Effect of gold nanoparticle incorporation into oil-swollen surfactant lamellar membranes

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ABSTRACT
An oil-swollen surfactant membrane is employed to measure the effects of incorporated hydrophobically functionalized gold nanoparticles (AuNPs) on the structure and dynamics of the membranes. While maintaining an average AuNP diameter of approximately 5 nm, the membrane thickness was varied from 5 nm to 7.5 nm by changing the amount of oil in the membrane. The membranes become softer as the proportion of oil is increased, while the thickness fluctuations become slower. We attribute this to an increased fluctuation wavelength. Incorporation of AuNPs in the membrane induces membrane thinning and softening. Oil molecules surround the nanoparticles in the membrane and help their relatively homogeneous distribution. AuNPs significantly alter the membrane’s structure and dynamics through thinning of the membrane, increased compressibility, and possible diffusion of AuNPs inside the membrane.

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I. INTRODUCTION
In biomedical applications, nanotechnologies provide unique capabilities for drug and gene delivery, diagnosis and treatment of various diseases, biosensors, imaging, and disease therapy. The most clinically established system at the nanometer scale is liposomes, which are single or multi layered lipid bilayer compartments. Since liposomes are biocompatible, biodegradable, along with the capacity for surface and size modification, they are considered an almost ideal drug-carrier system. More importantly, the system can contain both hydrophilic and hydrophobic drugs for delivery, while encapsulation of chemical agents can also reduce toxicity. These systems have been intensively studied due to these potential biomedical applications, and many review articles appear in the literature. A new generation of liposome applications, external or environmental stimuli responsive liposomes, has been designed and prepared for targeted drug release with various imaging capabilities. For such applications, organic, inorganic or metal nanoparticles (NPs) are encapsulated into liposomes to probe new functionality potential.

Among other nanoparticle technologies, gold nanoparticles (AuNPs) are particularly interesting due to their size, shape-, and geometry-controlled synthesis, along with their receptiveness to surface modification. Furthermore, they have distinct electronic properties with tunable optical and x-ray absorption. These properties have various applications in catalysis, optoelectronics, biosensors, drug delivery, cancer therapy, imaging, and diagnostics. As the AuNPs can have either a hydrophilically or hydrophobically functionalized surface, these particles can be dissolved in either polar or non-polar conditions. This characteristic allows the nanoparticle, when incorporated into living organisms for example, to have the ability to accumulate in the cytosol or to insert itself into the cell membrane. Understanding the interaction between cell membranes and AuNPs is, thus, an emerging interest.

According to a coarse-grained molecular dynamics simulation, AuNPs with size smaller than a lipid bilayer thickness are captured in the bilayer, while larger NPs with larger lipid-NP interactions, escape from the bilayer. During this escape, the AuNPs capture some of the
lipid molecules from the bilayer, which is a potential reason for cytotoxicity from resulting changes to the membrane composition. The smaller incorporated AuNPs remain in the membrane unless they cluster, whereupon the membrane distortion due to AuNPs in the bilayer is revealed. These modifications to the membrane shape can induce changes in the membrane deformation energy. This consists of two contributions from energy penalties associated with membrane bending and compression. When spontaneously curved bilayers are deformed the bending free energy increases, which is proportional to the bending elastic modulus. Similarly, changes to the lipid bilayer thickness are associated with an increase in the compression free energy, which is proportional to the membrane’s compressibility modulus. The insertion of AuNPs in a lipid bilayer is known to change the deformation free energy by modifying the distribution of the alkyl tails of the lipid molecules around the inserted AuNPs, thus, altering these elastic constants.

The membrane’s elastic constants dictate the thermal fluctuations of membranes. When AuNPs are attached to giant unilamellar vesicles, Montis et al. observed slowdown of the lipid molecular motions which was considered as a rigidification of the lipid bilayers. More direct observation of membrane dynamics using neutron spin echo (NSE) spectroscopy showed a softening of lipid membranes when silica nanoparticles are attached to the lipid bilayers. Lipid bilayers with incorporated hydrophobically functionalized AuNPs were studied in terms of the structural and thermal analysis and von White and colleagues suggested a rigidification of the lipid vesicles, while dynamics measurement by NSE showed softening of the membrane. The authors for the latter speculated that the membrane softening is induced by increased membrane compressibility resulting from the incorporation of AuNPs in the bilayer. Although it is known that the interactions between membranes and NPs affect not only the elastic properties of the membranes, but also their fluidity, the influence of NPs on membrane dynamics is not fully understood. It is likely that changes in the conditions, such as charge, particle size, and lipid geometry will affect the dynamical behavior of such membranes.

Lipid bilayers are one of the ideal targets for membrane structure and dynamics studies; however, they form metastable structures, i.e., multilamellar or unilamellar, and the phase behavior of these lipid membranes may be affected by the incorporation of NPs. In order to apply the NSE technique to measure membrane’s elastic properties, large unilamellar vesicles are used after undergoing the extrusion procedure. It has proven difficult to incorporate nanoparticles into membranes due to phase transition of lipid membranes and the aggregation of nanoparticles both inside and outside the membrane. Furthermore, the weak scattering intensity from unilamellar vesicles has limited detailed studies of the membrane dynamics.

