Nanoplasmonic Biosensing for Soft Matter Adsorption: Kinetics of Lipid Vesicle Attachment and Shape Deformation

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ABSTRACT: An indirect nanoplasmonic sensing platform is reported for investigating the kinetics of attachment and shape deformation associated with lipid vesicle adsorption onto a titanium oxide-coated substrate. The localized surface plasmon resonance (LSPR) originates from embedded gold nanodisks and is highly sensitive to the local lipid environment. To interpret the corresponding results, we have extended treatments of diffusion-limited adsorption kinetics and adsorbate-related LSPR physics, identified the expected scaling laws for the LSPR-tracked kinetics measured at different lipid concentrations and/or nanometer-scale vesicle sizes in the case when vesicle deformation is negligible, and scrutinized experimental deviations accordingly. After adsorption, the smallest 58 nm diameter vesicles were found to maintain shape on the time scale of adsorption at high lipid concentrations in solution, and shape deformation became more appreciable at lower lipid concentrations. Higher saturation coverage was observed with increasing lipid concentration, which is attributed to the difference in relative time scales of vesicle attachment and deformation. For larger vesicles between 80 and 160 nm diameter, deviations associated with their shape deformation and correlations with the location of gold nanodisks became more apparent at moderate and high coverages. Taken together, the results obtained support that the quantitative measurement capabilities of nanoplasmonic biosensing should be considered for applications demanding highly surface-sensitive characterization of soft matter adsorption and related phenomena at liquid–solid interfaces.

INTRODUCTION

Owing to their nanoscale size, wide range of controllable properties, and deformability, lipid vesicles represent an ideal model system to investigate the adsorption of soft matter at the liquid–solid interface.1,5,6 Clarifying the behavior of vesicle adsorption may provide insights into the structural transformations associated with cellular activities such as membrane transport and vesicle fusion.3,4 An understanding of the mechanisms of such processes may also improve the fabrication of artificial lipid bilayer5,6 and native cell membrane platforms6 on solid supports. Combined with various surface-sensitive experimental techniques, these platforms enable quantitative tracking of interactions between lipid membranes and biomacromolecules, including peptides, proteins, enzymes, and nucleic acids.5,9 To this end, one of the key goals of vesicle adsorption studies has been tracking the corresponding kinetics and delineating how control over experimental parameters can be exploited for model membrane fabrication. Depending on properties of the solid support (e.g., atomic composition,10 crystallinity,11 topography12), vesicles (e.g., size,13 lipid composition,14 lamellarity,15 osmotic pressure16), and environmental conditions (e.g., ionic strength,17 solution pH,18 temperature,19 divalent cations20), vesicle adsorption may lead to the formation of a single layer of intact vesicles or to vesicle rupture and the formation of a planar lipid bilayer via one or more possible channels that are mediated by vesicle–substrate, vesicle–vesicle, and vesicle–rupture product interactions (see ref 1 and references therein). In all cases, adsorbed vesicles experience shape deformation,21–25 which is a prerequisite of rupture and the extent of which is influenced by the balance between the vesicle–substrate interaction and vesicle bending energy. Phenomenologically, the relative role of these two factors is determined by a dimensionless parameter which is defined as $p \equiv W\rho^2/k$, where $W$ is the contact energy per unit area, $k$ is the membrane bending rigidity, and $\rho$ is the vesicle radius.26 With increasing $p$, the shape of an adsorbed vesicle may vary appreciably from spherical (at $p \cong 2$) to boule-
shaped (p up to 10). With increasing coverage, the shape of vesicles may also depend on their mutual interaction.

Globally, the kinetics of vesicle adsorption is controlled by diffusion almost up to saturation because vesicle size is relatively large and the diffusion coefficient is small (see, e.g., the kinetics observed without and with vesicle rupture on gold27 and silicon oxide,27–30 respectively; for comparison, note that the rate of adsorption of proteins is often controlled by diffusion as well31 despite their smaller size). To what extent vesicles are deformed at low and moderate surface coverage and what happens near saturation are still open for debate. The uncertainty in the behavior of adsorbed vesicles is related to the low sensitivity of conventional experimental techniques to the shape of adsorbed vesicles. In particular, surface plasmon resonance (SPR) measurements detect changes in the refractive index near a metal surface within the evanescent field usually on a length scale larger than vesicle size and hence are sensitive only to the total amount of adsorbed lipid mass.28 The interpretation of quartz crystal microbalance with dissipation (QCM-D) measurements is complicated by trapped water,28 and there is poor correlation between the QCM-D response and the surface coverage of adsorbed vesicles.

Recently, direct observation of vesicle adsorption and rupture on glass was reported by using interferometric scattering microscopy (iSCAT), which combines the surface sensitivity of interferometry and lateral resolution of scattering microscopy.32 The findings provided the first direct evidence of several, previously suggested phenomena related to planar bilayer formation and were generally consistent with a model in which the formation process is governed by the balance between edge tension and the lipid–substrate interaction. In such measurements, vesicles are visualized as diffraction-limited spots due to scattering based on an interference contrast and vesicle deformation can hardly be accurately tracked. Consequently, the iSCAT approach is most appropriate for tracking object movement and structural transformations.

