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Abstract: Molecular transfer between nanoparticles is presumed to be a key factor influencing nanoparticle stability. We recently reported a significant enhancement (150-fold) in the spontaneous lipid transfer rate between discoidal bicelles, compared to that of vesicles. To investigate the mechanism behind this enhanced transfer, lipid transfer rates were measured as a function of bicelle size and temperature. It was noted that smaller bicelles and higher temperatures resulted in faster lipid transfer. Analysis of the data indicated that lipid transfer is entropically favorable, but enthalpically unfavorable, with an activation energy that is independent of bicelle size. Molecular dynamics simulations revealed a lower energy cost for lipid dissociation near the interface boundaries between long- and short- chain lipids, compared to the energy cost of a bilayer composed of only the long-chain lipid. Together, these results suggest that the enhanced lipid transfer observed in bicelles arises from interfacial defects caused by the hydrophobic mismatch between the long- and short-chain lipid species.

Biological membranes allow for unique, compartmentalized biochemical processes to take place. The structural dynamics of biomembranes, and the transfer kinetics of their molecular constituents – primarily lipids, sterols and proteins – are among the most important physical parameters affecting these biochemical processes. For example, malfunctions in lipid transfer can lead to cardiovascular[1] and autoimmune diseases,[2] Parkinson's disease,[3] obesity,[4] and diabetes,[5] to name just a few. Physical studies are sometimes stymied by the chemical complexity of biological membranes, and tractable model systems are therefore used to investigate the physicochemical properties and dynamic behavior of lipid bilayers. Of special value are unilamellar vesicles (ULVs) and discoidal bicelles, owing to their ease of preparation, well-characterized morphologies and defined size distributions.[6]

It has been reported that bicelles composed of a mixture of long- and short-chain phosphatidylcholines (PC) have a uniform diameter and thickness, and spontaneously form under certain environmental conditions.[7] The long-chain lipids constitute the planar bilayer disk, while the short-chain lipids are sequestered to the disk's rim. Although the structural properties of bicellar mixtures have been extensively studied,[8] their kinetic properties (i.e., stability[9] and lipid transfer rates[10]) have attracted the attention of researchers. We recently reported a 150-fold enhancement in the interparticle lipid transfer rate constant, k_{inter}, of dimyristoyl-PC (DMPC) bicelles (0.156 ± 0.011 hr^{-1}) compared to that of DMPC ULVs [$(1.01 \pm 0.06) \times 10^{-3}$ hr^{-1}].[11] Although an interesting result, the molecular origin of this difference in lipid transfer rate was not elucidated.

To understand the mechanism responsible for this difference, we have performed a systematic study of lipid transfer between dipalmitoyl-PC (DPPC) and dihexanoyl-PC (DHPC) bicelles. In addition, 5 mol% of the negatively charged dipalmitoylphosphotidylglycerol (DPPG) was added to these bicelles in order to minimize bicelles fusing with each other. We first looked at the temperature dependence of lipid transfer between bicelles, with a long-to-short-chain lipid molar ratio, Q = 3 ($Q \equiv \frac{[DPPC]+[DPPG]}{[DHPC]}$), using time-resolved small-angle neutron scattering (TR-SANS) (see Material and Methods in SI).[12] A different set of bicelles composed of either DPPC or deuterated DPPC-d62 (H-bicelles and D-bicelles, respectively) were prepared in an H_2O/D_2O mixture, such that the neutron scattering length density (NSLD) of the aqueous solvent matched that of an equimolar DPPC/DPPC-d62 (H/D-bicelle) mixture. This is known as the "contrast-matched" condition (Table S1). Hence, a complete exchange between H- and D-bicelles would result in a dynamic equilibrium, where $\Delta\rho = \rho_{bicelle} - \rho_{solvent} = 0$ (Fig. S2 and Tables S2-S4). Fig. 1 shows the time evolution of the NSLD contrast between the bicelles and water. k_{inter} was obtained by fitting the data to a single exponential decay, as only monomeric lipid transfer takes place under these experimental conditions.[11] The Arrhenius analysis on k_{inter} yields an activation energy, E_a, of 119 $\pm$ 9.8 kJ mol^{-1} with the previously obtained k_{inter} at 10 °C.[11]