Here, in order to overcome these difficulties, we have used more simplified membranes, composed of a nonionic surfactant, oil, and water. These membranes are thermodynamically stable and the membrane thickness can be easily controlled by varying the amount of oil in the membrane while maintaining the overall lamellar structure. In addition, the scattering intensity is in general strong in the surfactant systems, which allows determination of dynamical parameters with better counting statistics. Hydrophobically modified AuNPs are easily dispersed in the oil phase, so encapsulation in the membrane is straightforward. Yet, at the same time, the surfactant membrane dynamics in this system are fundamentally similar to lipid vesicles.

Thin elastic sheet theories are well applicable to both these surfactant and lipid membranes, and therefore, the present system well serves as a model membrane for studies into the applications of NP incorporation.

II. METHODS
A. Material

Pentaethylene glycol monododecyl ether, C_{12}E_{8}, with a purity of >98% was purchased from Nikko Chemicals Co., Ltd. In order to control neutron scattering contrast to emphasize scattering signal from different parts of membranes, both protiated and deuterated solvents were used in the present experiments. The n-octane-d_{18} (C_{8}D_{18}), with a purity of 99% and the deuterium oxide, D_{2}O, with a purity of 99.9% were purchased from Cambridge Isotope Laboratories, while the n-octane (C_{8}H_{18}) with a purity of ≥99% was purchased from Sigma Aldrich. The octadecylamine (C_{18}H_{35}N; ODA) functionalized AuNPs were synthesized in order to facilitate hydrophobic character of the AuNPs. Transmission electron microscopy (TEM) observation showed a mean AuNP diameter of (49 ± 12) Å. A 95% confidence interval of the diameter is from 45.7 Å to 52.3 Å. Thermal gravimetric analysis showed about 20% mass of the AuNPs was from ODA, which allowed us to estimate the grafting density of ODA on 49 Å AuNPs of about 10 molecules/nm^{2}.

Dynamic Light Scattering (DLS) measurements were conducted on 0.1% volume fraction solutions of AuNP dissolved in C_{8}H_{18} being measured for 10 acquisitions of 50 s at 28 °C. The particle diameter and degree of polydispersity in solution were extracted as 69 Å and 16%, respectively. The estimated hydrodynamic radius from DLS suggests that the ODA extends into the octane phase and the hydrodynamic radius of the AuNP becomes larger than that estimated from the TEM image where the greatest contrast is from the Au core. As seen subsequently, the thickness of the oil-swollen surfactant membrane is roughly the same dimension as the diameter of the AuNPs.

In the present experiments, we have used an oil-swollen lamellar phase to incorporate AuNPs in the membranes as we can easily test the hydrophobic mismatch between the membrane and the AuNP, without changing the size of the particles. The ODA coated AuNPs were successfully dissolved in octane as DLS measurements showed monomodal particle size distribution, and as such, we expect that the nanoparticles are preferentially dispersed in the oil region in the oil-swollen membranes. According to previous experiments on C_{12}E_{8}/octane/water systems, the oil-swollen lamellar phase is stable across a wide range of oil content in the membrane when the surfactant volume fraction $\phi_{s}$ is fixed at $\phi_{s} = 0.041$ and the amount of oil controls the bilayer thickness. In the present experiments, we changed the bilayer thickness by changing the amount of oil in the membrane to see the effects of membrane-AuNP hydrophobic mismatch on the membrane’s structure and dynamics. The values of $\Psi = \phi_{s}/\phi_{o}$ were selected from 0.3 to 1.25, while $\phi_{o} = 0.041$ was fixed for the present measurements, where $\phi_{o}$ is the volume fraction of octane. The Au to C_{12}E_{8}, molar ratios, $X_{Au} = [Au]/[C_{12}E_{8}]$, ranged from 0 to 1 x 10^{-3}. For example, the mass composition of each component for $\Psi = 0.3$ and $X_{Au} = 1 \times 10^{-3}$ was AuNP of 19 mg, C_{12}E_{8} of 38 mg, C_{8}D_{18} of 9.74 mg, and D_{2}O of 1.0475 g. This corresponds roughly to a volume fraction of AuNPs in the solution on the order of 10^{-6}, while the
lamellar membrane is about 2%. Therefore, the scattering intensity is dominated by the lamellar structure and we safely neglect the intensity contribution from the AuNPs in the scattering experiments.

