Modeling of Transient Trapping of Fatty Acid Tails in Phospholipids

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ABSTRACT

We explore the dynamics of the bilayer in liposomes in relationship to the motion of individual lipid molecules. Monitoring the dynamics at the length-scale of single lipids is important because it may relate to cellular signaling and regulatory processes. Our experiments prove a very localized relaxation, which we experimentally confirmed as a trapped motion of the fatty acid tails in a very crowded environment, by analyzing results from neutron spin echo spectroscopy on fully and partially deuterated lipids. This motion is visible over several orders of magnitude in time- and length-scale and a comprehensive scientific study require several neutron spectroscopic techniques. Analyzing experiments on protonated and partially deuterated lipids indicate the statistical independence of tail-motion and height-height correlation of the membrane. This permits to deduct a theoretical model that describes neutron spectroscopy data of liposomes. Our results are best compatible with lipid tails relaxing in potential with cylindrical symmetry. Our modeling approach satisfactorily describes the dynamics of different phospholipids, including mixtures of different lipids, and partially deuterated systems. Despite substantial differences in the chemistry of the fatty acid tails, the observed motion seems to behave rather universal. The existence of the fast-localized tail motion and the slow translational diffusion of liposomes limit
the time range in which the height-height correlation function can be monitored and analyzed. Taking into account these time constraints, we determined bending moduli between 8 k_BT (SoyPC) to 22 k_BT (DSPC). These values are slightly lesser than determined in the earlier literature, i.e., without including explicitly lipid tail motion. Thus, the new analysis shows that these membranes are a bit more elastic than observed before.

1 INTRODUCTION

Phospholipids are an essential part of cell membranes. Many recent studies focus on lipids and their impact on the proper functioning of membrane proteins. Many relaxation processes emerge from the motion of objects in the environment of densely packed lipid bilayers. At the molecular or lipid length-scale we observe lipid rotational and lateral motions with an additional slow flip-flop lipid exchange across the two monolayers. The rotational diffusion of the lipid plays an important role in transport of proteins, whereas, the lipid flip-flop motion is important for maintaining the stability and composition of the inner and outer monolayers of the membranes. At intermediate length scale, the membrane as a whole can undergo out-of-plane thickness and bending fluctuations or undulations. Such motions are responsible for cellular uptake or release and pore formations in membranes. At larger length scales the translational diffusion, _D_t_, of the liposome becomes dominant. The Brownian nature of the translational dynamics is important for characterizing the size of the liposomes used in bio-engineering and drug delivery applications.

Among the many techniques, dynamic light scattering (DLS) is a well-established tool to determine the translational diffusion coefficient, size and size distribution of liposomes. Microscopic techniques at smaller length scales, e.g., fluorescence recovery after photobleaching (FRAP) and single particle tracking (SPT) with fluorescent labelling can be utilized to
determine the lateral diffusion coefficient and mean square displacement of lipids over short distances. Most microscopic techniques are limited due to their spatial and temporal resolution and/or may require a fluorescence dye that may lead to additional effects, especially when tracking particle trajectories. More importantly, due to their fast motion at the ps to sub-μs time-scale, studying the dynamics of fatty acid tails is impossible by microscopy and outside the length-scale window of DLS.

Several, non-invasive neutron scattering techniques exist that are very useful to explore the structure and dynamics at the appropriate length and time scales of the living cells in their natural state. In order to understand the functionality of the living organism, we can obtain the specific structural and dynamical details of the individual components of the cell by selective deuteration and performing contrast variation neutron scattering experiments. Due to their importance, thickness fluctuations at the intermediate length-scale have been intensely studied by neutron spin echo spectroscopy (NSE), including selective isotopic labeling to especially important to distinguish head and tail group motion and to understand the phenomena of pore formation and the dynamics of lipid rafts in lipid bilayer.

In this context the time dependent mean-squared displacement (MSD or \( \langle r^2(t) \rangle \)) is one of the most fundamental means of statistical physics to describe the molecular dynamics of a molecule or a whole system. Since the MSD provides valuable information it is often used to track molecular motions or changes due the influence of interactions and spatial confinements in crowded biomacromolecules. Recently, we utilized NSE to explore the motion at the time scale \( \approx 50 \text{ ps to} \approx 200 \text{ ns} \). We compared four different phospholipid samples, DOPC (1,2-dioleoyl-sn-glycero-3-phosphocholine), DSPC (1,2-dioleoyl-sn-glycero-3-phosphocholine),
DMPC (1,2-dimyrisoyl-sn-glycero-3-phosphocholine) and SoyPC (L-α-phosphatidylcholine), in their fluid phases.

We calculated $\langle \Delta r(t)^2 \rangle$ from the measured dynamic structure factor $S(Q,t)$.\textsuperscript{30, 31} We used a cumulant expansion to obtain $\langle \Delta r(t)^2 \rangle$ and the non-Gaussianity parameter, $\alpha_2(t) = \frac{d}{d+2} \frac{\langle \Delta r(t)^4 \rangle}{(\langle \Delta r(t)^2 \rangle)^2} - 1$, which is defined by the quotient of the fourth $\langle \Delta r(t)^4 \rangle$ and the second moment squared $\langle \Delta r(t)^2 \rangle^2$ and $d = 3$, the dimension in space. Our study revealed the existence of the complex dynamic organization of the lipid bilayer in terms of localized anomalous motions.\textsuperscript{29} By a detailed calculation of the time evolution of $\langle \Delta r(t)^2 \rangle$, we obtain three distinct power-law behaviors. We found $t^1$ at longer Fourier times, followed by $t^{0.66}$, and $t^{0.26}$, at intermediate and shorter Fourier times, respectively. The $t^1$ contribution is attributed to the center of mass diffusion of the liposomes, whereas the $t^{0.66}$, originates from the thermal undulations of the membrane as defined by Zilman-Granek (ZG),\textsuperscript{32} and also by the anomalous diffusion predicted by Monte Carlo simulations.\textsuperscript{33} We identified the $t^{0.26}$ as region which corresponds to the lateral motion of lipids, while undergoing a dynamic transient trapping within a certain range that is on the order of the size of the lipid molecules or less. The appearance of the non-Gaussianity $\alpha_2(t)$ in $\langle \Delta r(t)^2 \rangle$ and the transient trapping is suggested by the predictions of the continuous time random walk (CTRW) model and can account for the inhomogeneities in the crowded lipid bilayers.\textsuperscript{34}

However, in addition to the CTRW model some recent studies have pointed to the importance of fractional Brownian motion (FBM) in the observed lipid crowding in the bilayer showing anti-persistence non-Gaussian behavior.\textsuperscript{35} The non-Gaussianity in protein crowded membranes is assumed to arise from the dynamic intermittence of lipids in the bilayer. The small exponent in the anomalous diffusion may point towards such behavior. In this context a
distribution of diffusion coefficients can affect the non-Gaussianity of the MSD.\textsuperscript{36} Such kind of motions are found to be Brownian but non-Gaussian.\textsuperscript{37}

The recent Molecular Dynamics (MD) simulations and mode-coupling theory calculations by Flenner et al.\textsuperscript{38} support that our experimental observation of a trapped motion is associated with the motion lipid tail of the fatty acid. Additional Monte Carlo (MC) simulations by Pandey et al.\textsuperscript{33} find a dependence of the power-law on the specific strength of interactions, ranging from 0.17 ($\Delta F^o > 0$) to 0.34 ($\Delta F^o < 0$), with, $\Delta F^o$, the membrane-membrane interaction energy.