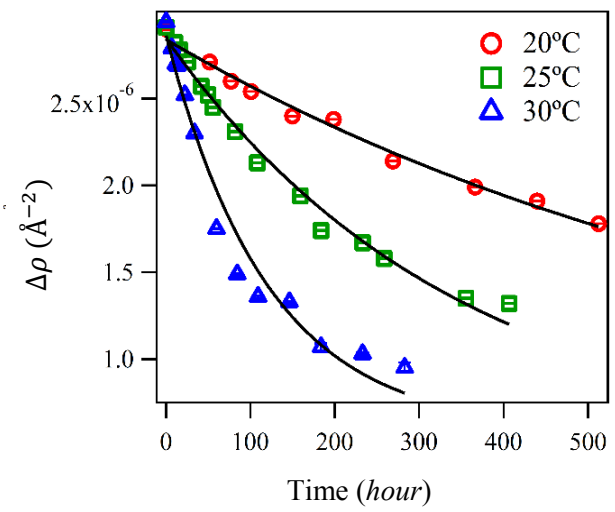

Figure 1 NSLD contrast decays of equimolar deuterated and protiated DPPC/DHPC/DPPG bicelles in contrast-matched water.

Next, we used Transition State Theory (TST) (which differs from Arrhenius analysis) to obtain the thermodynamic parameters for lipid transfer.[13] Specifically, the temperature dependence of the transfer rate constant is given by the Eyring-Polanyi equation,

$$k_{inter} = \frac{k_B T}{h} \exp\left(-\frac{\Delta H^{\ddagger} - T\Delta S^{\ddagger}}{RT}\right), \qquad (1)$$

where k_B, h and R are the Boltzmann, Planck and universal gas constants, respectively. The transition state[14] of dissociating lipid molecules from bicelles is enthalpically unfavorable [$\Delta H^{\ddagger} \equiv (E_a - RT) \sim 116$ kJ mol^{-1}], reflecting the energy barrier for transferring hydrophobic acyl chains to the water phase.[15] However, the chain disordering that occurs when a lipid leaves the bilayer and enters into water results in a favorable entropic contribution to the activation energy ($T\Delta S^{\ddagger} = 8.7$ kJ mol^{-1}).

The energy landscape of lipid transfer in bicelles is similar to that of lipoprotein-stabilized nanodiscs ($T\Delta S^{\ddagger} > 0$),[16] where compared to DMPC ULVs, a 20-fold increase in k_{inter} for DMPC nanodiscs was observed at 27 °C. The enhanced lipid transfer for nanodiscs was attributed to an enhanced packing of lipids caused by the rim tension induced the lipoprotein 'belt'.[16] However, this rationale does not apply to bicelles whose lateral tension can be minimized through bicelles fusing [9, 17] or ULV formation,[18] rather than enhanced lipid transfer. Here, we propose a different mechanism to account for the large increase in k_{inter}, namely the presence in bicelles of an interface separating DPPC-rich from DHPC-rich domains.

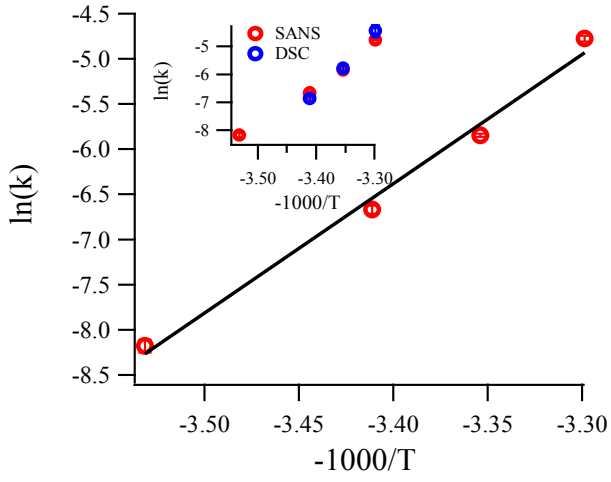

Figure 2 Arrhenius plot of k_{inter} for DPPC/DHPC/DPPG (Q = 3) bicelles obtained from SANS measurements. DSC and SANS data are in good agreement with each other (inset).

The first evidence for this proposed mechanism is the invariant activation energy for lipid transfer in bicelles at different Q values. As will be described below, k_{inter} can be obtained by differential scanning calorimetry (DSC) using an approach similar to TR-SANS, since the melting transition temperature, T_M, of DPPC and DPPC-d62 differ by 4°C (Fig. S6). Furthermore, T_M for a mixture of DPPC and DPPC-d62 varies linearly with composition. Specifically, a mixture of H- and D-bicelles initially exhibits two distinct T_M peaks that, as a function of time, move toward each other and eventually merge as lipids are exchanged between the two populations (see TR-DSC data in Fig. S5 and Tables S7-S15).[19] The difference in T_Ms (ΔT_M) as a function of time exhibits an exponential decay, from which k_{inter} is determined, as shown in Fig. 3 and Table 1, where k_{inter} increases with decreasing Q (i.e., increased DHPC molar ratio).