Another emerging highly surface-sensitive measurement technique suitable for studies of vesicle adsorption is based on the localized surface plasmon resonance (LSPR) of metallic nanostructures33–35 (e.g., nanowells,36 nanocubes,37 and nanodisks38). It detects changes in the local refractive index near the surface of such structures. In each particular case, there is a specific extinction wavelength maximum, \( \lambda_{\text{max}} \), which depends on nanoparticle characteristics, including size, shape, interspacing, and local dielectric environment. The latter can be used for biosensing on lipid membrane platforms located within a nanoparticle’s evanescent field. In particular, nanometric holes within thin gold films on glass were employed to house planar lipid bilayers36 and single tethered lipid vesicles.39 Asymmetric nanomembrane structures (i.e., nanocones with a coarse shape) with two plasmonic resonances originating from the special geometry were also reported for sensing of single tethered vesicles.40

In addition, the LSPR technique has been employed to track the kinetics of vesicle adsorption and rupture.38,41–43 On nanohole-containing gold and silver films with silicon oxide coats41,42 a monotonic increase in \( \Delta \lambda_{\text{max}} \) observed during the initial stage of adsorption was followed by an acceleration of the increase in \( \Delta \lambda_{\text{max}} \) due to vesicle rupture and formation of a planar lipid bilayer. The faster growth of the signal was related to higher sensitivity to lipids located in the bilayer due to their proximity to the substrate. A similar LSPR response was reported for vesicle adsorption and rupture on silicon oxide-coated gold nanodisks.38 Vesicle adsorption and rupture have also been observed on topographically flat silicon oxide substrates with embedded nanodisks.43 Altogether, there is strong evidence that the kinetics of vesicle adsorption can be tracked via LSPR monitoring, with highly sensitive detection of the local environment of lipids near the substrate.

However, to date, LSPR measurements focused on strictly vesicle adsorption have not been reported. Considering the experimental limitations of conventional surface-sensitive measurement techniques as described above, the high surface-sensitivity of the LSPR technique offers a compelling motivation to perform such experiments in order to clarify the kinetics of vesicle adsorption with respect to vesicle deformation. Herein, LSPR monitoring was employed to track vesicle adsorption onto titanium oxide-coated surfaces containing plasmonic gold nanodisks (Figure 1). The titanium oxide substrate was selected because it is a dielectric material suitable for indirect nanoplasmonic sensing,44 and zwitterionic lipid vesicles typically adsorb and remain intact, eventually reaching saturation coverage.13 Two governing parameters were chosen: lipid concentration and vesicle size.

In Figure 1, we show overview of lipid vesicle adsorption with indirect nanoplasmonic sensing. (a) Adsorption of lipid vesicles onto titanium oxide-coated gold nanodisks was tracked by time-resolved LSPR measurements. Left: schematic of measurement chamber. Right: vesicle adsorption onto deposited titanium oxide-coated gold nanodisks on glass substrate. (b) Atomic force microscopy imaging of deposited nanodisks on glass substrate. The scale bar is 200 nm. The inset is the height profile of a representative coated gold nanodisk. (c) There is a specific extinction wavelength maximum, \( \lambda_{\text{max}} \), which is associated with the plasmon resonance. When vesicles adsorb onto the nanodisks, there is a change in the local refractive index which causes a peak shift, \( \Delta \lambda_{\text{max}} \), and is also sensitive to the lipid morphology near the substrate.
deformation in relation to coverage. Just near saturation, the deviations are expected to relate primarily to the transition to the kinetically limited regime of adsorption. The LSPR measurements allowed us to clarify the latter regime as well.

## THEORETICAL ASPECTS

### Diffusion-Limited Adsorption Kinetics

As already noted in the Introduction, adsorption of vesicles is usually limited by diffusion almost up to saturation. Such kinetics are well-known to depend on the channel geometry and flow conditions. Mathematically, the diffusion limitation means that the adsorption rate can be identified with the diffusion flux toward the surface, and this flux can be calculated by assuming the vesicle concentration near the surface to be equal to zero (to be specific, we discuss vesicle adsorption). With this boundary condition, the concentration of vesicles in the measurement cell is described by using the diffusion equation complemented by the term taking the flow conditions in the cell into account. Phenomenologically, the diffusion flux identified with the adsorption rate can be represented as

\[ J = Dn/\delta \]  

where \( D \) is the vesicle diffusion coefficient, \( n \) is the number concentration of vesicles in solution, and \( \delta \) is the characteristic diffusion length. The gradients of the vesicle concentration take place near the surface within the distance comparable with \( \delta \). Far from the surface, the gradients are negligible, and the vesicle concentration is equal to \( n \).

In general, the diffusion equation describing adsorption should be integrated numerically. Two generic situations allowing analytical treatment are realized when the flow is negligible and appreciable. The equations corresponding to the “no-flow” case are well-known.\(^{25}\) In our experiments, we used a measurement cell with a simple geometry (Figure 1a) and relatively high flow rate simplifying interpretation of the results. Hence, we are interested in the situation when the flow oriented along the cell is appreciable. In this case, the kinetics is analytically tractable provided that the flow cell (of length \( L \)) is long and the channel cross section is rectangular (of dimensions \( h \times l \), where \( h \) and \( l \) are the channel height and width, respectively, and \( h \ll L \) and \( l \ll L \)) or circular. Under these conditions, the velocity distribution is nearly independent of the coordinate along the channel.