B. Small-angle neutron scattering

The solution microstructures were quantitatively characterized by small-angle neutron scattering (SANS) measurements which were conducted on the NGB-30m and NG7–30m SANS instruments at the NIST Center for Neutron Research (NCNR). Data were collected over a momentum transfer, \( q \), range between 0.003 Å\(^{-1}\) and 0.55 Å\(^{-1}\) using neutrons with a wavelength of \( \lambda = 6 \) Å and a wavelength distribution \( \Delta\lambda/\lambda \approx 0.14 \), where \( q = 4\pi \sin (\theta/2)/\lambda \) and \( \theta \) is the scattering angle. The temperature was maintained at a constant 28 °C using a water circulation bath with accuracy greater than 0.1 °C, while the samples were contained within quartz banoj cells with a 1 mm path length. In order to emphasize the membrane structure, \( {\text{C}}_{8}{\text{D}}_{18} \) was used to form the oil layer so that the neutron scattering contrast of the surfactant–surfactant correlations was enhanced. Maintaining a constant \( \Psi \), a series of samples containing progressively greater mole fractions of AuNP were prepared. The \( X_{\text{Au}} \) selected for the SANS measurements was 0, 2.5 \times 10^{-5}, 1 \times 10^{-4}, 2 \times 10^{-4}, 4 \times 10^{-4}, and 1 \times 10^{-3}. The raw two dimensional data were corrected for background scattering, azimuthally averaged, and normalized to an absolute intensity using the SANS data reduction software developed at NIST. The fit of the experimental SANS data was performed using the SASview software employing the DREAM algorithm to evaluate better parameter correlations and uncertainties.\(^{32}\)

C. Neutron spin echo

Neutron spin echo (NSE) spectroscopy offers a unique way of examining membrane motions on the nanometer and nanosecond scales, which is well suited to measure collective membrane fluctuations of these systems. The measurements were conducted on the NGA-NSE spectrometer at the NCNR\(^{11,14}\) using \( \lambda = 6 \) Å, 8 Å, and 11 Å with a wavelength spread of \( \Delta\lambda/\lambda \approx 0.18 \). The \( q \) range was covered from 0.04 Å\(^{-1}\) to 0.25 Å\(^{-1}\), while the measurement time scale \( t \) was between 0.1 ns and 100 ns. The samples were contained in demountable titanium cells using quartz windows with a 1 mm path length, and the temperature was maintained at 28 °C using an oil circulation system. By selectively deuterating the system, we could focus on different types of collective membrane fluctuations. The \( \text{C}_{12}\text{E}_{5}/ \text{C}_{8}\text{H}_{18}/\text{D}_{2}\text{O} \) system provides the scattering contrast of the entire membrane with respect to the solvent \( \text{D}_{2}\text{O} \) and is thus sensitive to the membranes’ undulation fluctuations in the measured \( q \) and \( t \) ranges. On the other hand, the \( \text{C}_{12}\text{E}_{5}/\text{C}_{8}\text{D}_{18}/\text{D}_{2}\text{O} \) system provides additional scattering contrast to see the correlations between surfactant monolayers in an oil-swollen surfactant membrane. Thus, this contrast is sensitive to thickness fluctuations in addition to the undulation fluctuations.\(^{32}\) The NSE data were corrected for the polarization of the incoming neutron beam, the resolution function determined by scattering from carbon powder (used as a standard elastic scatterer), and the contribution from the scattering of the bulk \( \text{D}_{2}\text{O} \). This was all done using the Data Analysis and Visualization Environment (DAVE) reduction software.\(^{45}\)

III. RESULTS

A. Structure

1. Effects of oil incorporation

Figure 1(a) represents the \( \Psi \) dependence of SANS profiles for membranes without incorporated AuNPs. Although the clear elastic scattering peak from the inter-lamellar spacing, which is typically seen in high concentration or well defined lamellar structures, was not observed in the present SANS experiments, the lamellar phase for each sample was confirmed by its optical anisotropy using crossed polarizers prior to the neutron experiments. When \( \Psi \) is increased at \( X_{\text{Au}} = 0 \), the features seen at high \( q \) that originate from the form factor of the lamellar membranes shift to lower \( q \). This result indicates that the bilayer thickness increases with increasing the amount of oil in the oil-swollen surfactant membrane, which is consistent with the previous results.\(^{33}\) The high \( q \) region was fitted to a form factor model for lamellar bilayers derived by Nallet et al.\(^{36}\) which permitted quantification of the average bilayer thickness, \( d_{m} \). This model was developed to fit the scattering intensity for a randomly oriented lyotropic lamellar phase in solution. The solid curves in the figure indicate the respective fit results with fit parameters of the surfactant and oil layer thicknesses,

\[ d_{m} = \frac{2}{\pi} \frac{q^{2}}{\lambda^{2}} \]
and $d_w$, the scattering contrast of the oil layer, $\rho_o$, the oil layer thickness polydispersity, $\rho_d$, scale factor, $I_o$, and the incoherent background level, $I_{inc}$.