Table 1 Lipid transfer rate constants (hr^{-1})

	20 °C (DSC)	20 °C (SANS)	25 °C (DSC)	25 °C (SANS)	30 °C (DSC)	30 °C (SANS)
Q = 2.5	0.0012 ± 7×10^{-5}		0.0041 ± 6.8×10^{-4}		0.012 ± 4.4×10^{-4}	
Q = 3.0	0.0010 ± 1.1×10^{-4}	0.0013 ± 5×10^{-5}	0.0031± 4.8×10^{-4}	0.0029 ± 5×10^{-5}	0.012 ± 0.00245	0.0084 ± 2.6×10^{-4}
Q = 3.5	0.00053 ± 5×10^{-5}		0.0021± 2.1×10^{-4}		0.0051 ± 7.8×10^{-4}	

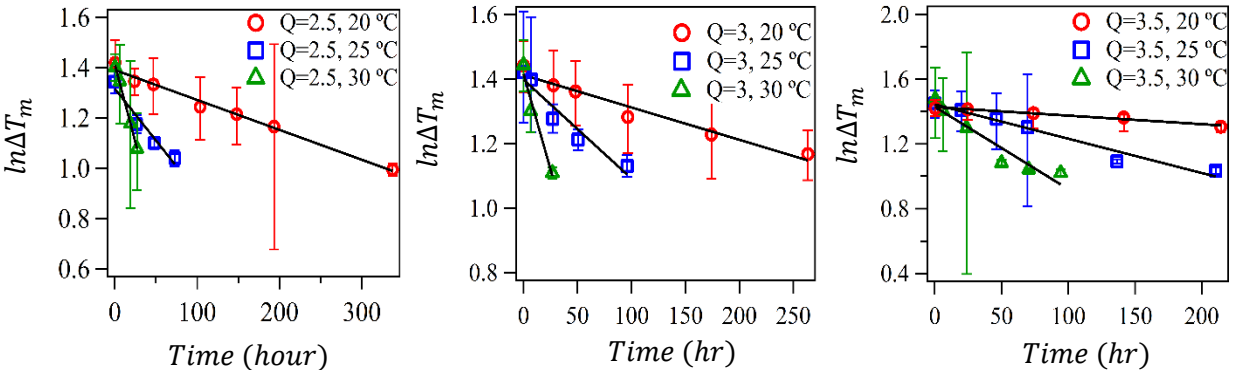

Figure 3 DSC data of $ln\ \Delta T_m$ as a function of time in different Q DPPC/DHPC/DPPG bicelles at T = 20, 25 and 30 °C.

E_a for the transfer process obtained from the Arrhenius analysis (Fig. 4) is independent of Q (E_a = 173.2 ± 4.5, 179 ± 13 and 168 ± 20 kJ/mol for Q = 2.5, 3 and 3.5 bicelles, respectively), revealing that the energy barrier for DPPC dissociating from bicelles into the water phase is independent of DHPC concentration. The increase in k_{inter} observed with decreasing Q can thus be attributed to an increasing interface between DPPC-rich and DHPC-rich domains, rather than a fundamental change in the energy landscape.

The proposed mechanism of lipid transfer is further validated by analyzing the SANS data using a disk model. Analysis shows higher-Q samples result in larger diameter bicelles (radii of 73 ± 8, 82 ± 7 and 95 ± 6 Å for Q = 2.5, 3 and 3.5 bicelles, respectively, Fig. S7 and Table S18). For bicelles, the interface between DPPC-rich and DHPC-rich domains is mostly localized at the disk rim, although some mixing of DPPC and DHPC may exist in the bilayer plane (Fig. 5). The fraction of interfacial DPPC (~ $4\pi R_{bicelle}$) to total bilayer DPPC (~$2\pi R_{bicelle}^2$) is proportional to $(R_{bicelle})^{-1}$. Lower-Q (smaller) bicelles therefore have a greater total interfacial area, leading to an enhanced lipid transfer. The reduced enthalpy of the DPPC gel-to-L_α transition at lower Q further supports this notion (Table S17), suggesting the presence of fewer liquid ordered DPPC molecules, i.e., more liquid disordered DPPC molecules found at the interface. Further evidence is the 40-50% increase in k_{inter} in bicelles doped with 5 mol% of distearoyl-phosphatidylethanolamine-PEG2000 (DSPE-PEG2000) compared to that of neat bicelles (Fig. S3) – although DSPE-PEG2000-doped bicelles are more stable due to steric effects.[20] This increase in k_{inter} is most likely underpinned by the same mechanism found in neat