Let us first consider that the channel cross section is rectangular with \( h \ll l \) and adsorption takes place on one (or both) of the \( l \times L \) walls. In such a channel, the velocity distribution along the coordinate \( (0 \leq y \leq h) \) perpendicular to this wall is well-known to be represented as

\[ v(y) = 6v_0 \left[ \frac{y}{h} - \left( \frac{y}{h} \right)^2 \right] \]

where \( v_0 \) is the average flow velocity. Near the surface (at \( y/h \ll 1 \)), the second term in the right-hand part of 2 can be neglected, i.e.

\[ v(y) \cong 6v_0 y/h \]  

and then the solution of the diffusion equation yields\(^{29,46}\)

\[ J = 0.97 \left( \frac{v_0 D^2}{hx} \right)^{1/3} n \]  

where \( x (0 \leq x \leq L) \) is the coordinate along the channel. The corresponding expressions for the surface concentration and diffusion length are given by

\[ C = Jt = 0.97 \left( \frac{v_0 D^2}{hx} \right)^{1/3} nt \]  

\[ \delta = 1.03 \left( \frac{Dhx}{v_0} \right)^{1/3} \]  

where \( t \) is time.

Equations 2–6 describe the kinetics of adsorption provided the saturation is negligible along the whole channel. During this stage, the diffusion length is small near the channel entrance (at \( x \approx 0 \)) and becomes larger with increasing \( x \) as shown in the upper part of panel a in Figure 2. The adsorption rate is accordingly large near the entrance and becomes smaller with increasing \( x \). Physically, it is clear that with increasing time the adsorption at the area near the entrance will rapidly reach saturation, the whole diffusion front will be shifted inside the channel, and the adsorption rate will there increase. Although this stage of the kinetics was analyzed in the literature by

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solving numerically the diffusion equation (see, e.g., ref 47 and references therein), understanding of the corresponding kinetics is still limited. To clarify the physics behind this regime, we give here its analytical description by using the iterative procedure (the applicability of this procedure is validated a posteriori).

As the first iteration, we use eq 5 up to reaching saturation, i.e., up to $C = C_s$, where $C_s$ is the maximum surface concentration. According to eq 5, the saturation at given $x$ is reached at $AT/x^{1/3} = C_s$, where $A = 0.97(v_0D^2/h)^{1/3}n$. This means that at given $t$ the shift of the beginning of the diffusion front along the channel is $(AT/C_s)^{1/3}$, i.e., the surface is saturated at $x \leq (AT/C_s)^{1/3}$, while at $x > (AT/C_s)^{1/3}$ the diffusion flux can be calculated in analogy with eq 5 taking, however, into account that the front is shifted inside the channel as shown in the lower part of panel a in Figure 2. Following this line, we introduce this shift during the second iteration into eq 5 at $x \geq (AT/C_s)^{1/3}$ as $0 \leq y \leq h$

$$J = A/[x - (AT/C_s)]^{1/3}$$

Taking into account that $dC/dt = J$, we have

$$\frac{dC}{dt} = \frac{A}{[x - (AT/C_s)]^{1/3}}$$

To integrate this equation, it is convenient to use the dimensionless time, $\tau \equiv AT/(x^{1/3}C_s)$, for each $x$. Using this new variable, we rewrite eq 8 as

$$1 \frac{dC}{C_s} \frac{dr}{[1 - (\tau^{1/3})^{1/3}}$$

Thus, at any given $x$, the surface concentration depends on time as

$$\frac{C(\tau)}{C_s} = \int_0^\tau \frac{dz'}{[1 - (z')^{1/3}]^{1/3}}$$

According to this expression, the adsorption kinetics do become somewhat faster near saturation (Figure 2b), but this effect is modest (this justifies the iterative procedure).

If the channel cross section is circular, the velocity distribution is represented as

$$v(r) \equiv 4v_0 \left[1 - \left(\frac{r}{R}\right)^3\right]$$

where $r$ ($0 \leq r \leq R$) is the radial coordinate, $R$ is the channel radius, and $v_0$ is the average flow velocity. Near the surface ($y = R - r \ll R$), this expression is reduced to

$$v(y) \equiv 4v_0 y / R \equiv 8 \eta v y / d$$

where $d = 2R$ is the channel diameter playing the same role as $h$ in eq 3. Comparing eqs 3 and 12, one can notice that the difference between them is in the factor of 4/3. This factor is close to unity. Taking also into account that the dependence of the flux on velocity and accordingly on this factor is weak (due to the cubic root in eq 4), it can be neglected. If the channel cross section is rectangular (as treated in the case of our experiments), the situation is similar; i.e., we can use eqs 2–10.

In summary, our analysis (Figure 2b) indicates that under the flow conditions the diffusion-limited adsorption kinetics is nearly linear up to relatively high coverage, $C/C_s > 0.8$. This stage of the kinetics is described by eq 5, which was long derived in the literature. In the context of SPR, it was earlier used to describe the kinetics of protein adsorption. Our treatment clarifies the limits of applicability of eq 5. Specifically, we have shown that, at $C/C_s > 0.8$, the diffusion-limited kinetics taking into account the shift of the diffusion front inside the channel is expected to be somewhat faster compared to that predicted by eq 5. Comparing the deviations predicted with those observed experimentally may help to identify what happens near saturation (at $C/C_s > 0.8$). To employ the equations above, we recall that according to hydrodynamics the vesicle diffusion coefficient is represented as

$$D = \frac{k_B T}{6\eta \rho}$$

where $\rho$ is the vesicle radius in solution, $\eta$ is the viscosity, $T$ is the temperature, and $k_B$ is the Boltzmann constant.