According to the simulations, the existence of anomalous diffusion seems to coincide with an increasing disorder for the lipids, e.g., due to raising temperature or adding cholesterol.\textsuperscript{39} Similar observation was noted in natural membranes where proteins are present to transport ions or genetic code across the membrane.\textsuperscript{35} In such crowded environments significant inhomogeneities were observed in single particle trajectories resulting in non-Gaussian diffusion.\textsuperscript{55}

This novel discovery of anomalous diffusion at the length scale of a few Angstrom and a time-scale < 5 ns deserves further supported by experiments and by more detailed analysis to unravel the origin of the $t^{0.26}$ power-law dependence and to expand the fundamental understanding. Hereafter, we will provide experimental and theoretical evidence that this fast relaxation is caused by the fatty acid tails that undergo a highly constrained motion. We further the unique importance of the accurate analysis to understand the fundamental processes in liposomes, which are important to understand the behavior and function of cell membranes.

2 \textbf{MODELING DYNAMICS OF LIPOSOMES}

This section introduces our model newly developed based on the data presented below and helps to understand the molecular dynamics of liposomes. We continue with a short discussion of
the data analysis including a comparison with existing models in the literature, especially detailing
the difference and length- and time-scales in which the different models can be used.

2.1 Derivation of a new model for the dynamics of liposomes

The experiments show at least three processes, tail motion, lipid motion and translational
diffusion of liposome contribute to the time dependent mean squared displacement (MSD or
\langle r^2(t) \rangle) within the length and time window of the NSE. From our recent paper,\textsuperscript{29} we know that
the translational motion of the liposome is independent of the lipid motion, at least within a very
good approximation.

Using partially deuterated lipids where the lipid tail is contrast matched with the solvent,\textsuperscript{6} it
is evident that the lipid head motion can be described by the Zilman-Granek (ZG) approximation
for membrane undulation, which we identify with the height-height correlation function.\textsuperscript{32} The
ZG approximation does neglect the lateral motion of lipids and more local motions to \( S(Q, t) \).

Our experiments presented below demonstrate that the time-scales are well separated and the
fast-local relaxation of lipids and the height-height correlation of membranes can be treated as
statistically independent functions. Therefore, we assume the faster lipid tail motions are not
affected by the slower ZG dynamics.\textsuperscript{29} Thus, the intermediate scattering function of the liposome
\( S_{\text{liposome}}(Q, t) \), can be written as

\[ S_{\text{liposome}}(Q, t) = S_{\text{lipid}}(Q, t) \times S_{\text{height}}(Q, t) \times S_{\text{thickness}}(Q, t) \times S_{\text{trans}}(Q, t) \]  \hspace{1cm} (1)

Here, the lipid bilayer motion is given by the height-height correlation of the membrane
represented by \( S_{\text{height}}(Q, t) \), and the bilayer thickness fluctuation, \( S_{\text{thickness}}(Q, t) \). The localized
motion of the lipid in the bilayer is introduced by \( S_{\text{lipid}}(Q, t) \), whereas the translational motion of
the liposome is given by \( S_{\text{trans}}(Q, t) \). Following pages 102 and 309 in the book of Higgins and
Benoit, this approach is strictly valid, if the different motions are statically independent. Our experiments suggest that this assumption should be fulfilled at least to a very good approximation. Equation 1 permits to include multiple processes, including rotational diffusion of liposomes and lipids. These processes are beyond the scope of the present work.

Equation 1 is very similar to version described by Zilman and Granek, which especially includes translational diffusion, height-height correlations describing the motion in a plane perpendicular to the flat membrane surface and in plane lateral motion. Below, we show the importance of diffusion for our analysis and compare it to theoretical assumptions by Zilman and Granek. Unlike the approach by Zilman and Granek, we use the term $S_{\text{lipid}}(Q, t)$ to describe the localized motion of lipids, without limiting it to lateral motions. Hereafter, we further advance the equation and generalize this contribution, which finally leads to a more detailed understanding of the respective correlation function.

The dynamic structure factor $S_{\text{height}}(Q, t)$ represents the relaxation by following the height-height correlation function, while $S_{\text{thickness}}(Q, t)$ is related to thickness fluctuations between the bilayers. These processes are treated as statistically independent in the respective literature.

The diffusive motion of liposomes can be expressed as a function time using the momentum transfer $(Q)$ and the of the translational diffusion coefficient ($D_t$) as:

$$S_{\text{translation}}(Q, t) = \exp(-D_t Q^2 t)$$ (2)

Zilman an Granek discuss the impact of translational diffusion on the dynamic structure factor and introduce $D \sim k_B T/\eta R$, with the thermal energy $k_B T$ compared with the product of the viscosity, $\eta$, and the size of the liposome, $R$. They mention for $QR \gg 1$ the contribution of diffusion on
\( S(Q, t) \) is negligible for \( t \ll \eta R^3 / \kappa \). This discussion includes that the relaxation of the dynamic structure factor \( S(Q, t) \) diminishes to vanishingly small value for \( t \gtrsim \eta R^3 / \kappa \), which could make the contribution of the diffusion barely visible. As suggested by Zilman and Granek we have replaced the plaquettes size \( \xi \) by the liposome radius \( R \).

The height-height correlations have been extensively studied in the literature. Most studies use

\[
S_{\text{height}}(Q, t) = A \exp \left[ -\left( \Gamma_Q t \right)^{2/3} \right] \tag{3}
\]

The frequency of fluctuations is given by, \( \Gamma_Q = \Gamma_{ZG} \), that represents the ZG decay rate or the undulation frequency, and \( A \) the amplitude.