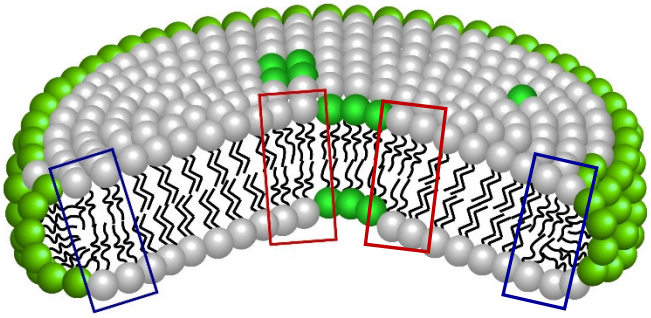

Figure 5 Schematic of DPPC lipids (grey headgroups) at the interface between the gel and L_α DPPC in the vicinity of the DHPC (green headgroups), represented by blue (rim) and red (plane) rectangles, respectively.

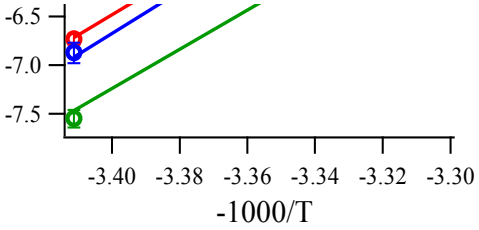

Figure 4 Arrhenius plots of DPPC/DHPC/DPPG bicelles at different Qs obtained from DSC data.

bicelles, since the size of DSPE-PEG2000-containing bicelles is smaller than that of neat bicelles (Fig. S4).

Molecular dynamic (MD) simulations (see Fig. S1 in SI) were performed to assess the energies of DPPC lipid dissociation from a planar DPPC bilayer, or a DPPC-DHPC interface (near a DHPC forming bilayer hole; insets in Fig. 6).[21] A free energy penalty of 83.7±0.42 kJ/mol was incurred in the case of planar DPPC bilayers, while the energy required for pulling DPPC at the DPPC-DHPC interface was only 65.7 ± 0.42 kJ/mol (Fig. 6). The difference (18.0 kJ/mol) in the dissociation energy from MD simulations gives us a molecular understanding of the experimentally determined k_{inter}, which is enhanced in the case of bicelles compared to vesicles. Moreover, in our simulations we observed that pulling one lipid molecule from the interface, appears to "drag" surrounding lipids out of the bilayer, inducing a local membrane curvature. Furthermore, the simulation results imply that the intrinsic lipid transfer rate constant at interfaces does not depend on DHPC concentration, consistent with the Arrhenius analysis.

In conclusion, TR-SANS, TR-DSC and MD simulations suggest that the interface between hydrophobic mismatched DPPC and DHPC molecules accounts for the faster lipid transfer observed in bicelles, compared to vesicles (as high as two orders of magnitude). This observation enabled a molecular understanding as to how defects can substantially alter the physical characteristics of a system. The mechanism underpinning it can also be applied to other biologically relevant and polymeric systems.

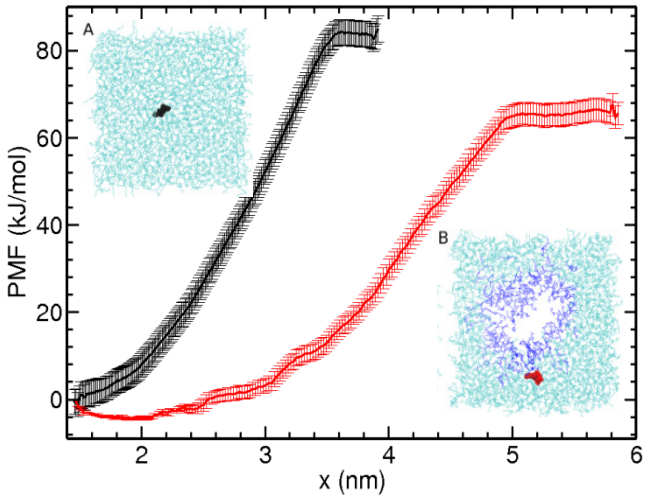

Figure 6 PMFs as a result of pulling one DPPC lipid out of a bilayer (black symbols) in the vicinity of the DHPC domain (red symbols). The lipid pulled out from the DPPC bilayer (black) and the DPPC-DHPC interface (red) are shown in insets A and B, respectively. DPPC and DHPC lipids are represented by cyan and blue sticks, respectively.

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