**Contribution of Vesicles to the LSPR Signal.** In conventional SPR spectroscopy, the excitation of plasmons in a metal film is influenced by the surrounding media. The measured sensor response, $\Delta R$, represents a change in the angle of the incident light required to maintain surface plasmon excitation upon adsorbate-induced changes in the interfacial refractive index and is expressed in dimensionless units. The adsorbate is usually modeled as a film of thickness $H$, and the dependence of $\Delta R$ on $H$ is described as

$$\Delta R \propto 1 - \exp(-2H/L_d)$$

where $L_d$ is the decay length of the evanescent electromagnetic field. Physically, this expression can be obtained by assuming that the contribution to $\Delta R$ of the region located at the distance from $z$ to $z + d$ is proportional to the square of the electric field or, more specifically, to $\exp(-2z/L_d)$ and then integrating over $z$ from 0 to $H$ ($z = 0$ corresponds to the metal film).

In the context of LSPR, an approach similar to that outlined above was suggested in general and used to interpret the kinetics of the formation of a planar lipid bilayer on silica-coated gold nanodisks via vesicle rupture. The kinetics were tracked by measuring $\Delta \lambda_{max}$. In that particular case, the decay length of the field extending from the silica surface was estimated to be $L_d \approx 5$ nm. Here, we modify and extend the treatment in two directions. First, we notice that the electric field around metal nanoparticles does not decay exponentially. In general, the dependence of this field on coordinates may be complex and should be calculated numerically. In the case of nanodisks, the geometry is, however, relatively simple. As already earlier noticed, a physically reasonable approximation here is to replace a nanodisk by an ellipsoid (another version of this term is “spheroid”). Outside a metal nanosphere, in analogy with spherical nanoparticles, the external periodic field is well known to induce an electric field proportional to $1/r^3$, where $r$ is the radial coordinate (with respect to the center of a nanosphere). This field generates in turn the dipole moment in the medium surrounding a metal nanoparticle. The moment generated in volume $dv$ is proportional to $dv/r^3$. The latter moment in turn influences electrons in a metal nanoparticle due to the dipole–dipole interaction. The corresponding contribution to the plasmon frequency or $\Delta \lambda_{max}$ shift is proportional to $dv/r^3$. The whole shift can be obtained by integrating this contribution. Our arguments behind this prescription are heuristic. Physically, they are based on the fact that the polarization of gold nanodisks is much higher than that of lipid in vesicles. In a
more straightforward but simultaneously cumbersome way, it can be validated by directly analyzing various induced dipole–dipole interactions (see, e.g., eq 10 in ref 55 and take into account that the nanoparticle frequency shift is there determined by the denominator of the first term on the right-hand side; in particular, the shift is proportional to \( V_0^2 \), where \( V_0 \) is a geometric factor that is in turn proportional to \( 1/r^3 \)).

The second ingredient of our analysis is related to the shape of vesicles. As a first-order approximation, let us consider that the substrate-induced deformation of a vesicle is not significant and its shape is nearly spherical. If \( z \) is the coordinate perpendicular to the support, the area segment of a vesicle is known to be \( 2\pi p \, dz \) (this mathematically exact expression was used in the Supporting Information for ref 56 in the context of total internal reflection fluorescence microscopy and in the Supporting Information for ref 57 in the SPR context). The corresponding volume element is proportional to the area segment, i.e., \( dv \propto 2\pi p \, dz \). Thus, in the LSPR (or SPR) context, a vesicle can be viewed as a uniform island with thickness \( 2\pi p \).

The contribution of vesicles to \( \Delta \lambda_{\text{max}} \) can accordingly be estimated as

\[
\Delta \lambda_{\text{max}} \propto C\rho \int_0^d \frac{dz}{(R_n + z)^6} \tag{15}
\]

where \( R_n \) is the length scale characterizing the distance between the center of a metal nanoparticle and adjacent vesicles contributing to \( \Delta \lambda_{\text{max}} \) (by definition, \( R_n \) includes the dielectric layer covering the nanoparticles; taking into account that the substrate layer covering metal particles is relatively thin, \( R_n \) is expected to be comparable to the average length scale of a metal nanoparticle; note also that \( R_n \) is different for different vesicle–nanoparticle pairs due to the nonplanar and non-spherical geometry).

The power-law term \( 1/(R_n + z)^6 \) rapidly decreases with increasing \( z \). For this reason, the integration in eq 15 can be extended up to infinity. In this approximation, the integral in eq 16 is independent of \( \rho \), and accordingly we have

\[
\Delta \lambda_{\text{max}} \propto C\rho \tag{16}
\]

Taking eqs 5 and 13 into account, we can rewrite eq 16 as

\[
\Delta \lambda_{\text{max}} \propto \rho^{1/3} nt \tag{17}
\]