As $\Psi$ increases, we see an increasing trend of the surfactant layer thickness, $d_s$. However, the reliability of the estimated single layer thicknesses is less than that of the total bilayer thickness $d_m$, which is calculated as $d_m = 2d_i + d_s$, and shown in Fig. 1(b). The solid line is a linear fit and the dashed lines indicate the range of the 95% confidence interval. The $C_{12}E_5$ bilayer thickness at $\Psi = 0$ with a 95% confidence interval was estimated to be $(34.9 \pm 3.1) \text{ Å}$, which is consistent with the previous estimates.33,47

2. Effects of AuNP incorporation

Figures 2(a)–2(d) show a series of SANS profiles for $\Psi = 0.3$, 0.6, 0.9, and 1.25, respectively, with varying $X_{Au}$. The data display similar scattering behavior with increasing AuNP content. A notable feature of the SANS scattering data is the shift to higher $q$ values of the dip position that occurs at the length scale corresponding to the average bilayer thickness, $d_m$. Each plot in Figs. 2(a)–2(d) is also displayed with the corresponding fit to the Nallet model.46 The shift of the form factor dip location to higher $q$ means the bilayer thickness decreases with increasing AuNPs.

The estimated values of $d_m$ are shown in Fig. 3(a). At small $\Psi$ ($\Psi = 0.3$), where bilayers are less swollen by oil, the value of $d_m$ is almost constant. However, as we increase the amount of oil in the membrane, $d_m$ starts to depend on $X_{Au}$. The membrane gets thinner as we increase the amount of AuNP. This contrasts with studies of AuNP loaded lipid bilayers when an increase in the bilayer thickness with increasing AuNP has been observed.27,28 Chakraborty et al. also pointed that the lipid bilayer thickness changes depend on the particle size, thickening when the particle size is relatively large with no significant change in the thickness for smaller particles.28 As we observed thinning effects of AuNPs, the existence of oil in the membrane clearly affects the membrane structure with incorporated AuNPs.

![Fig. 2. Small-angle neutron scattering profiles for oil-swollen surfactant system forming lamellar structure with incorporated AuNPs in the membrane for both $\Psi$ and $X_{Au}$ dependencies for (a) $\Psi = 0.3$, (b) $\Psi = 0.6$, (c) $\Psi = 0.9$, and (d) $\Psi = 1.25$, where $I_{inc}$ was subtracted from the observed scattering intensity. The arrow around the dip location suggests a general shift of the dip location to higher $q$. Solid lines are the results of the fit to a form factor model lamellar membrane. The parenthesis in the legend shows a shift factor applied to the scattering intensity for clarity. Some error bars are smaller than the symbols.](image-url)
Furthermore, we have estimated the oil layer thickness polydispersity \(pd\) by fitting the data assuming a Gaussian distribution of the bilayer thickness as shown in Fig. 3(b). The values of \(pd\) for \(\Psi = 0.3\) samples are significantly bigger than the values estimated for the other samples. This suggests that the sample at \(\Psi = 0.3\) has a significantly large heterogeneity in terms of the oil distribution. In addition, the scattering contrast of the deuterated oil region is not significant enough, and estimation of the oil layer and its polydispersity could be more difficult compared to the others. The inset to Fig. 3(b) presents a close-up of the \(X_{Au}\) dependence of the \(pd\) for \(\Psi = 0.6\) to 1.25 samples. In general, the polydispersity becomes slightly smaller if not constant as we add more AuNPs in the membrane. We expect the results of the \(X_{Au}\) dependencies of \(dm\) and \(pd\) to relate to the distribution of AuNPs in the membrane, which will be discussed later in this paper.

B. Dynamics

1. Undulation fluctuations

Figure 4(a) displays typical results of the NSE experiments, which measure the normalized intermediate scattering function \(I(q, t)/I(q, 0)\) of the system for \(C_{12}E_5/C_8H_{18}/D_2O\). The solid lines in the figure show fit results according to a single membrane fluctuation model proposed by Zilman and Granek (ZG),\(^48,49\)

\[
\frac{I(q, t)}{I(q, 0)} = \exp \left[-\left(\Gamma_{ZG} t\right)^{2/3}\right],
\]

where \(\Gamma_{ZG}\) is the relaxation rate for the single membrane undulation. ZG theory predicts that a non-interacting thin sheet depicts a relationship between the relaxation rate of the bending fluctuations, \(\Gamma_{ZG}\), and the bending modulus, \(\kappa\), as,

\[
\Gamma_{ZG} = 0.025\gamma_A \sqrt{\frac{k_B T}{k_B T}} \gamma_A \eta_{eff}^{2/3},
\]

where \(\gamma_A\) is the bending rigidity of the membrane.

