**SUPPORTING INFORMATION for**

# $^1$H NMR Shows Slow Phospholipid Flip-Flop in Gel and Fluid Bilayers

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**Section S1: Materials**

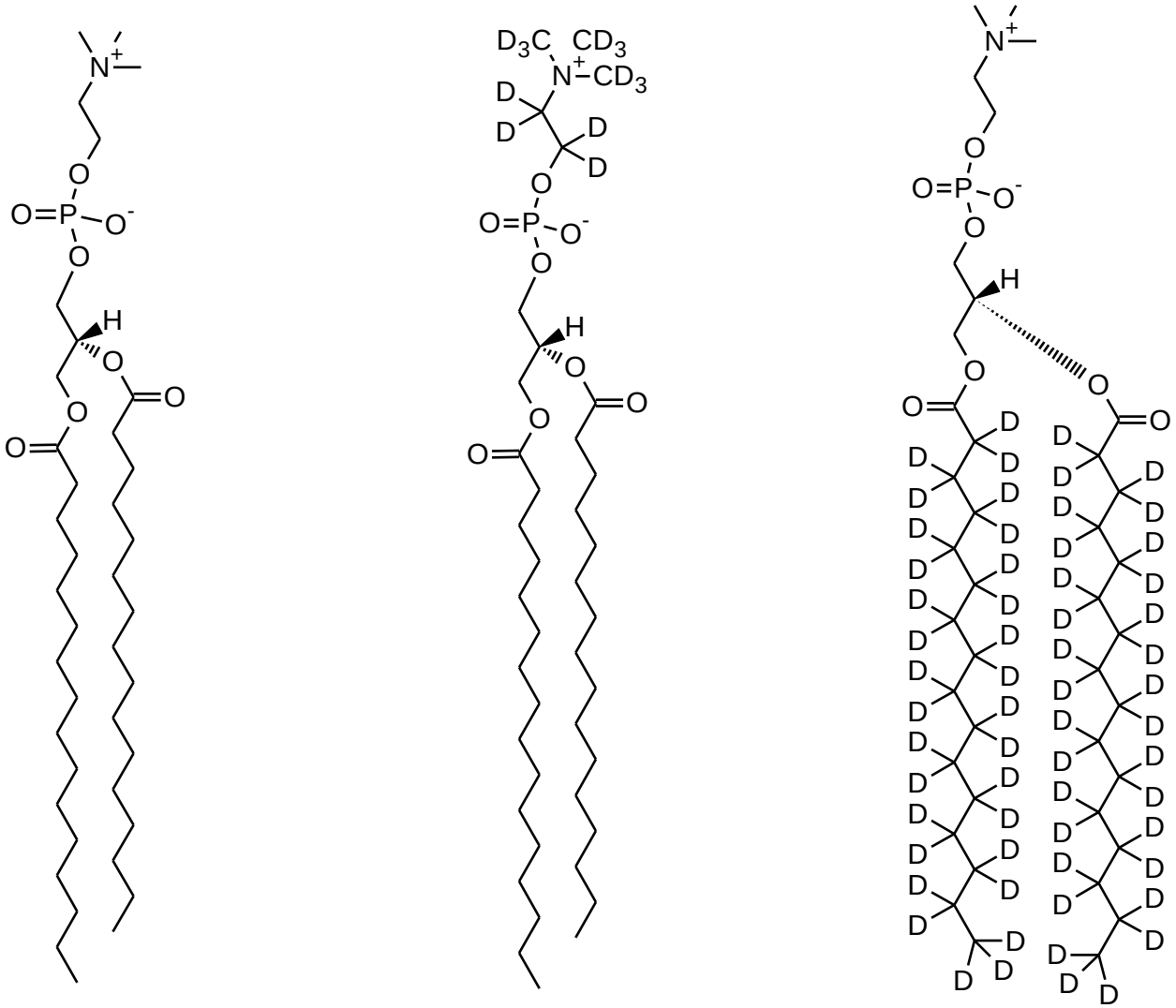



**Figure S1:** Chemical structures of DPPC and the deuterated variants used in this study. The label underneath each lipid shows our naming convention for this manuscript where DPPC is protiated, DPPC-dH is headgroup deuterated, and DPPC-dC is chain perdeuterated.