Concerning these expressions, we have four remarks. (i) The observed shift, \( \Delta \lambda_{\text{max}} \), is an average over different vesicle–nanoparticle pairs. The fact that \( R_n \) is different for different pairs influences the coefficient of proportionality between \( \Delta \lambda_{\text{max}} \) and \( C\rho \) (or \( \rho^{1/3} nt \)) but does not influence the proportionality itself. (ii) The scale of the interval making the main contribution to integral (15) is \( \Delta z \cong R_n/5 \) (this is clear from the shape of the function under the integral; in particular, the integral \( \int_0^d dz/(R_n + z)^6 \) approaches \( \int_0^d dz/(R_n + z)^6 \) already at \( \Delta z \cong R_n/5 \)). In our case, \( R_n \approx 75 \text{ nm} \), and accordingly \( \Delta z \cong 15 \text{ nm} \). (iii) In our analysis, we have referred to an ellipsoid approximation. In fact, the final expressions 16 and 17 are, however, applicable for an arbitrary shape of nanoparticle because the only condition one needs to derive these expressions is that the electric field near nanoparticles rapidly drops. (iv) In our treatment, vesicles are considered to be spherical. The case of appreciably deformed vesicles can be described using the formalism similar to that employed recently in the SPR context.

Expression 17 can be used in order to interpret the dependence of \( \Delta \lambda_{\text{max}} \) on \( \rho \). Let us, for example, consider that the vesicles are prepared under conditions such that the lipid mass is constant. In this case, we have \( n \propto 1/\rho^2 \). Substituting this relation into eq 17 yields

\[
\Delta \lambda_{\text{max}} \propto t/\rho^{5/3} \tag{18}
\]

This expression describes the transition to saturation. At saturation, the surface concentration of nondeformable vesicles scales as \( C \propto 1/\rho^2 \). Substituting the latter relation into eq 16 results in

\[
\Delta \lambda_{\text{max}} \propto 1/\rho \tag{19}
\]

Comparing the scaling predicted by eq 17, 18, or 19 with those measured experimentally, one can judge whether the deformation of an attached vesicle is negligible.

An additional interesting aspect of the manifestation of vesicle attachment in the LSPR signal is related to their contribution to the full width at half-maximum (fwhm). The average shift is proportional to the average number, \( N \), of vesicles contacting a nanoparticle. The fwhm is in turn proportional to \( (\Delta N)^{1/2} \). The size of vesicles is generally comparable to the size of the coated metal nanoparticles (ca. 150 nm diameter; see Figure 1b) used as the sensing elements. This means that \( N \) is small, and accordingly the contribution of vesicles to fwhm can be smaller than \( \Delta \lambda_{\text{max}} \) only by a factor of \( \sim 2 \).

\section*{Materials and Methods}

\subsection*{Vesicle Preparation.}

Vesicles composed of 1-palmitoyl-2-oleoyl-sn-glycerol-3-phosphocholine (POPC) (Avanti Polar Lipids, Alabaster, AL) were prepared by the extrusion method. Briefly, lipids dissolved in chloroform were treated with nitrogen air to form a dried lipid film. The film was rehydrated in aqueous buffer solution (10 mM Tris [pH 7.0] with 200 mM NaCl) at a nominal lipid concentration of 5 mg/mL, followed by vortexing. Extrusion was then performed in order to control the vesicle size distribution. Track-etched polycarbonate membranes of varying sizes (30, 50, 100, or 200 nm diameter) were used depending on the sample. Vesicles were diluted in buffer solution immediately before experiment and used within 24 h of preparation. All aqueous solutions and buffers were prepared with Milli-Q-treated water with a minimum resistivity of 18.2 MΩ·cm (Millipore, Billerica, MA).

\subsection*{LSPR Measurements.}

LSPR measurements were conducted via indirect nanoplasmonic sensing (INPS). 44 In the INPS approach, changes in the dielectric properties of a coated nanoplasmonic device are tracked. The coating can be essentially any dielectric material, in this case titanium oxide. The main requirement of the coating is to be sufficiently thin so that the surface processes being tracked are within the nanoplasmonic evanescent field. For all measurement in this study, the sensor chip (Insplorion AB, Gothenburg, Sweden) was a glass slide containing deposited gold nanodisks with random arrangement (surface coverage \( \sim 8\% \)), as prepared by hole-mask colloidal lithography. 45 The sensor chip’s surface was coated by a sputtered titanium oxide film (thickness \( \sim 10 \text{ nm} \)). The individual coated nanodisks had an average height and diameter of 22 and 150 nm, respectively (see Figure 1b for an AFM image of a representative coated nanodisk structure). The bulk sensitivity of the coated nanodisks was approximately 120 nm per refractive index unit based on a series of measurements performed using water/glycerol mixtures.

Ensemble-averaged LSPR measurements were performed in optical transmission mode by using an Insplorion XNano instrument (Insplorion AB). Briefly, a white light beam entered the measurement chamber, passed through the sensor chip (\( \sim 4 \text{ mm}^2 \) circular spot), and exited through a quartz glass window. The transmitted light was collected by a spectrophotometer, and data analysis was performed.
with the Insplorer software package (Insplorion AB). The time resolution was 1 Hz. The spectral resolution of the plasmon resonance was determined by high-order polynomial fitting, and the centroid position was calculated from the fit.\textsuperscript{36} For the titanium oxide-coated sensor chips, the LSPR plasmon peak recorded in aqueous buffer solution was around 760 nm (see, e.g., Figure 1c). The full width at half-maximum (fwhm) was also determined from the spectral data.