![FIG. 3. Fit parameters from the SANS measurements. The \(X_{Au}\) dependence of (a) thickness \(dm\) and (b) its polydispersity \(pd\) for the oil-swollen surfactant membrane. The inset to (b) is to show the dependences from \(\Psi = 0.6\) to 1.25 samples. Some error bars are smaller than the symbols.](image)

![FIG. 4. (a) Typical example of the intermediate scattering function, \(I(q, t)/I(q, 0)\), measured by NSE for \(C_{12}E_5/C_8H_{18}/D_2O\) membrane to measure bending fluctuations. The sample is for \(\Psi = 0.3\) and \(X_{Au} = 0\). The solid lines are the fit results to the Zilman and Granek theory [Eq. (1)]. (b) The observed relaxation rate, \(\Gamma\), for the sample. The solid line indicates the fit to a power law with the power of 3. Some error bars are smaller than the symbols.](image)
where \( \eta_{sl} \) is the effective solvent viscosity and \( \gamma_k \) accounts for the orientational averaging between the membrane plaquettes and scattered neutrons. When \( \kappa/k_BT \gg 1 \), \( \gamma_k \) approaches unity. However, when \( \kappa \approx k_BT \), it has been suggested that the full form of \( \gamma_k \approx 1 - 3 \ln (q \zeta_k) k_BT/4\pi k \), where \( \zeta_k \) is a correlation length, be used. \(^{48}\) Furthermore, Monkenbusch and colleagues proposed to analyze NSE data from surfactant membranes to solve numerically the original ZG model so that the estimation of the values of \( \kappa \) is more reliable. \(^{50-52}\) In the present treatment, though, we set \( \gamma_k = 1 \) and apply an effective solvent viscosity to be three times the D\(_2\)O viscosity \(^{53,54}\) in order to simplify the discussion. Also, this treatment is the same as our previous study on a similar lamellar system. \(^{31-33}\) Although the absolute values of \( \kappa \) may have some uncertainties, the relative change between different samples is not affected by such different treatments.

The fit results according to Eq. (1) are shown in Fig. 4(a) by solid lines and the fits are, in general, great. This quality of fit was obtained using the ZG equation [Eq. (1)] of the ZG model so that the estimation of the values of \( \kappa \) is more reliable.\(^{50-52}\) In the present treatment, though, we set \( \gamma_k = 1 \) and apply an effective solvent viscosity to be three times the D\(_2\)O viscosity \(^{53,54}\) in order to simplify the discussion. Also, this treatment is the same as our previous study on a similar lamellar system. \(^{31-33}\) Although the absolute values of \( \kappa \) may have some uncertainties, the relative change between different samples is not affected by such different treatments.

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**FIG. 5.** (a) Observed bending modulus \( \kappa \) and (b) area compressibility modulus, \( K_{\text{Aund}} \), calculated from \( \kappa \). Some error bars are smaller than the symbols.
solid lines in the figure, and the relaxation rate $\Gamma$ was extracted, which are shown in the form of $\Gamma/q^3$ in Fig. 6(b). If the dynamics solely originate from the undulation fluctuations as discussed earlier, the $\Gamma/q^3$ plot should be a constant. However, we clearly see an enhancement in the $\Gamma/q^3$ data. The peak like feature that appears at $q \approx 0.06$ Å$^{-1}$ has been attributed as thickness fluctuations and fitted with a Lorentz function as

$$\Gamma/q^3 = \Gamma_{2D}/q^3 + (\tau_{TF} q_0)^{-1} \frac{1}{1 + (q - q_0)^2/\xi^2}, \tag{4}$$

where $\tau_{TF}$ is the relaxation time of the membrane thickness fluctuations, $q_0$ denotes the position of the peak maximum for the $\Gamma/q^3$ plots, and $\xi$ denotes the half width at half maximum of the Lorentz function.

The first term of Eq. (4) originates from the bending fluctuations as shown in the previous subsection. As $q_0$ is determined by our SANS experiments, only two fit parameters $\Gamma_{2D}$ and $\xi$ are quantified. It is noted here that the upturn in $\Gamma/q^3$ observed at high $q$ in Fig. 6(b) is due to other internal membrane dynamics, such as the lateral diffusion of surfactant molecules in the membranes, and thus, is excluded from the fitting procedure.

Figure 7 shows the estimated fit parameters from the thickness fluctuations; Fig. 7(a) for $\tau_{TF}$ and Fig. 7(b) for the fractional change in the thickness fluctuation amplitude, $\sigma_d = \xi/q_0$. When $\Psi$ is increased, both $\tau_{TF}$ and $\sigma_d$ also increase. As the membrane thickness $d_m$ also increases with the amount of oil, this indicates an increase in the amplitude of the thickness fluctuations with $\Psi$ since the amplitude is expressed as $d_m \xi/q_0$. Together, these results show that the thickness fluctuations become slower with increasing amplitude as $\Psi$ is increased.

Incorporation of AuNP in the membrane slightly increased $\tau_{TF}$ with $X_{Au}$, thus the membrane thickness fluctuations become slower as more AuNP is incorporated in the membrane. On the other hand, an increase in $\sigma_d$ is clearer for the membranes with $\Psi \geq 0.6$, while at low oil content, it decreases with increasing AuNP. This indicates that the presence of the AuNPs with only a small amount of oil has a damping effect on the fluctuations of the membrane, whereas if the amount of oil between the monolayers is sufficiently large, the fluctuation amplitude is enhanced.

