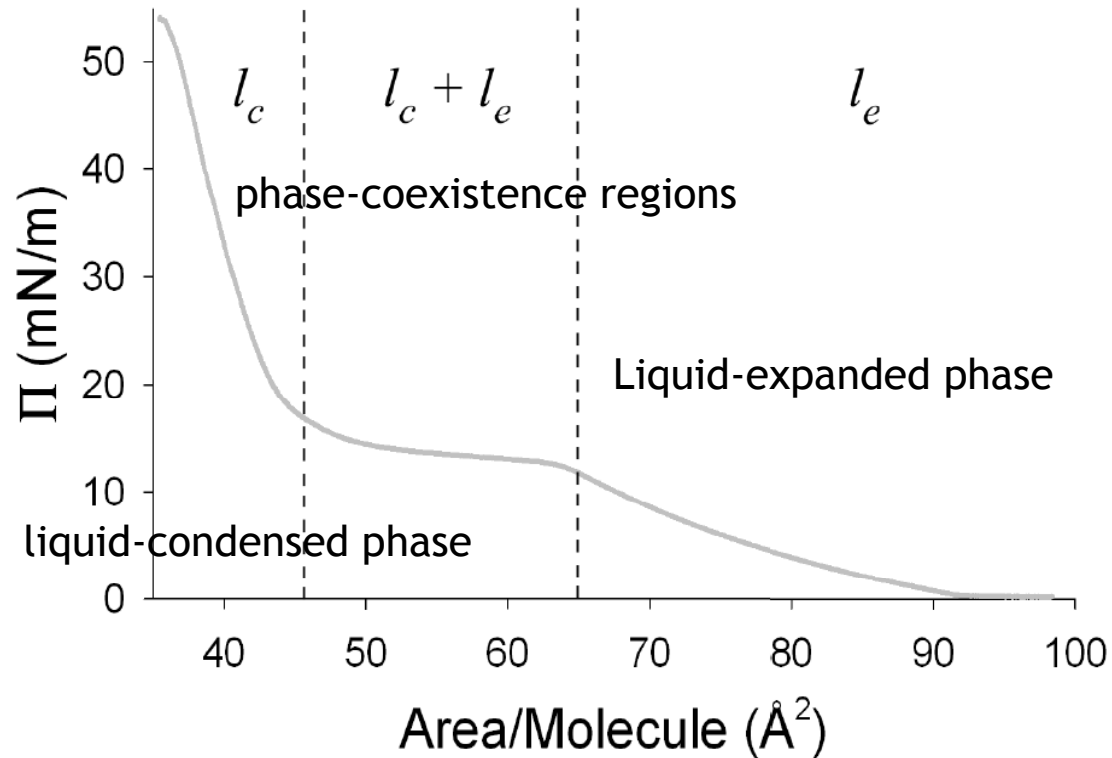


samples were prepared in the $l_c$ phase

Sequential deposition of DPPC and DPPC-$d_{62}$ on a hemicylindrical fused silica prism using the LB/LS method.

Sample pressure-dependent SFVS $CH_3$ $\nu_s$ intensity decays measured at 2876 cm$^{-1}$ for asymmetric DPPC/DPPC-d$_{62}$ bilayers recorded at 37C.



Decay rates and half-lives as a function of deposition pressure

| $\Pi$ (mN/m) | $k$ (s$^{-1}$) x 10$^4$ | $\ln(k)$ | t$_{1/2}$ (min) |
|---|---|---|---|
| 28 | 10.93 $\pm$ 0.12 | -6.82 $\pm$ 0.07 | 5.28 $\pm$ 0.06 |
| 31 | 5.61 $\pm$ 0.07 | -7.49 $\pm$ 0.09 | 10.3 $\pm$ 1.3 |
| 34 | 7.47 $\pm$ 0.06 | -7.20 $\pm$ 0.06 | 7.73 $\pm$ 0.06 |
| 36 | 2.02 $\pm$ 0.01 | -8.51 $\pm$ 0.04 | 28.7 $\pm$ 0.6 |
| 37 | 3.22 $\pm$ 0.02 | -8.04 $\pm$ 0.05 | 17.9 $\pm$ 0.1 |
| 40 | 1.31 $\pm$ 0.01 | -8.94 $\pm$ 0.07 | 44.1 $\pm$ 0.3 |
| 42 | 1.03 $\pm$ 0.01 | -9.18 $\pm$ 0.09 | 56.0 $\pm$ 0.5 |

Applying the concepts of

transition state theory (TST) to the process of flip-flop

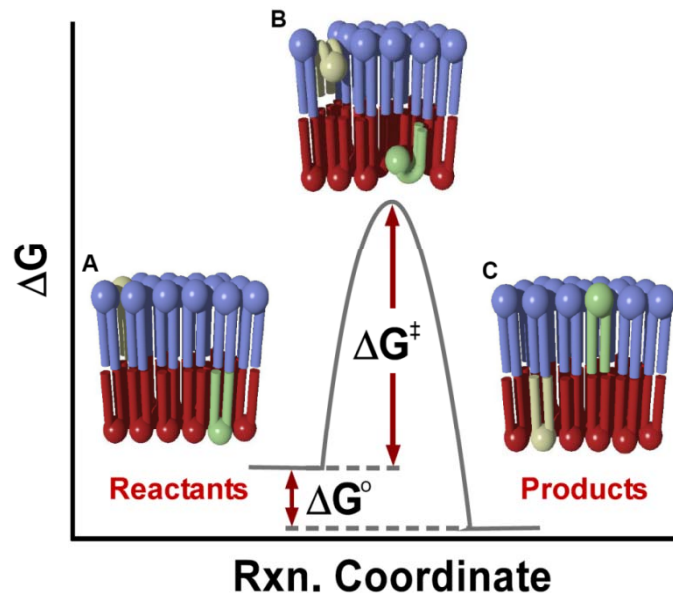( tool for the thermodynamic analysis of phospholipid flip-flop)