The parameter \( \Gamma_{ZG} \) introduces a \( Q \)-dependent decay rate, from which we derive the intrinsic bending modulus, \( \kappa_\eta \), by

\[
\frac{\Gamma_Q}{Q^3} = \frac{\Gamma_{ZG}}{Q^3} = 0.0069 \frac{k_B T}{\eta} \sqrt{\frac{k_B T}{\kappa_\eta}} \tag{4}
\]

Here \( \eta \) is the viscosity, \( k_B \) the Boltzmann constant, \( T \) the temperature, and \( \gamma \) is a weak, monotonously increasing function of \( \kappa_\eta / k_B T \). In case of lipid bilayers usually \( \kappa_\eta / k_B T \gg 1 \), leading to \( \gamma = 1.6, 7, 32, 42, 43 \). Equation 4 represents the elastic bilayer component and can be derived from a modified ZG theory that includes intermonolayer friction. A detailed discussion is beyond the scope of the present work but can be found in the literature. In previous studies a prefactor 0.0058 in equation 4 was used to better explain the NSE data, however this leads to the assumption that the neutral surface from the bilayer midplane is located inside the headgroup region of the bilayer. This was corrected by using the prefactor 0.0069. Details are reviewed by recent literature.
Bilayer thickness fluctuations where monitored more in detail by NSE utilizing contrast matched fatty acid tails. The importance of introducing a second term, approximated by a Lorentzian function to describe the observed relaxation rate, $\Gamma_Q$, was noted\(^6\).\(^{42}\) 

$$
\frac{\Gamma_Q}{Q^3} = \frac{\Gamma_{ZG}}{Q^3} + \frac{(\tau_{TF}Q_0^3)^{-1}}{1 - (Q - Q_0)^2\xi^2}
$$

Where, $\tau_{TF}$ is the relaxation time, and, $\xi^{-1}$ is the half width half-maximum of the Lorentzian at the thickness fluctuation peak momentum transfer, $Q_0$.

Bingham \textit{et al.}\(^{46}\) introduced a theoretical relation between thickness fluctuations and viscoelastic properties of membranes. Following that prediction Nagao \textit{et al.}\(^{42}\) included both the elastic and viscous components in the Lorentzian expression of equation 6 which leads to

$$
\frac{\Gamma_q}{Q^3} = \frac{\Gamma_{ZG}}{Q^3} + \frac{K_A k_B T}{\mu Q_0^3 k_B T + 4\mu Q_0 K_A A_0 (Q - Q_0)^2}
$$

Here $K_A$ is the area compressibility modulus, $A_0$ is the area per lipid molecule and $\mu$ is the local viscosity of the membrane.

Nagao \textit{et al.}\(^{42}\) inserted equation 5 or 6 in equation 3. Since both equation 5 and 6 represent a summation over two contributions, height correlation and thickness fluctuations, equation 3 can be divided into the product of two contributions, which we named $S_{height}(Q, t) \times S_{thickness}(Q, t)$.

Thus, the model of Nagao is mathematically equivalent to the factorization approach in equation 1, except translational diffusion of the liposome and local processes. More details about the thickness fluctuations are beyond the scope of the present work. More details can be found in the recent publication by Nagao \textit{et al.}\(^{42}\).
A study on partially deuterated liposome suggests that the dynamics of the lipids can be separated from the undulation. Neutron scattering experiments require to take into account the contrast/number of protons deuterons that are involved. We use a simple approach and write

\[
S_{\text{lipid}}(Q,t) = \left( n_{H,\text{head}} + n_{H,\text{tail}} \left( \mathcal{A}(Q) + (1 - \mathcal{A}(Q)) \exp \left( - \left( \frac{t}{\tau} \right)^\beta \right) \right) \right)
\]  

(7)

Here, \(n_{H,\text{head}}\) and \(n_{H,\text{tail}}\) are the fractions of the total number of protons in the head and in the tail, respectively.

Equation 7 has the same form as would be used to analyze the results from quasielastic neutron scattering (QENS). If we would analyze QENS experiments, \(\mathcal{A}(Q)\) would be identical to the elastic incoherent structure factor (EISF). However, we emphasize the fact that NSE measures the coherent dynamics. Therefore, analyzing the data by the EISF is not possible. The reason why it looks similar is that a simple stretched exponential is used to model the relaxation of the lipid tail. For such a simple assumption, \(S_{\text{lipid}}\) has a very similar coherent, \(S_{\text{coh}}\), and incoherent, \(S_{\text{inc}}\), dynamic structure factor. This approach is very similar to existing data analysis, e.g., for the diffusion of particles. Below we test this assumption critically by comparing the results of NSE and QENS studies.

If \(\mathcal{A}(Q)\) has a similar meaning then the EISF, then we can utilize techniques introduced for QENS analysis. We utilize the advantage that for simple for simple cases closed equations exist, e.g., for a particle diffusing in a sphere

\[
\mathcal{A}(Q) = \left[ \frac{3j_1(QR)}{(QR)} \right]^2 = \frac{9}{(QR)^6} (\sin(QR) - QR \cos(QR))^2
\]

47 Here, \(j_1\), is the first order spherical Bessel function, and \(R\) is the radius of the sphere that localizes the motion of the particle. This approach is very common and has been successfully used for polymers with side-chains that have a similar number of carbons as in the
l lipid tails. The crowded environment within the bilayer may impose a stronger constraint which can be better described by a cylinder symmetry. By considering the lateral, \( A_0(Q_Z) = \left[ j_0(\frac{Q R_L \cos(\theta)}{(Q R_L \cos(\theta))}) \right]^2 \), and perpendicular diffusion, \( B_0^0(Q_\perp) = \left[ \frac{3j_1(Q L \sin(\theta))}{(Q L \sin(\theta))} \right]^2 \frac{1}{2} \int_0^{\pi} \sin(\theta) d\theta \), we obtain the \( A(Q) = A_0(Q_Z) B_0^0(Q_\perp) \). Here, \( j_0 \), is the zeroth order spherical Bessel function, \( R_L \), and, \( L \), are the radius and length of the cylinder, respectively.

At the first glance with increasing the complexity of the models we seem to introduce more degrees of freedom. However, we combine several independent experimental techniques to acquire the results independently, which reduces the number of free parameters substantially. For example, we use DLS to determine the translational diffusion coefficient of the liposome, which avoids free parameters in the analysis of NSE data. In addition, we have well separated time- and length-scale contributions, which allow a simultaneous fit. Thus, the results are very reliable. More importantly, in a next step we include the sensitivity of neutron to independently determine the different contributions to equation 1.

In a first step towards the understanding of the molecular dynamics in liposomes, we analyze NSE experiments on partially deuterated lipids, in which the fatty acids were contrast matched by the solvent. Following the motion of the heads, confirms the original assumption that the tails determine the measurement signal in case of fully protonated samples. The following considerations improve the discussion by Zilman and Granek, because it generalizes their statement of the lateral motion of lipids and relates it directly to the molecular potential.