Statistical mechanics predicts that the area compressibility modulus relates to the fractional change in area $\sigma_d$. If the volume conservation law applies, the fractional change in area is compensated for by the fractional change in thickness. This assumption works well for lipid bilayers both in the presence and absence of oil. Then, $\sigma_d = \sigma_A$, and the area compressibility modulus can be estimated as

$$K_{Aund} = \frac{k_BT}{\sigma_A^2}, \tag{5}$$

where $A_1$ is the area per molecule. The NSE data include the contribution from the effects of finite instrumental $q$ resolution, which is not trivial to take out in the present case, so we normalize the value of $K_{Aund}$ to $K_{Athick}$ estimated for $\Psi = 0.3$ and $X_{Au} = 0$, and the relative change is compared, as shown in Fig. 8(a). With the exception of $\Psi = 0.3$, $K_{Aund}$ and $K_{Athick}$ show similar trends in terms of both $\Psi$ and $X_{Au}$. As $\Psi$ increases, $K_A$ decreases significantly. On the other hand,
Structural Dynamics

The values of \( \eta_m \) for the oil swollen surfactant membrane without AuNPs were also estimated from bulk rheology measurements, where the solution composition was set from \( \Psi = 0 \) to 1.37. The measured bulk viscosity, \( \eta_f \), was then multiplied by the bilayer thickness, \( d_m \), to calculate \( \eta_m = \eta_f d_m \). The comparison between the NSE and rheology measurements is shown in Fig. 8(c). The rheologically determined \( \eta_m \) shows a slight increase with \( \Psi \) around 0.1 nPa \( \cdot \) s \( \cdot \) m. The order of the values estimated by the NSE experiments is consistent with those estimated from the macroscopic rheology measurements. However, the NSE result clearly shows a much steeper increase in \( \eta_m \) with \( \Psi \). A potential explanation of this discrepancy is given in Sec. IV.

IV. DISCUSSION

A. Effects of oil in the membrane

In the present experiments, we observed decreases of the bending modulus \( k \) as well as the area compressibility modulus \( K_A \) with increasing \( \Psi \). Since the bilayer thickness \( d_m \) increases with \( \Psi \), the softening of the membrane thus originates from the decrease in \( K_A \). Recently, Nagle \(^{68} \) suggested that the bilayer’s area compressibility modulus should be considered like a spring for each monolayer and the bilayer compressibility modulus should be sum of the two as per the usual spring force transformation. If we use this concept, the present result cannot be explained, as the bulk modulus of oil is relatively high and the calculated area compressibility modulus of the oil layer is higher than those of surfactant monolayers. If the compressibility modulus was solely coming from the molecular volume compressibility, the oil-swollen surfactant membrane would be less compressible. However, as we have oil molecules in the membranes, they freely move within the pseudo-bilayer. Therefore, the area compressibility modulus of the oil-swollen surfactant bilayers should be considered together with molecular transport degrees of freedom within the membrane. Membranes are much more deformable in the normal plane without violating volume conservation law when oil molecules are in the membrane, i.e., more compressible membranes than those without oil. This trend is also confirmed by the increased thickness fluctuation amplitude, \( \sigma_b \), thus leading to similar conclusions from both the undulation and thickness fluctuation data sets.

As the bilayer thickness increases, the thickness fluctuation relaxation time \( \tau_{TF} \) gets larger. This indicates that the fluctuations become slower as the amount of oil in the oil-swollen bilayer is increased. This result sounds reasonable as larger objects (thicker membranes) usually move slower. However, this result leads to a contradiction in the estimation of the membrane viscosity \( \eta_m \). As the bulk rheology measurements did not give a significant oil concentration dependence, we do not expect a significant change in the membrane viscosity with increasing amount of oil in the membrane. The original theory by Bingham et al. \(^{36} \) predicts \( \tau_{TF} = (\eta_m + 2\eta_f/\Psi)/K_A \), where \( \eta_f \) is the fluctuation wavenumber. \(^{36} \) In addition to the contribution from the membrane viscosity, the solvent viscosity becomes important when the fluctuation wavelength becomes larger than \( l_{QP} \). By assuming that the estimate for \( \Psi = 0.3 \) is not affected by this contribution and the \( \eta_m \) does not change significantly with \( \Psi \), we can calculate the fluctuation wavelength \( \lambda_f \approx 2\pi/\eta_f \) at both \( \Psi = 0.3 \) and \( \Psi = 1.25 \). Due to the large wavelength fluctuations, the relaxation time of the thickness fluctuations become significantly longer at a large \( \Psi \). The discrepancy

\[ K_A \] slightly decreased with \( X_{Au} \) except for \( \Psi = 0.3 \), where \( K_{K_{Au}} \) is increased and it is not consistent with the trends observed for \( K_{K_{Au}} \). These results suggest that the incorporation of AuNPs in the oil-swollen surfactant membrane induces increased compressibility of the membrane at a relatively large \( \Psi \).

