Nanoparticle-Engendered Rupture of Lipid Membranes

Sean Burgess,‡,‡† Aleksey Vishnyakov,‡,‡ Christopher Tsovko, ‡ and Alexander V. Neimark*†,‡

‡Department of Chemical and Biochemical Engineering, Rutgers, The State University of New Jersey, 98 Brett Road, Piscataway, New Jersey 08854, United States
‡Skolkovo Institute of Science and Technology, Nobel st. 1, 121205 Moscow, Russia

Supporting Information

ABSTRACT: Tension-induced rupture of 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) lipid membranes with encapsulated hydrophobic nanoparticles is elucidated using dissipative particle dynamics simulations. The dynamics of hole formation is studied, and a nanoparticle size-dependent relationship is established for the probability of membrane rupture within a given time as a function of the membrane tension. Two mechanisms of hole formation are explored: homogeneous nucleation and heterogeneous nucleation at the nanoparticle surface. While the kinetics of homogeneous nucleation in unloaded membranes complies with the predictions of the classical Deryagin–Gutop (DG) theory, the heterogeneous nucleation causes progressively lower lysis tensions as the particle size increases. The thermodynamics of heterogeneous nucleation is treated by introducing an effective contact angle at the three-phase, solvent–membrane–solid boundary into the DG theory. The proposed approach helps quantitatively interpret the simulation results and predict the membrane stability in real experiments with significantly larger (by many orders of magnitude) observation times and spatial scales.

Interactions of nanoparticles (NPs) with lipid bilayers (LBs), which constitute the foundation of cell membranes, play an important role in emerging nanotechnologies, such as drug delivery, biomedical imaging, and design of bioinspired membranes for water purification.1−3 At the same time, an explosion in production of nanomaterials and their commercial applications raises multiple health-related concerns regarding nanoparticle toxicity.4 Adhesion of inhaled or digested nanoparticles may lead to membrane rupture and cell apoptosis.5 A better understanding of the nanoparticle-engendered mechanisms of membrane instabilities is required for developing new nanoparticle biotechnologies as well as for evaluating health threats related to nanoparticle manufacturing.

In this Letter, we explore tension-induced rupture of lipid membranes with encapsulated nanoparticles using dissipative particle dynamics (DPD) simulations. To compensate for the lack of relevant experimental data, we produced a set of data on the dynamics of hole nucleation in 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) membranes with encapsulated NPs of different sizes by using an original simulation setup, which mimics experiments performed with unloaded membranes. To explain the found correlation between the probability of rupture and the particle size, we developed a thermodynamic theory of the heterogeneous nucleation by introducing a notion of the contact angle at the three-phase, solvent–membrane–solid, boundary controlled by the balance of respective line tensions. These findings shed light on the mechanism of heterogeneous nucleation of holes in membranes triggered by adhered or encapsulated nanoparticles that may have important implications both for the therapeutic use of nanoparticles as drug and imaging carriers and for the assessment of cell membrane damage by commercial nanoparticles.

Lipid-based membranes are prone to rupture under stress or being affected by external stimuli. The basic thermodynamic theory of membrane rupture was suggested by Deryagin and Gutop (DG)6 based on the classical nucleation theory (CNT) applied to a homogeneous two-dimensional film. The DG model implies that film rupture is triggered by nucleation, due to thermal fluctuations, of an unstable hole (pore), which grows spontaneously upon achieving a certain critical size. The hole nucleation is controlled by the membrane surface tension, σ, and the line tension, Γ, which represents the excess free energy of the membrane edge per unit length. The work of formation of a round hole of radius r equals the difference between the work of formation of the hole edge of length 2πr and the work of extension of the membrane area by 2πr²σ.

\[ E(r, \sigma) = 2\pi r \Gamma - \pi r^2 \sigma \]

(1)

\[ E(r, \sigma) \] achieves a maximum at \( r_c = \Gamma / \sigma \), which represents the size of critical hole nucleus: holes of \( r < r_c \) may collapse, while holes of \( r > r_c \) expand irreversibly. The work of formation of the critical nucleus

\[ E_c = E(r_c, \sigma) = \pi r_c^2 \sigma = \pi \Gamma^2 / \sigma \]

(2)

represents the free energy, or nucleation (activation) barrier, of expanding hole formation, which is the main quantitative parameter for predicting the probability and kinetics of film rupture.

Received: May 31, 2018
Accepted: August 7, 2018
Published: August 7, 2018
rupture. The nucleation barrier determines the rate constant of film rupture

$$k_t = A_{k_0} \exp(-E_i/k_BT) = A_{k_0} \exp(-\pi \Gamma^2/\sigma k_BT)$$  \hspace{1cm} (3)

$A_{k_0}$ is the kinetic rate prefactor that depends on the film area and lipid mobility.\(^7\)

It is worth noting that the DG model is purely macroscopic and does not account for the molecular features of the lipid interfaces that are important at the nanoscale level of critical nuclei. Nevertheless, the DG model in its original and modified forms has been widely used for interpretation of the results of experimental\(^8-14\) as well as molecular simulation\(^7,15-20\) studies of the lysis tension defined as characteristic tension of membrane rupture at given conditions. In particular, Levadny et al.\(^11\) studied the tension-driven rupture of giant unilamellar vesicles (GUVs) and identified the lysis tension by the micropipette suction method.\(^21\) By repeating experiments, the authors determined the probability $P_f(\sigma, t)$ of the membrane rupture within given time $t$ at fixed tension $\sigma$. The rupture probability $P_f(\sigma, t)$ as a function of $\sigma$ was correlated with the predictions of the classical Kramers dynamics theory\(^22\) that implies the Poissonian stochastic process of hole formation with the rate constant $k_t$ determined by the energy barrier via eq 3

$$P_f(\sigma, t) = 1 - \exp(-k_t t)$$  \hspace{1cm} (4)

From this correlation, the authors calculated the line tension, $\Gamma$. This approach with various modifications was applied for interpretation of experimental studies of homogeneous lipid membranes of different type.