As illustrated by equation 7, the scattered intensity in neutron scattering experiments is very sensitive on the number of protons and deuterons. In case of fully hydrogenated lipids, all protons contribute to \( S(Q, t) \). The number of protons in the tails is much greater than the number of protons in the head group. For example, in case of DOPC \( N_{\text{tail}} = 66 \), and \( N_{\text{head}} = 18 \), which leads to the
fractions \( n_{\text{tail}} = 0.79 \), and \( n_{\text{head}} = 0.21 \), respectively. Contrast matching is the appropriate tool to distinguish head and tail motion. The signal from the contrast matched tails is completely suppressed and the relative fraction of protons in the tail, i.e. \( n_{h,\text{tail}} = 0.79 \), and \( n_{h,\text{head}} = 0.21 \), respectively. Contrast matching is the appropriate tool to distinguish head and tail motion. The signal from the contrast matched tails is completely suppressed and the relative fraction of protons in the tail, i.e. \( n_{h,\text{tail}} = 0.21 \), reflects the presence or absence of the dynamic contribution of the lipid head and tail in the relaxation spectra.

In summary, the dynamics of liposomes studied by NSE includes diffusion, membrane fluctuations, and trapped motion. By inserting equations 2, 3, and 7, in equation 1 we obtain:

\[
S_{\text{liposome}}(Q,t) = \left( n_{h,\text{head}} \right. \\
+ n_{h,\text{tail}} \left( A(Q) + (1 - A(Q)) \exp \left( -\left(\frac{t}{\tau}\right)^{\beta}\right) \right) \exp \left( -\left(\Gamma_{0}t\right)^{2/3}\right) \exp(-D_{t}Q^{2}t) \right)
\]

Having identified the motion of the head groups, the tail groups can be analyzed more in detail, using protonated samples.

2.2 Relationship to existing approaches

2.2.1 Cumulant approach

In NSE spectroscopy due to the spin flip one measures the normalized total signal as the sum of coherent and incoherent scattering. The coherent scattering probes the inter-particle pair correlation function and therefore can give structural and correlated dynamical information, like membrane undulation. Incoherent scattering probes the autocorrelation function that is related to the diffusive motions and density of states of correlated motions. However, one can measure the coherent scattering contribution over a specific \( Q \)-range where, \( \sigma_{\text{coh}} > \sigma_{\text{inc}} \), i.e.

\[
\frac{S(Q,t)}{S(Q)} = \frac{\sigma_{\text{coh}}S(Q,t)_{\text{coh}} - (1/3)\sigma_{\text{inc}}S(Q,t)_{\text{inc}}}{\sigma_{\text{coh}}S(Q)_{\text{coh}} - (1/3)\sigma_{\text{inc}}S(Q)_{\text{inc}}} \approx \frac{S(Q,t)_{\text{coh}}}{S(Q)_{\text{coh}}}
\]

Where, \( \sigma_{\text{coh}} \) and \( \sigma_{\text{inc}} \) are the coherent and incoherent scattering cross-sections.

Following pages 104 and 108 in the book of Higgins and Benoit, the expression for the intermediate incoherent translational scattering function can be simplified as
\[ S^\text{tr}_{\text{inc}}(Q, t) = N \exp(-Q^2D_{\text{eff}}t) \] (10)

And the intermediate coherent scattering function as
\[ S^\text{tr}_{\text{coh}}(Q, t) = N Z^2 \exp(-Q^2D_{\text{eff}}t) P(Q) \] (11)

Here \( N \) is the number of particles, \( Z \) is the number of segments that constitutes a polymer with form factor, \( P(Q) = (1/Z^2) \sum_{i \neq j}^Z \exp(-i\mathbf{Q} \cdot (\mathbf{r}_i - \mathbf{r}_j)) \). Here the correlation between the particles \( i \) and \( j \) at positions \( \mathbf{r}_i \) and \( \mathbf{r}_j \), respectively. In case of particle form factor, we can consider \( Z = 1 \). Comparing with equation 1, equation 2 can be implemented in the limit \( Q \to 0 \), assuming for a large molecule their rotations and conformational changes as a function of time. In that case, \( P(Q) \to 1 \) in the limit \( Q \to 0 \). This effectively means that at small \( Q \)'s the molecules are treated as point masses for which equations 1 and 2 can be applied. Now from the ZG prediction the Stokes-Einstein diffusion of a membrane sheet can be written as, \( D_{\text{eff}} \sim \frac{k_B T}{\eta} \sqrt{\frac{k_B T}{\eta} Q} \). However, one can follow a generalized approach to understand the overall mean squared displacement (MSD). For a Gaussian distribution one can follow equation 1 and 2 as
\[ \frac{S(Q, t)}{S(Q)} = A \exp\left[ -\frac{Q^2 \langle \Delta r(t)^2 \rangle}{6} \right] \] (12)

In general for a non-Gaussian distribution, \( S(Q, t) \) can be expressed by a cumulant expansion
\[ \frac{S(Q, t)}{S(Q)} = A \exp\left[ -\frac{Q^2 \langle \Delta r(t)^2 \rangle}{6} + \frac{Q^4 \alpha_2(t)}{72} \langle \Delta r(t)^4 \rangle \right] \] (13)

It includes the second order expansion in \( Q^2 \) and utilizes the non-Gaussianity parameter, \( \alpha_2(t) = \frac{d \langle \Delta r(t)^4 \rangle}{d+2 \langle \Delta r(t)^2 \rangle^2} - 1 \) to indicate deviations from the often assumed Gaussian approximation. The definition via the quotient of the fourth \( \langle \Delta r(t)^4 \rangle \) and the second moment squared \( \langle \Delta r(t)^2 \rangle^2 \) and \( d = 3 \), the dimension in space relates it directly to the molecular dynamics in a system. \(^{30, 31}\)
This approach effectively provide us with an estimation of the MSD from \( S(Q, t)/S(Q) \) for the limit \( Q \to 0 \). Therefore, it fulfills the criteria where equations 12 and 13 are equivalent.

Comparing equations 3 and 13 yields the dependence of the bending rigidity on time and mean-squared displacement

\[
\frac{\kappa_\eta}{k_B T} = \frac{t^2}{c(\eta, T)^3 \langle \Delta r(t)^2 \rangle^3}
\]  

(14)

with the pre-factor, \( c(\eta, T) = \frac{1}{6} \left( \frac{\eta}{0.0069 k_B T} \right)^{2/3} \). The ZG model implies \( \alpha_2 = 0 \) and the signature of membrane undulations is \( \langle \Delta r(t)^2 \rangle \propto t^{2/3} \). Inserting this proportionality in equation 5 yields \( \kappa_\eta/k_B T \propto t^2/t^2 = \text{const} \). Thus, the bending elasticity is independent of time within the framework of the ZG model. Any deviation from this behavior implies dynamics outside the underlying theory.