The baseline LSPR response was recorded in aqueous buffer solution. After stabilization of the signal, the buffer solution was replaced by that containing vesicles, and the vesicle adsorption process was tracked by measuring the plasmon peak shift (Δλ<sub>max</sub>) as a function of time. Typically, a red-shift occurred because lipid molecules have a higher refractive index than water. Hence, lipid adsorption was interpreted as an increase in the peak position. During experiment, liquid sample was continually introduced by peristaltic pump at a constant flow rate of 100 μL/min, and the average flow velocity was 50 mm/min. The channel cross section and length were 1 × 2 and 5 mm, respectively.

Sensor chips were used for consecutive measurements up to eight times. In between experiments, the measurement chamber (with the loaded sensor chip still in place) and tubing were rinsed thoroughly with 1 wt % sodium dodecyl sulfate (SDS) in water, water, and ethanol in sequential wash steps with a minimum duration of 10 min per step. After disassembling the measurement setup, the sensor chip was again rinsed with water and ethanol, followed by drying with a gentle stream of nitrogen air. Immediately before experiment, the sensor chip was treated with oxygen plasma at maximum radio frequency power for 1 min (Harrick Plasma, Ithaca, NY) and then reassembled in the measurement chamber. As an initial check, the wavelength of the plasmon peak of the sensor chip in aqueous buffer solution was verified and was consistently in the same range; for each subsequent experiment involving the same chip, there was typically a minor decrease in the peak wavelength of ∼0.3 nm. For each series of experimental runs (e.g., lipid concentration or vesicle size dependent study), one sensor chip was used to collect the reported data. The variation between sensor chips in general was minimal in terms of peak wavelength and sensitivity of the bulk refractive index, and the measurement trends were reproducible for different sensor chips.

\section*{RESULTS AND DISCUSSION}

\subsection*{Dependence on Lipid Concentration.}

Initial experiments were performed using extruded vesicles passed through 30 nm pore diameters (average vesicle diameter was 58 nm, as determined by dynamic light scattering). A series of lipid concentration-dependent measurements were conducted between 0.2 and 0.0125 mg/mL lipid (Figure 3a). Independent of lipid concentration, monotonic adsorption was observed until reaching saturation coverage. The coverage depended on lipid concentration, with greater final peak shifts corresponding to higher lipid concentration. In particular, the final peak shifts for 0.025 and 0.0125 mg/mL lipid concentrations were 3.28 and 3.10 nm, respectively. By comparison, the peak shift for 0.2 mg/mL lipid concentration was 4.07 nm.

With constant vesicle size, the diffusion-limited adsorption kinetics measured at different lipid concentrations are expected to depend on ct (c is the lipid mass concentration) and to be linear almost up to saturation (see Figure 2b). In fact, the linear dependence of the uptake on ct is expected for kinetically limited adsorption as well but only at relatively low coverages. The observed kinetics are largely in agreement with theory (Figure 3b). In particular, the kinetic curves constructed as a function of ct nearly coincide at low coverage. To verify that the measured adsorption kinetics are controlled by diffusion, the theoretical surface coverage of vesicles on the substrate, \( \theta \equiv \pi r^2 C \), was estimated. Specifically, for each individual experiment, we first determined the time interval corresponding to 85% saturation of the respective LSPR signal, i.e., the time between \( \Delta \lambda = 0 \) and \( \Delta \lambda = 0.85 \Delta \lambda_{\text{max}} \). By taking this time interval into account along with the specifics of the system (i.e., vesicle characteristics and environmental conditions), the theoretical surface coverage was then calculated according to eq 5 in order to obtain the packing fraction. At high lipid concentrations (0.1 mg/mL and above), the obtained value of the coverage was ∼0.62, which is consistent with the random-loose-packed (RLP) model if one assumes the vesicles are nondeformable. In the RLP model,\textsuperscript{60} the packing fraction of adsorbed particles in the two-dimensional case is 0.77 so the surface coverage for corresponding to 85% saturation of adsorbed vesicles would be 0.65, which is in near agreement with our theoretical estimate. Deviations from this model were observed for lower lipid concentrations.

Our estimations of the saturation coverage above confirm that the time scale of vesicle attachment is largely determined by diffusion at high lipid concentrations. The higher final peak shift observed with increasing lipid concentration is consistent
with more adsorbed vesicles, with the maximum signal corresponding to the ideal case of close-packed, nondeformable vesicles. The dependence of the final peak shift on lipid concentration further led us to consider that the relative time scale of vesicle attachment is much faster than the time scale of vesicle deformation at high lipid concentrations. By contrast, at lower lipid concentrations, the time scale of attachment is longer and, according to the experiments, appears to be comparable to the time scale of vesicle deformation. Indeed, one can notice, at lower lipid concentrations, that the dependence of the LSPR signal on time is not perfectly linear already at relatively low coverages. These deviations from the theoretical predictions for the diffusion-limited regime of adsorption of nondeformable vesicles (eqs 5 and 16) appear to be related primarily to deformation of adsorbed vesicles and can be classified as apparent. Physically, deformation of a vesicle at a given location on the surface is expected to be fast on the time scale of our experiments. The surface used in our study is, however, heterogeneous on the length scale comparable to vesicle size (Figure 1). For this reason, deformation of adsorbed vesicles may include (especially at appreciable coverage) the local changes of their location, and accordingly the whole deformation process may be relatively slow.