FIGURE 5   A general reaction coordinate diagram illustrating free energy as a function of reaction progress. Also shown are a series of illustrations depicting the translocation of a lipid starting with the initial state ($A$), followed by the transition state for phospholipid flip-flop ($B$), and ending in the final state after exchange ($C$). For the case of phospholipid flip-flop, $\Delta G^0 = \Delta H^0 - T\Delta S^0$ will reduce to $\Delta G^0 = -T\Delta S^0$, as there is no enthalpic driving force for mixing of the phospholipid components. This depiction is not meant to suggest cooperativity across the leaflets.

lipid molecules on the proximal leaflet of the bilayer may be thought of as ground-state reactants which must cross an energetic barrier via a flip-flop transition state to reach the distal leaflet (analogous to conversion to products)

It is well known that investigation of the pressure dependence of a reaction rate allows one to measure an activation volume ($\Delta V^{\ddagger}$) for a reaction

$$-RT\left(\frac{\partial \ln(k)}{\partial P}\right)_{T} = \Delta V^{\ddagger}$$

$\Delta V^{\ddagger}$ : the difference in volume occupied by the reactants in their ground state and at the transition state.
k : the rate constant for the reaction.
T : temperature.
P : pressure.
R : the gas constant.

For a two-dimensional film,

an activation area ($\Delta a^{\ddagger}$): $-RT\left(\frac{\partial \ln(k)}{\partial \Pi}\right)_{T} = \Delta a^{\ddagger}$
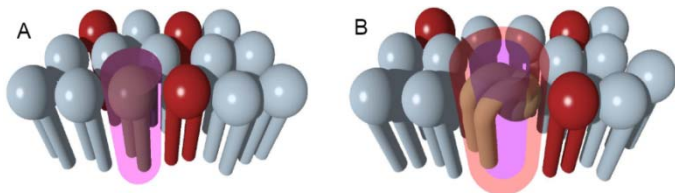


FIGURE 6 An illustration of the activation area for flip-flop according to one possible transition-state geometry. The area occupied in the ground state is shown in purple (A), with the net area at the transition state shown in pink (B). The difference between the two areas represents the activation area for phospholipid flip-flop.
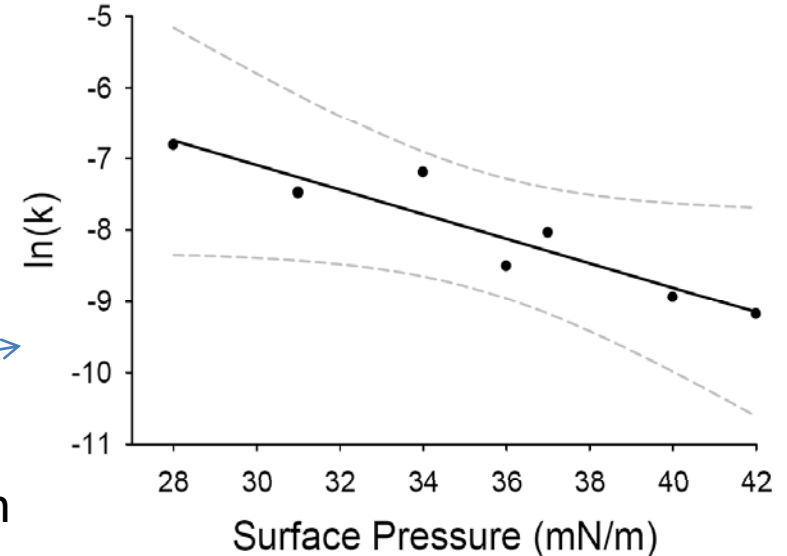
total area at the transition state:

$$a\ddagger = a_{initial} + \Delta a\ddagger,$$

where    $a\ddagger$ : the total area per molecule at the transition state
        $a_{initial}$ :  the initial area in the ground state.

FIGURE 7    A plot of $\ln(k)$ versus surface pressure ($\Pi$) for DPPC/DPPC-$d_{62}$ bilayers at 37°C. Also shown is the linear least-squares fit to the data (*solid line*) and confidence bounds at 95% (*shaded dashed line*).



From the slope of the resulting line, the activation area for flip-flop of DPPC at 37ºC in a PSLB is calculated to be 73 $\pm$ 12 A$^{\circ 2}$/molecule.

activation area  is 73 $\pm$ 12 A$^{\circ 2}$/molecule
initial equilibrium area is ~ 40 A$^{\circ 2}$/molecule

At the transition state,
the area is 113 $\pm$ 12 A$^{\circ 2}$/molecul

# Conclusion

- To our knowledge, this is the first report of an activation area for phospholipid flip-flop.

  - Shows promise
    For the study of lateral pressure-dependent kinetics and thermodynamics in a wide number of model lipid systems.

  - The results presented here open the way for a complete thermodynamic description of the transition state for flip-flop in planar-supported membranes

  - it is our hope that
    a general thermodynamic description of phospholipid flip-flop may be achieved