Recently we experimentally observed \( \alpha_2 \neq 0 \) for \( t \leq 3 \text{ ns} \), which seems to be very generic and applies to many phospholipids, like DOPC, DMPC, DSPC, DPPC and SoyPC.\(^{29} \) Equation 15 can therefore be used to illustrate deviations from the behavior underlying the ZG model.

### 2.2.2 Milner-Safran (MS) model

The Milner-Safran (MS) model was introduced to study undulations in microemulsion droplets and vesicles by expanding the intermediate scattering function in spherical harmonics\(^{51, 52} \).

\[
S(Q, t) \approx \exp(-D_T Q^2 t) \left[ 4\pi j_0^2(QR) + \sum_i F_i(u_{i0}(t)u_{i0}(0)) \right]
\]  

(15)

The idea behind this factorization is that each bending mode, \( l \), contributes to \( S(Q, t) \).

Here the autocorrelation function, \( \langle u_{i0}(t)u_{i0}(0) \rangle \), is weighted by a factor, \( F_i(z) = (2l + 1)[(l + 2)j_l(z) - zj_{l+1}(z)]^2 \), that contains the Bessel functions, \( j \), of order \( l \) and \( l + 1 \).
It was recently observed that the MS model successfully describes $S(Q,t)$ of small microemulsion droplets for sizes on the order of 5 nm. It fails in case of unilamellar vesicles of radii with around 20 nm, because it yields unphysical values of the membrane rigidities. The ZG model seem to be better if unilamellar vesicles of radii $> 20$ nm are analyzed. This is consequence of the derivation. As detailed in the literature, the ZG model should provide a better estimate of $S(Q, t)$ for $QR > 1$ that region, in which our experiments were conducted.

2.2.3 Summation approach
Following the classical Milner-Safran (MS) theory equation 15 can be simplified. The literature often uses

$$S(Q, t) = \exp(-D_t Q^2 t) \left\{ A + (1 - A) \exp \left[-(\Gamma_Q t)^{2/3}\right]\right\}$$  \hspace{1cm} (16)

For our experimental case $QR \gtrsim 1$ is important. Here the contribution of the fluctuation modes is much stronger than that of the translational dynamics, i.e. $\Gamma_Q \gg D_t Q^2$. Thus, equation 8 can be approximated by an apparent bimodal function which is frequently used for the analysis

$$S(Q, t) \approx A \exp(-D_t Q^2 t) + (1 - A) \exp \left[-(\Gamma_Q t)^{2/3}\right]$$ \hspace{1cm} (17)

Both equations 16 and 17 were used to successfully model experimental data. The analysis of the NSE data shows that $D_t Q^2$ and $\Gamma_Q$ determined with equations 16 or 17 are within the same within the experimental accuracy.

At the first glance, the summation approach, e.g., equation 16, appears to be the same as new model uses for the lipid tail motion (equation 7). However, the MS model is describing the motion of the whole liposome without the contribution of head and tail separately and therefore are not connected to our new model.
2.2.4  Hybrid approach  
In general, a factorization of intermediate scattering functions, as in equation 1, is mathematically exact if the processes are statistically independent. Though, it is very common, because it simplifies the analysis, it may not be fulfilled for all cases where additional modes contribute to the NSE signal.\textsuperscript{11} The hybrid approach was used to understand the relation between membrane bending and local reorganization of the bilayer material undergoing intermonolayer sliding.\textsuperscript{53} In the hybrid model the membrane curvature and dilation was coupled with the ZG membrane fluctuation along with the translational diffusion in $S(Q,t)$ by\textsuperscript{53,56}

$$
S(Q,t) \approx \exp(-D_T Q^2 t) \left[ A_T(Q,R) + (1 - A_T(Q,R)) \right] \left[ a_{\text{bend}} \exp\left(-\left(\Gamma_Q t\right)^{2/3}\right) + a_{\text{hyb}} S_{\text{hyb}}(Q,t) \right]
$$

(18)

Where $A_T(Q,R) = 4\pi j_0(Q,R)^2$, with $j_0$, the zeroth order spherical Bessel’s function, and $\Gamma_Q = \Gamma_{\text{ZG}}$, the Zilman-Granek relaxation rate. The internal mode is given by $A_{\text{int}} = 1 - A_T(Q,R)$. For a rigid membrane, $S_{\text{hyb}}(Q,t) = 1$, however, for highly elastic membrane the hybrid mode is given by a single exponential decay $S_{\text{hyb}}(Q,t) = \exp(-\Gamma_{\text{hyb}} t)$. The model can describe the experimental data reasonably well for rigid membranes, however for elastic membranes it fails.\textsuperscript{53} The model predicts a systematic faster relaxation at longer times than that was observed experimentally.\textsuperscript{53} However, the hybrid model in equation 18 does to include the dynamic contribution from the lipid head and tail as was defined in our model in equation 8.

3  EXPERIMENTAL SECTION  
3.1  Sample preparation  
Sample preparation procedures are detailed in previous work.\textsuperscript{29} In brief, for the convenience of the reader, the lipid powder is dissolved in chloroform. Solvent was removed using a rotary evaporator and further dried under vacuum overnight. The dried lipid was hydrated using ultrapure D\textsubscript{2}O and
then subjected to freeze-thaw cycling by immersing the flask in water at around 50 °C and then by placing in a freezer at -20 °C. Finally, the solution was extruded using a mini extruder (Avanti Polar Lipids, Alabaster, AL, USA) through a polycarbonate membrane with pore diameter of 100 nm (33 passes) to obtain unilamellar vesicles. All the samples that are studied in the present work were obtained commercially from Avanti Polar Lipids, Alabaster, AL, USA.

3.2 Experiments
The Neutron Spin Echo (NSE) experiments were conducted at BL15 at the Spallation Neutron Source of the Oak Ridge National Laboratory, Oak Ridge, TN and at the NGA-NSE at the NIST Center for Neutron Research (NCNR) of the National Institute of Standards and Technology (NIST). We used Hellma quartz cells at BL15-NSE and titanium cells at NGA-NSE, in both cases 4 mm neutron path length was used. The data reduction was performed with ECHODET (SNS-NSE) and Dave (NGA-NSE). Wavelengths of 8, 11 and 15 Å were used at NG5-NSE and 8 Å was used at BL15-NSE. D2O was measured separately and was subtracted as the background.

4 RESULTS and DISCUSSION
Results from NSE for h-DOPC, h-DMPC and h-SoyPC in D2O are presented in Figure 1. The solid lines illustrate a comparison of the height correlation model, $S_{height}(Q,t)$, (Figure 1 (a-c), equation 3) with the factorization approach (Figure 1 (d-e), equation 8). In the fitting routine the relaxation amplitude in equation 3 is kept as a free parameter rather than fixing it to $A = 1$. The reason for this procedure will become obvious below.