To illustrate the deviations from the theoretical predictions in more detail, it is instructive to show the differential rate of the LSPR signal as a function of \( ct \) (Figure 4). If vesicles are not deformable and the adsorption rate is controlled by diffusion (eq 5), then the time derivative of the LSPR signal is expected to be constant almost up to saturation. At high lipid concentrations (0.1 mg/mL and above), the experimentally tracked kinetics are largely in agreement with this prediction. However, at lower lipid concentrations, the experiments showed deviations from the theoretical prediction. At 0.05 mg/mL lipid concentration, the rate gradually reached its maximum value and then decreased also gradually. An appreciable drop then occurred at high coverage, until the rate eventually became negligible. At lower lipid concentrations, the adsorption profiles again showed three stages corresponding to similar coverages. Hence, the LSPR measurement response to vesicle adsorption had up to three stages depending on the lipid concentration. Across the range of lipid concentrations, the maximum rate of adsorption was also nearly proportional to \( ct \).

Based on these experimental results, a few general conclusions can be drawn. Vesicles adsorb onto the substrate largely independently of already adsorbed vesicles until reaching \( \sim 30-40\% \) of the saturation coverage. In this stage, each vesicle contributes equally to the LSPR measurement response. As the surface coverage becomes more appreciable, adsorbing vesicles are influenced by already adsorbed vesicles. Specifically, newly and already adsorbed vesicles may undergo shape deformation. There are three competing effects in this case: (i) lipids in a deformed vesicle are, on average, closer to the surface, and hence this effect would lead to a greater LSPR shift per vesicle; (ii) with increasing coverage, the already adsorbed vesicles may prevent full deformation of newly arrived vesicles and in turn may themselves be deformed by the latter vesicles; and (iii) deformed vesicles have a higher surface coverage per vesicle, and therefore the total number of adsorbed vesicles at maximum coverage would be smaller. Experimentally, it was observed that the rate of the increase in the peak shift becomes smaller with increasing coverage, and the individual contribution of each vesicle to the LSPR signal decreases accordingly. At \( \sim 73\% \) of the saturation coverage, the rate of vesicle adsorption begins to decline appreciably, followed by a final drop at \( \sim 90\% \) of the saturation coverage. An increase in the diffusion-limited adsorption rate at high coverage (see, e.g., Figure 2b) was not observed. The end of diffusion-limited adsorption kinetics at this fraction of the saturation coverage (\( \sim 85-90\% \)) is consistent with previous reports.\(^{27,30}\) Taken together, the LSPR response not only indicates diffusion-limited kinetics of vesicle adsorption but also clearly shows deviations that are consistent with mutual vesicle deformation when the time scales of vesicle attachment and deformation are comparable. Hence, the data support that the predominant effect of vesicle deformation on the LSPR signal is related to higher surface coverage per adsorbed vesicle in the adlayer. We may also remark that the observed deviations are inconsistent with vesicle rupture to form two-dimensional planar bilayers as the rupture process would involve an acceleration\(^{41}\) in the LSPR signal response above the diffusion-limited adsorption rate and does not occur under the experimental conditions\(^{25}\) used in this study.

**Dependence on Vesicle Size.** The influence of vesicle size was also tested at a fixed lipid concentration of 0.1 mg/mL. Extruded vesicles were prepared through 30, 50, 100, or 200 nm pores and had average diameters of 58, 80, 122, and 160 nm, respectively, as measured by dynamic light scattering. Upon vesicle addition, small vesicles (80 nm diameter or below) immediately showed an increase in the peak shift that is characteristic of vesicle adsorption. However, larger vesicles (122 nm diameter and above) showed an initial small decrease in the peak shift before the peak shift began to also increase (Figure 5a). Previous reports\(^{30,41}\) have also noted an initial blue-shift. Although the reason for this shift is not fully clear, our results complement the previous findings and show that this effect is only apparent for large vesicles, at least in our

![Figure 4. Adsorption stages leading to saturation coverage of vesicles. The derivative of the time-resolved shift in extinction wavelength maximum (nm/min) scaled according to \( ct \) is presented for five lipid mass concentrations in solution. In general, the adsorption profile showed two or three stages corresponding to (i) diffusion-limited adsorption of nondeformed vesicles at low coverage (at \( \sim 0.15 \text{ min} \times \text{mg/mL} \leq \text{ct} \leq 0.15 \text{ min} \times \text{mg/mL} \)), (ii) diffusion-limited adsorption of deformed vesicles at intermediate coverage (only present at low lipid concentrations), and (iii) kinetically-limited adsorption at high coverage (\( \text{ct} \geq 0.15 \text{ min} \times \text{mg/mL} \)). The adsorption profile of the 0.1 mg/mL lipid concentration case shows a nearly constant rate up to high coverage, which is consistent with theoretical predictions for diffusion-limited adsorption.](image)
measurement setup. In all cases, vesicles adsorbed until reaching saturation coverage (Figure 5a). The final peak shift for large vesicles (80 nm diameter or greater) was similar (between 3.20 and 3.31 nm). By contrast, the final $\Delta \lambda_{\text{max}}$ for 58 nm diameter vesicles was 3.71 nm. Hence, vesicle size influenced the LSPR response associated with saturated vesicle adlayers in accordance with eq 19, which predicts that the maximum peak shift would decrease with increasing $\rho$ for nondeformable vesicles.