## Section S2: Differential Scanning Calorimetry

**Table S1**: Pre-transition ($T_p$, $L_{\beta'} \rightarrow P_{\beta'}$) and main transition ($T_M$,$P_{\beta'} \rightarrow L_\alpha$) temperatures and transition widths ($T_{1/2}$) determined by differential scanning calorimetry on MLVs.

| | $L_{\beta'} \rightarrow P_{\beta'}$ | | $P_{\beta'} \rightarrow L_\alpha$ | |
|---|---|---|---|---|
| **Lipid** | **$T_p$ [°C]** | **$T_{1/2}$ [°C]** | **$T_m$ [°C]** | **$T_{1/2}$ [°C]** |
| DPPC | 35.5 | 2.4 | 42.0 | 0.7 |
| DPPC-dH | 34.7 | 2.1 | 41.8 | 0.5 |
| DPPC-dC | 32.2 | 2.7 | 37.8 | 0.4 |

**Table S2**: Pre-transition ($T_p$, $L_{\beta'} \rightarrow P_{\beta'}$) and main transition ($T_m$,$P_{\beta'} \rightarrow L_\alpha$) temperatures and transition widths ($T_{1/2}$) of an aLUV composed of DPPC-dH enriched inner leaflet and a DPPC-dC-enriched outer leaflet. Parameters were determined by modeling $T_p$ with a single Gaussian and modeling $T_m$ with 3 Gaussians.

| | $L_{\beta'} \rightarrow P_{\beta'}$ | | $P_{\beta'} \rightarrow L_\alpha$ | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Lipid** | **$T_p$ [°C]** | **$T_{1/2}$ [°C]** | **$T_m$ [°C]** | **$T_{1/2}$ [°C]** | **$T_m$ [°C]** | **$T_{1/2}$ [°C]** | **$T_m$ [°C]** | **$T_{1/2}$ [°C]** |
| DPPC | 36.0 | 7.1 | 39.4 | 1.7 | 40.7 | 1.3 | 41.8 | 1.8 |

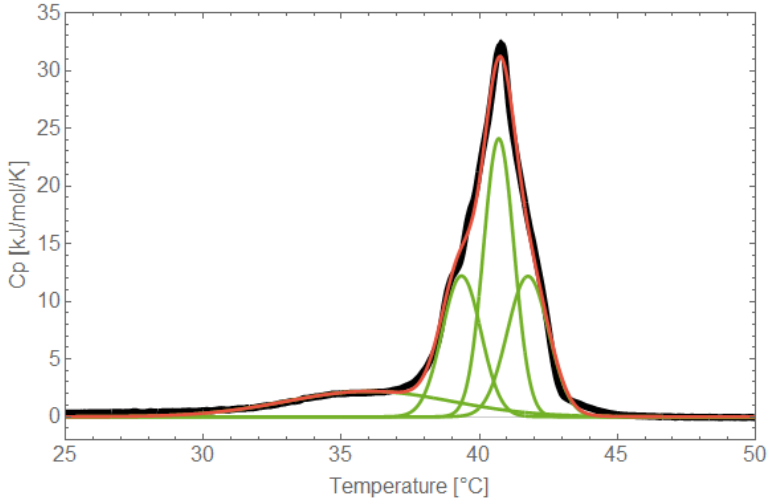



**Figure S2:** DSC exotherm for aLUV with a DPPC-*dH*-enriched inner leaflet and a DPPC-*dC*-enriched outer leaflet. Shown are the experimental exotherm (black line) and fit (red line) as a sum of four Gaussians (green).