We note that the calculated $S_{height}(Q,t)$ shows deviations for $t < 5$ ns (h-DOPC), $t < 3$ ns (h-DMPC) and at $t < 10$ ns (h-SoyPC), even more pronounced at higher momentum transfers, $Q$’s. Earlier we illustrated that diffusion affects $S(Q,t)$ at higher Fourier times, thus cannot explain the deviations. Following the ZG formalism, the effect of diffusion should be negligible for $t \ll \tau$.
\[ \eta R^3 / \kappa = 4.4 \, \mu s, \text{ radius of liposome (DOPC), } R \approx 66 \text{ nm in } D_2O \text{ with viscosity, } \eta_{D2O} = 1.25 \text{ mPa}\cdot s, \text{ and } \kappa = 20 \text{ k_BT}. \]

The model calculations with equation 9 describe the experimental data very well, including lower Fourier times. In the data modelling the fraction of the relative fractions of protons in the head is kept fixed to, \( n_{H,\text{head}} = 0.21 \), for h-DOPC, \( n_{H,\text{head}} = 0.23 \) h-SoyPC, and, \( n_{H,\text{head}} = 0.25 \) for h-DMPC. Due to the contrast conditions, the head group correlations are not visible. Thus, \( S_{\text{thickness}}(Q, t) \) is not visible in the experimental data.

Figure 1: Lin-log representations of the normalized dynamic structure factor, \( S(Q,t)/S(Q) \), as a function of Fourier time, \( t \), for different \( Q \)'s, for, (a,d) 5 % protonated DOPC at 20 °C (data from reference 29), (b,e) 5 % protonated DMPC at 37 °C (data from reference 8) and (c,f) the 5 % protonated Soy-PC sample at 30 °C (data from reference 29), each dispersed in \( D_2O \). The same data sets are analyzed by fits using the (a-c) Zilman Granek model (ZG) (equation 3) and (d-e) the
full model that starts from equation 1 and includes diffusion and trapped motion (equation 8). The error bars representing one standard deviation.

The NSE data for tail contrast matched samples are presented in Figure 2 (a) and (b) for DPPC and for a DMPC - DSPC binary mixture, respectively. In these partially deuterated samples the tail is contrast matched by D$_2$O. For this case $n_{H,\text{head}} = 1$ and $n_{H,\text{tail}} = 0$, i.e., the contribution of $S_{\text{lipid}}(Q,t)$ is expected to disappear. As Figure 2 (a) and (b) illustrate the model describes the experimental $S(Q,t)$ very well. This indicates the absence of the short time contribution to the signal and connects the short time dynamics observed on the fully protonated lipids with the motion of the fatty acid tails.

![Figure 2](image)

**Figure 2:** Normalized dynamic structure factor, $S(Q,t)/S(Q)$, as a function of Fourier time, $t$, for different $Q$'s. (a) for mixture of protonated and deuterated tail DPPC in D$_2$O sample at 50°C and (b) for the 100 mg/ml of equimolar mixture of tail contrast matched deuterated DMPC and DSPC.
at 65 °C, each 10% lipid mass fraction. The data is fitted using our modified ZG model, equation 9, with \( n_{H,\text{head}} = 1 \), and, \( n_{H,\text{tail}} = 0 \). Experimental data adopted from the literature \(^6,^{23}\).

Apparently, Figure 1 and 2 can be well described by the modeling concept. Hereafter, we use the MSD to illustrate the different contributions. Using the cumulant expansion in equation 13 and superimposing the MSDs in the ZG regime we obtain \( \langle \Delta r(t)^2 \rangle_N \). The results are illustrated in Figure 3 (a) and compared with our previous results for h-DOPC, h-DSPC, h-DMPC and h-SoyPC samples.\(^{29}\) The results from MD simulations of h-POPE (palmitoyl-oleoyl-phosphatidylethanolamine) are also included (grey circles).\(^{34}\)

The MSDs from lipids with contrast matched tails – dt-DPPC and dt-DMPC/DSPC mixture (open circles)\(^6,^{23}\) – do not show the \( t^{0.26} \) regime. This does not imply the absence of the process in these samples, but rather reflects hiding the contribution of the tails for neutrons by contrast matching. More importantly, it shows the universal height-height correlation in pure lipids and in lipid mixtures. It experimentally connects the emergence of the \( t^{0.26} \) regime with the dynamics of the fatty acid tails. In other words, if the neutrons cannot see the tails, the ZG region extends to smaller Fourier times and covers the entire region, as one observes in the analysis of single membrane layers, e.g. from microemulsions.\(^{39}\) The absence of the \( t^{0.26} \) adds further evidence to the argument on the hidden lipid tail motion in tail contrast matched samples. We have incorporated the relaxation spectra from equation 6 to calculate the effective MSD similar to the cumulant analysis and have included that in Figure 3 (a) for comparison. They are illustrated by the black and green solid lines for dt-DPPC and dt-DMPC/DSPC, respectively. It describes the impact of membrane thickness fluctuations on the NSE data for the tail contrast matched samples (dt-lipids).\(^6,^{23,42}\) It overlaps the experimental data (open circles) where the deviation at \( t < 10 \text{ ns} \) is missing.
The corresponding non-Gaussianity, $\alpha_2(t)$, is presented in Figure 3 (b). For all fully protonated samples we observe finite non-Gaussianity, $\alpha_2(t) > 0$ for low Fourier time. If the tail is contrast matched, we find $\alpha_2(t) = 0$ for the full-time window of our NSE experiment. In other words, non-Gaussianity is directly related with the motion of the tail groups.

Figure 3 (a) Normalized mean square displacement, $\langle (\Delta r(t))^2 \rangle_N$, vs. Fourier time, $t$, for 0.1%, 1% and 5% h-DOPC, 10% h-DSPC, 1% h-DMPC and 5% h-SoyPC samples, adopted from our previous study. The data for 10% dt-DMPC/DSPC mixture and 10% dt-DPPC are calculated using $S(Q,t)/S(Q)$ from the literature. The dashed lines represent the experimental power-law dependence, filled circles from MD simulation for h-POPE. The solid lines represents the calculation for thickness fluctuation from equation 6 for dt-DPPC (black) and dt-DMPC/DSPC (green), as explained in the text. (b) The corresponding non-Gaussian parameter $\alpha_2$.