Bingham et al. \(^{36} \) predicted that the relaxation time of thickness fluctuations, \( \tau_{TF} \), depends on both \( K_A \) and the membrane and solvent viscosities, \( \eta_m \) and \( \eta_f \). As was originally discussed, for the fluctuations wavelengths shorter than Saffman–Delbrück length, \( l_{SD} = \eta_f/\eta_m \), the in-plane monolayer viscosity dominates and the damping is independent of wavelength, \(^{36} \) and in this case the relation is expressed as \( \tau_{TF} \approx \eta_m/K_A \). Figure 8(b) shows the results of calculation of \( \eta_m \) using either \( K_{K_{Au}} \) or \( K_{K_{Au}} \).

![Figure 8](image_url)

**FIG. 8.** (a) Comparison of area compressibility modulus \( K_{A_{Au}} \) and \( K_{A_{Au}} \) and (b) the membrane viscosity \( \eta_m \). (c) \( \Psi \) dependence of the membrane viscosity measured by rheology (\( \eta_f \)) and NSE (\( \eta_m \)). Some error bars are smaller than the symbols.
between rheology and NSE in estimating the membrane viscosity, thus, may originate from the fact that we did not consider a change of fluctuation wavelength, specifically at large $\Psi$, which might have led to an overestimation of $\eta_m$ for the NSE data.

B. AuNP distribution in the membrane

It has been found for lipid systems\textsuperscript{62} that the method of loading the NPs into the membrane can affect the distribution of the NPs inside the membrane. For the present measurements, the AuNP was dissolved into the oil phase and added to the surfactant before bilayer formation, and therefore, we believe the distribution of the AuNPs in the membrane is homogeneous.

The deformation free energy due to the inclusion of hydrophobic nanoparticles into membranes has been described, where the bending and stretching or compression components were considered.\textsuperscript{70} Such a deformation free energy model depicted that a condensed (aggregated) nanoparticle phase in a membrane has the minimum deformation free energy, while there is an energy barrier to reaching a homogeneously distributed (dispersed) nanoparticle state.\textsuperscript{23,67} When the hydrophobic mismatch between membrane and nanoparticle is large, i.e., membrane is thicker than the incorporated nanoparticle size, the deformation free energy gets smaller.

Rasch et al.\textsuperscript{27} found that the free energy change required to insert a hydrophobic sphere into a hydrophobic membrane from water, $\Delta G_{\text{def}}$, is roughly an order of magnitude greater than the free energy penalty incurred in deforming a lipid bilayer by inserting the hydrophobic sphere into its center, $\Delta G_{\text{def}}$. A coarse grained molecular dynamics calculation suggested that hydrophobic NPs which are smaller than lipid bilayer thickness are captured in the membrane, unless the NPs are clustered.\textsuperscript{27} In the lipid case, it has been proposed that the mechanism of incorporation involves the lipid hydrocarbon tail of each monolayer leaflet unzipping in order to accommodate the hydrophobic sphere. This creates an energetically unfavorable void space around the NPs. However, in the present oil swollen systems, the oil can fill the void space around each embedded particle and reduce the $\Delta G_{\text{def}}$ penalty without increasing the deformation of the bilayer hydrocarbon chain conformations, thus we expect that incorporation of AuNPs in the membrane is more preferable.

The present SANS and NSE results support the membrane thinning and the increased compressibility of membranes with $X_{\text{Au}}$. Therefore, we believe that the observed trends of decreasing $d_m$ and $p_d$ with $X_{\text{Au}}$ suggests that the AuNPs do not form a small number of large clusters but rather are dispersed through the membrane individually, or in small clusters, as $X_{\text{Au}}$ increases. This contrasts to a pure surfactant bilayers such as lipid bilayers, in which it can be supposed that a bilayer would favor the condensed state with the AuNPs clustered together to minimize the energy penalty from the void space.

C. Modification of membrane dynamics by AuNPs

It has been widely thought that the insertion of a rigid additive into a soft membrane should stiffen the membrane.\textsuperscript{1-7} There have been many attempts to confirm the effect of inclusions on the membrane stiffness. Incorporation of cholesterol in lipid bilayers is a typical system that sees stiffening of the membrane.\textsuperscript{62,74-77} n-Alkane and mixed lipid domains in the gel phase are other examples of membrane stiffening.\textsuperscript{76,79} However, n-alkanes and mixed lipids in the fluid phase show the opposite trend, i.e., softening of the bilayer.\textsuperscript{57,63} Moreover, various proteins and peptides also display softening of membranes.\textsuperscript{80-85} There are a couple of theoretical considerations that explain softening of membranes by incorporation of rigid inclusions. Leibler suggest that the presence of diffusing particles coupled to the local curvature of the membrane can reduce the rigidity.\textsuperscript{86} This may be the case for the present system of AuNPs at low $\Psi$. Another proposal is that there is a modification of the structural arrangement of lipid molecules around the inclusions.\textsuperscript{87} In a previous NSE experiment on lipid vesicles, it was shown that hydrophobically functionalized AuNPs soften the lipid bilayers,\textsuperscript{88} which may be caused by the modification of structural arrangement of lipid molecules. However, in the present oil-swollen lamellar membranes, this mechanism is not a likely origin of the softening as the present system already disrupted the conformation of the surfactant molecules due to the existence of the oil molecules. A likely mechanism is a change of membrane compressibility due to rearrangement of oil molecules surrounding the incorporated AuNP.