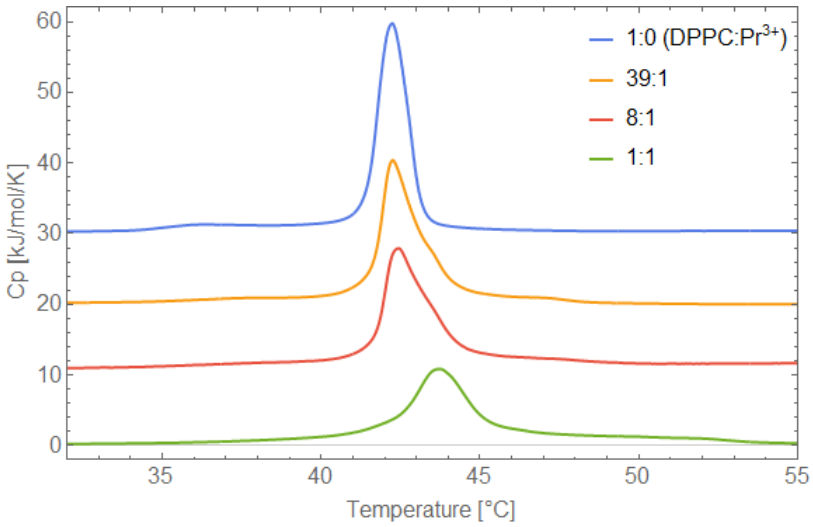



**Figure S3:** DSC exotherms for DPPC LUVs with increasing $Pr^{3+}$ concentration (from top to bottom): 0 mM, 0.08 mM, 0.08 mM and 1.5mM. Thermograms are offset for clarity.

**Section S3: Thermodynamic parameters.** To better understand the different thermodynamic contributions to $E_a$, we used transition state theory[1,2] to determine free energy of activation ($\Delta G^{\ddagger}$), enthalpy of activation ($\Delta H^{\ddagger}$) and entropy of activation ($\Delta S^{\ddagger}$) from $E_a$. The results of these calculations are summarized in Table S3, which includes previously reported values obtained in SSBs.[3]

**Table S3**: Thermodynamic quantities for DPPC translocation. All units are kJ $mol^{-1}$.

| **Phase** | **Geometry** | **$\Delta G^{\ddagger}$** | **$\Delta H^{\ddagger}$** | **$T\Delta S^{\ddagger}$[a]** | **$E_a$** |
|---|---|---|---|---|---|
| $L_{\beta'}$ | SSB[b] | 100.7 ± 0.3 | 245 ± 10 | 143 ± 8 | 224 ± 9 |
| | LUV[c] | *not determined (slow translocation)* | | | |
| $L_{\alpha}$ | SSB[b] | *not determined (fast translocation)* | | | |
| | LUV[c] | 116 ± 14 | 119 ± 14 | 3 ± 0.6 | 122 ± 14 |

[a]calculated at 20˚C.; [b]solid supported bilayer data from Anglin et al.[3]; [c]100 nm large unilamellar vesicle data from this study.