The representation of $S(Q,t)$ by $\langle r^2(t) \rangle$ and its power-law dependence, $\langle (\Delta r(t))^2 \rangle \propto t^x$, ($x = 0.26$ or 0.66 in the time region displayed) emphasize the fact that at least three different processes contribute to the relaxation within the length- and time scale of the NSE experiments. The absence
of the \(t^{0.26}\)-region if the tails not visible to the neutrons (contrast matched) is an experimental evidence that the associated \(S(Q,t)\) is connected solely with the dynamics fatty acid tails. The appearance of three different regions in \(\langle r^2(t)\rangle\) emphasizes the importance to analyze the data with a function that goes beyond the simple height-height correlation model traditionally used in the respective literature.

With the experimental evidence of the existence of the fast-local tail motion that determines the fast relaxation we can analyze the experimental results more in detail. In a next step we will explore the motion of the tail group more in detail. More precisely, we display \(A(Q)\) as obtained from the fit of the experimental data by equation 7. Additionally, we included \(A(Q)\) or the equivalent EISF from the QENS data.\(^8\) In both cases, a stretched exponential function was utilized to describe the dynamics of individual lipid tails, but not the pair correlation function of lipid tails. Thus, a comparison is possible, at least as a first attempt to accomplish a broader understanding.

**Figure 4** (a) presents the results for NSE. We modeled the data by a sphere and by a cylinder. The fit values are listed in **Table 1**. However, only a dynamic Guinier plateau is visible in our NSE data. This is to be expected, because the bilayer thickness fluctuations correspond to \(Q_0 \approx 0.091 \, \text{Å}^{-1}\). From this value we estimate a dynamic length \(2\pi/Q_0 = 69 \, \text{Å}\).\(^6\, 23\) Equation 1 assumes the motion of a single lipid tail, which is less than half of the distance between the heads in the inner and outer leaflets. In other words, \(Q_0\) at least doubles, which indicates that our NSE experiments did not reach the dynamic Porod region or even the transition to the dynamic Porod region. The appropriate length-scales are accessible by QENS experiments, which easily access \(Q > 0.2 \, \text{Å}^{-1}\). Therefore Figure 4 (b) includes QENS data.

The data in Figure 4 (a) is modeled using the ESIF for a particle confined in a sphere and for a cylinder. Both equally well describe the experimental results. The corresponding fit
parameters are reported in Table 1. It should be noted that, for some the samples where the radius is less than equal to the length of the cylinder, a motion confined to a cylindrical potential could also be represented by an ellipsoidal symmetry. However, our experimental results do not permit to make such a detailed analysis.

Assuming a cylinder and realizing that the crossover to the diameter is far outside the NSE Q range, we can only determine the length of the cylinder, to be between 1.4 Å and 2.7 Å for the different lipids, whereas, the length of the individual lipid molecule, δT/2, is between, 11 Å and 21 Å (Table 1). This comparison indicates that the confinement is caused within ~ 1/8th the length of the lipid tail, which is approximately the size of the CH₂ or CH₃ part of the acyl group of the fatty acid.⁶¹

Figure 4: (a) The A(Q) obtained from modeling the NSE relaxation spectra following equation 6. The solid and dashed lines are fits using the EISF for a particle diffusion in a sphere and cylinder.
models, respectively. (b) The $\mathcal{A}(Q)$ for h-DMPC obtained from NSE and QENS study, over a broad $Q$-range. The data is modeled using models for a sphere, cylinder in comparison with three and two site jump models. The error bars representing one standard deviation.

In order to extend the length ($Q$-range) and time scale of the observed dynamic confinement Figure 4 (b) includes the EISF obtained from quasi-elastic neutron scattering (QENS) experiments. The data from NSE and QENS are modeled simultaneously.

The fatty acid tails are mobile objects. Thus, several processes could account for $\mathcal{A}(Q)$. A spherical potential, a lipid confined to a cylinder, a two-site jump model of the lipid tails, which is related to rotational diffusion of the head perpendicular to the bilayer, and three site jumps of the protons in the methyl group. The protons in the methyl group are highly likely, because a total of 5 methyl groups (2 in the tails, and 3 in the head group contribute to the signal). The results are displayed in Figure 4 (b), the fitting values are listed in Table 1.

We can describe the experimental data by a two-site jump model choosing a radius, of 1.5 Å (solid red line), whereas the three-site jump model is calculated for using 1.34 Å (solid blue line) and 0.99 Å (dashed blue line). The last value represent the distance from each H-atom of a methyl group to the center of gravity is 0.99 Å. These are the values where we find the closest match to the experimental results. However, we witness notable discrepancies. Therefore, despite these motions exits, their contribution does not strongly affect the experimental data.

The diffusion inside a cylinder with length $L = 3.72 \pm 0.2$ Å and radius $R_L$ set to 0.5 Å yields the best description. From the fit of the dynamic Guinier range only, we obtain $L = 3.73 \pm 0.4$ Å. These values are very close to an independent QENS study on h-DMPC by Wanderlingh et al. who report $L = 3.73$ Å and $R_L = 4.25$ Å. The diameter of the cylinder is very close to the
distance between two CH₃ groups in the fatty acid tail. However, we note that these values are only an estimate, because even the QENS experiment does not resolve the dynamic Porod region.

The length of the fully extended tail of h-DMPC is between, 11 Å and 13 Å (δ₁/2 in Table 1), our observed length of the cylinder ~ 1/3rd of that. This indicates a strong confinement inside the lipid bilayer. It should be noted that all these length scales correspond to a dynamic confinement length, rather than the static lengths. The dynamic length of a lipid is not expected to match the static value. These values agree very well. However, the well fitting results from NSE and QENS confirm our assumption that we can model A(Q) from NSE and QENS for this particular case of the lipid tail motion simultaneously.

The importance of the spherical confinement for the lipid motion has been extensively studied using QENS. Previous QENS study have revealed the existence of solvation cage for the whole lipid molecule in the fluid phase, whereas, the motion of the lipid tail is highly heterogeneous in nature. It was also suggested in combination of MD simulations and QENS that this dynamic heterogeneity originates from the fact that in a spherical confinement the proton diffusion is greater at the chain ends than at the glycerol backbone.