If the lipid concentration is constant with varying vesicle size and vesicles are not deformable, then the LSPR signal is also expected to scale as $\propto t/\rho^{5/3}$ (eq 18). This scaling is in agreement with the observed kinetics at low and moderate coverages (Figure 5b). As the coverage became appreciable, there are clearly deviations, which are particularly apparent for large (80 nm diameter or greater) vesicles as compared to small (58 nm diameter) vesicles. At the lipid concentration under consideration (0.1 mg/mL), the saturation coverage of vesicles estimated for the 58 nm diameter vesicles, as described in the previous subsection, generally agrees well with that predicted by the RLP model, while for larger vesicles there is less agreement. In the latter case, one can also see a slow increase of the LSPR signal at long times (at the 1 h scale). This seems to be related with slow relaxation of the whole vesicle adlayer which is observed in the case of large vesicles because they are more deformable. Another relevant factor is the generally wider size distribution for larger vesicles obtained via the extrusion process (see, e.g., ref 15) and filling of vacant areas at the late stage of adsorption by smaller vesicles from this distribution.

As already noted in the previous subsection, the time derivative of the LSPR response is more sensitive to the deviations compared to that predicted for diffusion-limited kinetics of nondeformable vesicles. In Figure 6, the corresponding results for each vesicle size are shown as a function of $t/\rho^{5/3}$. The ratios of the maximum rates observed at different vesicle sizes are in this case in agreement with those predicted for diffusion-limited adsorption. For the smallest vesicles (58 nm diameter), the dependence of the LSPR-signal derivative on time agrees well with the diffusion-limited adsorption of nondeformable vesicles. For larger vesicles, the deviations are, however, appreciable. The adsorption of 80 nm diameter vesicles reaches a maximum rate, and then the rate gradually declines until reaching saturation coverage. By contrast, the adsorption of 122 nm diameter vesicles shows a nearly constant rate at low and intermediate coverages. Moreover, one can observe a few peaks for the largest vesicles (160 nm diameter). This is expected to be related to the fact that the size of these vesicles is comparable with the size of the sensing metal nanodisks, and accordingly the LSPR signal may be sensitive not only to deformation of vesicles but also to correlations in the locations of vesicles and metal nanodisks. For example, the initial adsorption may be preferable near the nanodisks because the contact area is larger there, while at the later stages the adsorption may primarily occur on the top of or between the disks. Vesicle lamellarity may also be a related issue. Thus, with increasing vesicle size between 58 and 160 nm diameter, the deviations generally became more apparent and underscore the LSPR measurement technique’s capability to scrutinize the kinetics of vesicle adsorption and corresponding effects of vesicle size and metal nanodisk size on the LSPR response.

Additionally, the experimental results for large vesicles (80 nm diameter and above) obtained at saturation coverage are generally consistent with theory concerning the relative magnitudes of $\Delta \text{fwhm}$ (1.57 ± 0.29 nm) and $\Delta \lambda_{\text{max}}$ (3.26 ± 0.06 nm), the former of which may be smaller than the latter
only by a factor of \(\sim 2\). Deviations from theory were observed for smaller vesicles (58 nm diameter). In the latter case, the size of vesicles may no longer be comparable to the size of metal nanoparticles, and hence \(N\) is not sufficiently small.

■ CONCLUSION

In summary, we have investigated the kinetics of vesicle adsorption by indirect nanoplasmonic sensing. By utilizing a titanium-coated substrate that contains embedded, plasmonic gold nanodisks, adsorbed lipids near the substrate were detected via the LSPR signal. In contrast to alternative measurement techniques for tracking soft matter adsorption (e.g., SPR and QCM-D), the LSPR signal is highly sensitive only to the local environment immediately adjacent to the substrate. Concomitantly, in the present case, the LSPR signal uniquely tracks both vesicle attachment and shape deformation. This measurement capability allowed us to address outstanding questions related to the behavior of adsorbed vesicles at different surface coverages. In particular, experimental measurements were focused on the effects of lipid concentration and vesicle size. By extending treatments of diffusion-limited adsorption kinetics and vesicle-related LSPR physics, the expected scaling laws were derived for nondeformable vesicles in order to identify deviations in the experimental measurements. For small vesicles, a two- or three-stage adsorption model was identified as follows: (i) diffusion-limited adsorption without vesicle shape deformation at low coverage; (ii) diffusion-limited adsorption with deformation of adsorbed vesicles at intermediate coverage (observed only at sufficiently low lipid concentration); and (iii) kinetically limited adsorption at high coverage. The saturation coverage of vesicles in the adlayer depended on lipid concentration, demonstrating an interplay between the relative time scales of vesicle attachment and deformation. With increasing vesicle size, deviations from the expected LSPR kinetics became more apparent due to greater vesicle deformation, wider size distribution, and correlations in the locations of vesicles and plasmonic metal nanodisks, even at low vesicle coverage. Hence, vesicle deformation is related to vesicle–substrate interactions (particularly at low lipid concentrations), correlations (e.g., large vesicles at low coverage), and/or vesicle–vesicle interactions (e.g., all-size vesicles at intermediate or high coverage). Our findings clarify the behavior of adsorbed vesicles in different coverage regimes and motivate continued investigation of soft matter adsorption and related phenomena at liquid–solid interfaces by nanoplasmonic biosensing.

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Notes
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