On one hand, the change in the thickness fluctuation amplitude is consistent with the change in the area compressibility modulus of the oil-swollen surfactant membranes, but on the other hand, the change in the relaxation time $\tau_{\text{FP}}$ with $X_{\text{Au}}$ needs more detailed consideration. As the fluctuation wavelength is likely increased when the amount of oil in the membrane is increased, the AuNP inclusions can affect the fluctuation wavelength as well. If we assume that the change of $\tau_{\text{FP}}$ comes from the change in the fluctuation wavelength without modifying the membrane viscosity, we estimate an approximate 20% reduction in the fluctuation wavelength. For example, for $\Psi = 1.25$ samples, $\lambda_f \approx 0.8 \mu m$ is expected at the highest AuNP loading. This suggests that the fluctuation wavelength becomes shorter while thickness fluctuations get slower. The opposite trends from the observation of $\Psi$ dependence suggest that there may be another mechanism for inducing such modifications of the thickness fluctuation dynamics.

Another consideration is the effect of AuNP diffusion in the membrane, which also modifies the membrane dynamics. The AuNPs incorporated into membrane have a spherical shape, and applying the Saffman–Delbrück equation\textsuperscript{89} allows estimation of the diffusion constant of an AuNP particle in the membrane. The diffusion coefficient, $D_{\text{Au}}$, is given as

$$D_{\text{Au}} = \frac{k_B T}{4\pi \eta_m} \left[ \ln \left( \frac{\eta_m}{\eta_f} \right) - 0.5772 \right].$$

where $r$ is the radius of the cylindrical inclusion in the original model and defined here by the hydrodynamic radius, measured by DLS to be $r \approx 35 \AA$. At the lowest to the highest loadings for $\Psi = 1.25$, $D_{\text{Au}}$ changed from $2.9 \times 10^{-12} m^2/s$ to $4.3 \times 10^{-12} m^2/s$. This calculation suggests that the AuNPs in the membrane diffuse more quickly with increasing $X_{\text{Au}}$. In three dimensional diffusion of colloidal particles, it is known that the collective diffusion constant becomes larger when the inter-particle interaction is repulsive.\textsuperscript{86,89} Therefore, the increased $D_{\text{Au}}$ may be consistent with the structural observation where the AuNP particles are in a more dispersed condition rather than condensed state. This may be linked to the increased compressibility at higher AuNP loading as the inclusion mobility may affect the membrane compressibility modulus as well. Although speculative, an interesting outcome from the present experimental results is a suggestion
that the lateral diffusion of particles in membrane may affect the membrane dynamics. This point will be followed up in future studies.

Finally, we comment on the dependence of the structural and dynamics parameters for $\Psi = 0.3$. In order for ODA to swell in membranes, we anticipate that a minimum number of oil molecules are required to surround the AuNPs, which might have been violated at $\Psi = 0.3$. In such a case, the ODA around AuNPs may be in a shrunken state inside the membrane and the inclusions may behave differently than for the well swollen particles. In addition, the bilayer thickness for this sample is $d_{\text{bi}} \approx 40 \, \text{Å}$, which is similar to the size of the AuNP measured by TEM. However, the DLS measurement provided a hydrodynamic radius of 35 Å, and therefore, in order to incorporate the AuNP particles in the membrane, we expect a significant deformation of the bilayer. In addition, as we see from the polydispersity index data Fig. 3(b), the amount of oil in each membrane may have a significant distribution. This may lead to a partitioning of AuNPs in the membrane and affect the estimation of the dynamics parameters, specifically the thickness fluctuation amplitude. We believe that although these membranes do contain AuNPs, the estimated structural and dynamical parameters are the average of all the different environments of the incorporated AuNPs, and so not just the dynamics of a single membrane. Even so, the general trends of membrane softening and increased compressibility with increasing AuNP content are consistent with the thicker membranes with more homogeneously distributed nanoparticles.

V. CONCLUSIONS

In the present study, we have demonstrated that the incorporation of an AuNP into an oil-swollen surfactant bilayer has a significant effect on the dynamics of the bilayer and that the magnitude of these effects are dependent on the concentration of the added AuNP. An increase in $X_{\text{Au}}$ decreases the rigidity of the bilayer which could be interpreted as increased membrane compressibility. The increase in the thickness fluctuation relaxation time is considered to be a result of the increased rigidity and stiffness when the membrane thickness is increased. On the other hand, the inclusion of AuNPs may reduce the fluctuation wavelength. We have shown that NSE can successfully measure these changes to the bilayer dynamics, as well as suggesting that diffusion of inclusions may affect the membrane dynamics. We hope that the ability to successfully differentiate the change in dynamics with NP content can be used as a basis for further work on more complex NP—bilayer systems.

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DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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