**Section S4: $Pr^{3+}$ influence on lipid translocation.**

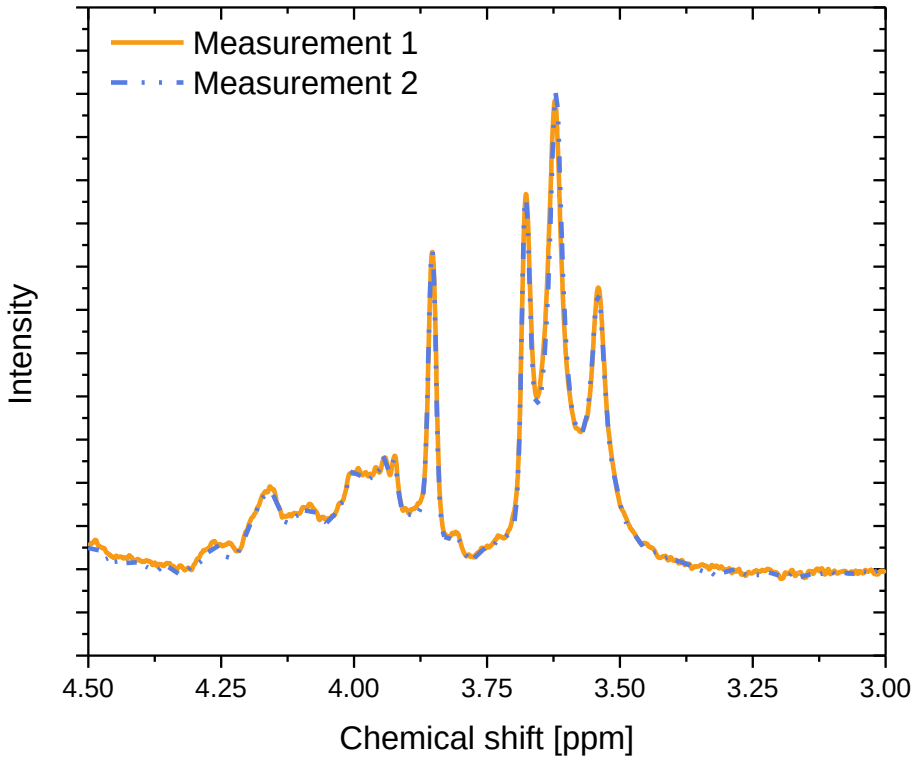
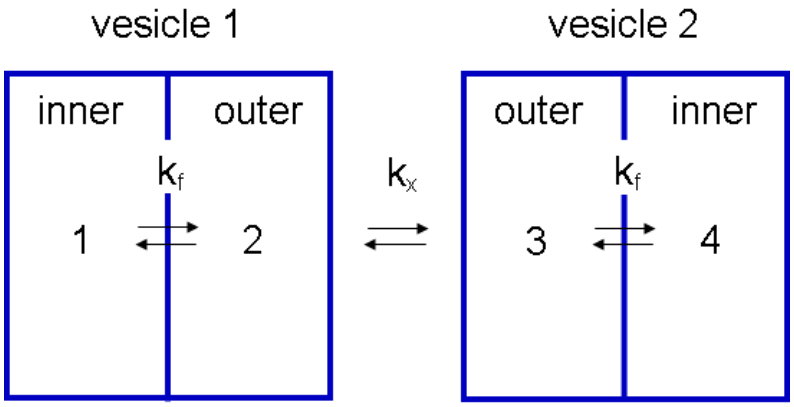



**Figure S4:** NMR spectra of DPPC-dC/DPPC-dH in the presence of 0.05 mM $Pr^{3+}$. Spectra 1 and 2 were successive measurements of the same sample, demonstrating $Pr^{3+}$ does not influence the lipid translocation over the duration of the NMR measurements.

**Section S5: Model of lipid transport.** The most general model considers two processes, namely exchange between vesicles (intervesicle lipid transport) and flip/flop (intravesicle transport). We consider four compartments corresponding to the inner and outer leaflets of *vesicle 1* (acceptor) and *vesicle 2* (donor):



Assuming that transport kinetics do not depend on isotopic variants, we write the following rate equations for the lipid concentration in each compartment:

$$dc_1/dt = -k_f c_1 + k_f c_2$$

$$dc_2/dt = k_f c_1 - (k_x + k_f) c_2 + k_x c_3$$

$$dc_3/dt = k_x c_2 - (k_x + k_f) c_3 + k_f c_4$$

$$dc_4/dt = k_f c_3 - k_f c_4$$

This is a first-order, homogeneous system of equations, rewritten in matrix form as:

$$M = \begin{bmatrix} -k_f & k_f & 0 & 0 \\ k_f & -(k_x + k_f) & k_x & 0 \\ 0 & k_x & -(k_x + k_f) & k_f \\ 0 & 0 & k_f & -k_f \end{bmatrix}$$