Table 1: The calculated values of κₜ as obtained from Figure 5 for a given temperature, T and comparison with literature values. The estimated trapping, τ, time from Figure 1. The gel-fluid transition temperature, Tm, of the lipids are reported in the literature. The A(Q) and EISF parameters for the radius sphere, R, the radius, Rₗ, and length, L, of the cylinder, respectively, as described in equation 8. Here δ_T is the thickness of the lipid tail regime across the bilayer, i.e. length of fully extended chain is δ_T/2.

| Samples | Tm (°C) | T - Tm (°C) | τ (ns) (estimated) | κₜ/k_BT (literature) | κₜ/k_BT (literature) | A(Q), EISF | EISF Cylinder | Rₗ (Å) | L (Å) | Lipid tail |
|---------|---------|-------------|-------------------|---------------------|---------------------|-------------|---------------|--------|-------|-----------|
|         |         |             |                   |                     |                     |             |               |        |       |           |
We note an obvious difference to bicontinuous microemulsions in which diffusion is absent. There, $\langle r^2(t) \rangle \propto t^{0.66}$ which indicates that only height-height correlations can be found. Thus, the analysis by the ZG model, or the asymptotic approach,\(^{69}\) or the more sophisticated MS model\(^{52}\) is valid. On the other hand, it becomes clear that our results indicate that the analysis by a simple ZG model (without taking into account additional effects) is not sufficient and necessarily leads to inaccuracies in the parameters. Since the ZG model is very common in the literature, we now attempt to estimate the errors involved in neglecting the local lipid motion.
For that purpose, we use equation 14 to determine the bending rigidity, \( \kappa_\eta/k_B T \), as a function of the Fourier time from \( \langle \Delta r(t)^2 \rangle_N \) in Figure 3. The results are illustrated in Figure 5. It is obvious that \( \kappa_\eta \) has a pronounced time dependence, initially proportional to \( t^{1.22} \), for \( \kappa_\eta/k_B T \propto t^{2-3x} \), \( x = 0.26 \). The constant full lines represent the expectations from the ZG model, \( t^0 \). We included those values from the analysis of our data by the ZG model and added the bending rigidities determined from the multiplicative approach (equation 8).

At the first glance even the more advanced model seems to have some discrepancies with the experimental data. However, this is related to the fact, that the calculated \( \kappa_\eta \) represents all motions, including the translational diffusion.

One can expect a constant value for \( \kappa_\eta/k_B T \) over the calculated time window. However, the strong deviation from the constant value at \( t < 5 \) ns is a result of the finite non-Gaussianity, \( \alpha_2(t) \neq 0 \). The average value of \( \kappa_\eta \) in the ZG regime is presented in Table 1. The deviation from the \( t^0 \) prediction of the ZG model suggests presence of additional dynamics.\(^{70,71}\)
Figure 5: The membrane rigidity calculated over the entire NSE time window from the MSD using equation 14. The data for protonated and partially deuterated lipids are presented for comparison. The error bars represent one standard deviation in a log-log plot. The NSE data for DMPC, DPPC and DSPC are adapted from the literature.6, 8, 23 The NSE data for DOPC, Soy-PC, DSPC are from our previous study.29

5 CONCLUSION

We presented experimental evidence of the existence of constrained local dynamics inside the lipid bilayer using neutron spin echo spectroscopy (NSE). A comparison of the MSD from fully protonated and tail contrast matched phospholipids shows the absence of the $t^{0.26}$ power law. Experimental result and analysis relate the fast time dynamics very strongly to the motion of the lipid tails. In addition, it demonstrates the need for the advanced model function derived in the present work. We demonstrated the limitation of the ZG model to a finite time range between a fast and a slow motion. The slow motion was identified to be the translational diffusion of liposomes. If not included then the overall relaxation behavior is not analyzed correctly, especially at long Fourier times. The analysis of the fast dynamics connects the dynamics of the lipid tails with a very confined motion. It cannot be described by the ZG model that assumes height-height correlations. Independently of its origin it needs to be included in the considerations, otherwise the fit provides wrong values for the bending elasticity. Furthermore, our results demonstrate that the need of a better understanding of neutron spectroscopic data, e.g., by including parameters like the translation diffusion of liposomes from dynamic light scattering. For example, if the time range of the NSE experiment is too limited, then DLS is the only means to determine the most accurate value, but NSE can utilize it to improve the accuracy of the result on the bending elasticity.
The simplest model that is compatible with our data at fast Fourier times is a potential with cylindrical symmetry. Our analysis emphasizes the importance of following the motion of the lipid tails over a broad range of length-scales. The present paper advances the understanding, by relating the term trapped motion to confined motion. This is the first experimental evidence that identifies the origin and the nature of the trapped motion in the bilayer over multiple length and time scale.

In addition, the availability of experimental data in a broad range could advance older literature, e.g., in which the confined motion of lipids was described by a spherical potential using a distribution of confinement sizes. In other words, the results strongly indicate that the lipids relax in a cylindrical confinement. It is very astonishing that the dynamic length scale represents only around about 1/3rd the length of the lipid tail.

The MSD shows power laws $t^n$ with $n < 1$. These so-called sub-diffusive motions are assumed to be important for cellular signaling and regulatory process. Transient trapping or the confined motion has a power law with $n = 0.26$. There are numerous examples that connect transient trapping to biophysical processes. (i) It has been reported that it is important for compartmentalization of mRNA into smaller subcellular regions in living cells. Clustering of “gene encoding interacting proteins” in this confined space facilitates a transfer of genetical information between living cells. (ii) It has been shown that the length scale associated with transient trapping corresponds to the distance that proteins move to find binding sites on DNA. (iii) A similar phenomenon has also been observed for transmembrane proteins that recognize specific adaptor molecules for binding. (iv) Recent studies on potassium channels of the plasma membrane of living cells have demonstrated the anomalous nature of the diffusion following a transient trap defined by CTRW model described by the observed non-Gaussianity.
It should be noted that following the CTRW model by Akimoto et al.\textsuperscript{34} the importance of dynamic heterogeneity behind the origin of transient trapping of the lipid tail, where the lipid tail in the fluid phase are disordered and randomly oriented, similar to that observed in colloids\textsuperscript{75} and glassy materials.\textsuperscript{76} The ability to identify the confined motion in experimental data, to analysis it and to study the impact of different environments is important and stimulates future studies.

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**Notes**

The authors declare no competing financial interest.

**ACKNOWLEDGEMENT**

The neutron scattering work is supported by the U.S. Department of Energy (DOE) under EPSCoR Grant No. DE-SC0012432 with additional support from the Louisiana Board of Regents. This paper was prepared as an account of work sponsored by an agency of the United States Government. We would like to acknowledge Dr. Antonio Faraone for assisting us with the neutron spin echo spectrometer from National Institute of Standards and Technology (NIST). Access to the neutron spin echo spectrometer was provided by the Center for High Resolution Neutron Scattering, a partnership between the National Institute of Standards and Technology (NIST) and the National Science Foundation under Agreement No. DMR-1508249. We would like to
acknowledge Dr. Piotr Zolnierzuk for assisting us with the neutron spin echo spectrometer from Spallation Neutron Source (SNS) at Oak Ridge National Laboratory (ORNL). Research conducted at the Spallation Neutron Source (SNS) at Oak Ridge National Laboratory (ORNL) was sponsored by the Scientific User Facilities Division, Office of Basic Energy Sciences, U.S. DOE.

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