The characteristic equation is given by:

$$\det[M - \lambda I] = 4k_x {k_f}^2 x + (6k_x k_f + 4{k_f}^2) x^2 + (2k_x + 4k_f) x^3 + x^4$$

Further assuming that *vesicle 1* and *vesicle 2* are identical, the initial conditions for the lipid concentration in each compartment are:

$$c1 = c4 = C_{in,0}$$

$$c2 = c3 = C_{out,0}$$

Solving for the inner and outer leaflet concentrations and normalizing to the total initial concentration ($C_{in,0} + C_{out,0}$) gives:

$$C_{in}(t) = \frac{1}{2}\left[1 + \left(\frac{C_{in,0} - C_{out,0}}{C_{in,0} + C_{out,0}}\right) e^{-2k_f t}\right] \qquad (1)$$

$$C_{out}(t) = \frac{1}{2}\left[1 - \left(\frac{C_{in,0} - C_{out,0}}{C_{in,0} + C_{out,0}}\right) e^{-2k_f t}\right] \qquad (2)$$

From the initial conditions (identical *vesicle 1* and *vesicle 2*), the vesicle—vesicle exchange kinetics ($k_x$) do not contribute to the observed kinetics, as expected. The concentrations of the inner and outer leaflet lipid (given by Eqs. 1 and 2) are proportional to the inner and outer leaflet peak areas obtained from NMR, normalized to the total area.

Taking the difference in peak areas normalized to the initial difference in peak areas gives:

$$\Delta C(t) \equiv \frac{C_{out}(t) - C_{in}(t)}{C_{out}(0) - C_{in}(0)} = e^{-2k_f t}. \qquad (3)$$

The flip-flop halftime is found by setting Eq. 3 equal to 0.5 and solving for $t$, which yields:

$$\mathrm{t}_{1/2} = \frac{\ln(2)}{2k_f}. \qquad (4)$$

With measurements at multiple temperatures, an activation energy ($E_a$) can be determined from an Arrhenius plot ($\ln(k)$ vs. $1/T$) using the Arrhenius equation (Eqn. 5). The Arrhenius equation relates the rate constant $k$ to $E_a$, temperature ($T$), the gas constant ($R$) and a pre-exponential factor ($A$).

$$k_f = Ae^{-\frac{E_a}{RT}} \qquad (5)$$

Other thermodynamic quantities such as the enthalpy of formation of the activated state, ($\Delta H^{\ddagger}$); the entropy of formation of the activated state, ($\Delta S^{\ddagger}$); and the free energy of activation, ($\Delta G^{\ddagger}$) were calculated from $E_a$ (Arrhenius plot) and the following relations originating from the Eyring equation:[1,2,4]

$$\Delta H^{\ddagger} = E_a - RT$$

$$\Delta S^{\ddagger} = R \ln\left(\frac{N_A h X}{RT}\right)$$

$$X = k_f e^{\frac{\Delta H^{\ddagger}}{RT}}$$

$$\Delta G^{\ddagger} = \Delta H^{\ddagger} - T\Delta S^{\ddagger},$$

where $h$ is Planck's constant and $N_A$ is Avogadro's constant.

## References


1 Homan,R.; Pownall, H. J. *Biochim. Biophys. Acta* 1988, 938, 155-166.

2 Dickerson, R. E.; Gray, H. B.; Haight, G. P. *Chemical Principles*, 3rd ed.; Benjamin/Cummings Pub. Co: Menlo Park, Calif., 1979.

3 Anglin, T. C.; Cooper, M. P.; Li, H.; Chandler, K.; Conboy, J. C. *J. Phys. Chem. B.* 2010, 114, 1903-1914.

4 Eyring, H. *J. Chem. Phys*. 1935, 3, 107-115.