Rupture of unloaded homogeneous membranes has been studied by various theoretical and molecular simulation methods\(^15-20,23,24\) attempting to identify the critical nucleation conditions and to calculate the membrane line tension. The problem of transformation of the results of molecular simulations for predicting the experimental conditions of membrane rupture remains open. The reported values of the lysis tension tend to be larger in simulated systems ($\sigma = 20-40$ pN/nm)\(^16,20,24\) when compared to experimental systems ($\sigma = 5-10$ pN/nm)\(^11,12,23-27\) because of significant, by many orders of magnitude, difference in observation time and system size. Because of a strong correlation between the line and surface tensions,\(^12,19\) the values of the line tension determined in simulations ($\Gamma = 12-40$ pN) are larger than those in experiments ($\Gamma = 4-28$ pN).

While the homogeneous hole nucleation in unloaded lipid membranes was studied in great detail,\(^9-12,16,24-28\) we are not aware of any experimental studies of heterogeneous hole nucleation in lipid membranes engendered by adhered or encapsulated NPs, which is the focus of our work. Various simulation studies monitored the process of particle-assisted membrane rupture;\(^28-31\) yet very few consider the NP effect on the nucleation barriers of hole formation.\(^32-34\) By using SCFT, Ting and Wang\(^34\) calculated free energy barriers for translocation of hydrophobic NPs across LBs. They showed different mechanisms of NP translocation: NP encapsulation followed by NP release and membrane relaxation and NP encapsulation followed by membrane rupture. It is worth noting that DPD simulations have been extensively used and found efficient for modeling various processes involving lipid membranes and nanoparticles.\(^35-37\) Doi et al.\(^37\) investigated the specifiers of water interlayers between lipid membrane and silica. Ganzenmuller et al.\(^38\) explored shock-wave rupture of lipid bilayers. Several papers considered relevant phenomena of NP translocation\(^34,35,42,43\) and wrapping by lipid bilayers;\(^32,33,40,44\) the latter problem is important for analyses of stability of NP dispersions.\(^45\) Yue et al.\(^32\) and Zhang et al.\(^33\) employed DPD simulations to study membrane rupture triggered by rotating NPs brought into contact with LBs. They determined a critical surface tension, above which the membrane ruptures. Although these studies shed light on the mechanisms of NP–membrane interactions, the problem of how particle encapsulation affects stability of lipid membranes under tension remained unexplored, and a robust theoretical model capable of predicting the dependence of the lysis tension on the NP size and chemistry is lacking.

In this work, we analyze the stability of NP-loaded membranes in silico and treat the heterogeneous nucleation in terms of an original theoretical model by introducing an effective contact angle on the three-phase, hole–membrane–solid boundary into the DG theory. To elucidate the specifics of tension-induced rupture of lipid membranes with encapsulated nanoparticles, we implement standard DPD simulation method in the form\(^46\) suggested by Groot and Warren.\(^47\) As an instructive example, we study the dynamics of hole formation in DMPC lipid bilayers with loaded hydrophobic nanoparticles ranging from 1 to 4 nm in radius upon incremental membrane stretching; the simulation details are presented in the Supporting Information, section I.

In DPD simulations, we mimic the classical experiments with a film placed of a frame with a movable plank, to which a constant force is applied to control the membrane tension (Figure 1); the details of the simulation setup are given in

![Figure 1. Snapshots (side and top views) illustrating the simulation setup for monitoring the dynamics of the tension-driven membrane rupture.](image-url)
100 independent repeats with 200,000 steps at each \( \sigma \) to determine the probability, \( P_r(\sigma, t) \), of membrane rupture within given time \( t \) at fixed tension \( \sigma \) (membrane rupture probability). A typical example of the evolution of heterogeneous hole nucleation is shown in Figure 2.

The results of simulations with spherical and cylindrical particles of different size are shown in Figure 3.

The right-most green data in the left image of Figure 3 corresponds to homogeneous nucleation in an unloaded membrane. The green solid line represents the predictions of the DG model according to eqs 3 and 4 with fitted membrane–solvent line tension \( \Gamma_{ls} = 38.7 \) pN and dynamic prefactor \( A_{0d} = 9.4 \times 10^{18} \) /s. The lysis tension \( \sigma^* \) (defined as the tension at which the rupture probability \( P_r(\sigma, t) = 0.5 \)) within given observation time of 176 ns (which is equivalent to 200,000 DPD time steps), is \( \sigma^* = 41 \) pN/nm. The obtained value of the line tension is within the range of reported simulation data.15,16,24

As seen from Figure 3, encapsulated NPs affect the membrane rupture and the lysis tension decreases as the NP size increases. The smallest NPs of 1 nm radius, which are completely immersed within the hydrophobic interior of the
membrane, do not affect the dynamics of hole nucleation significantly. The events of heterogeneous nucleation near the NP were not observed. In contrast, in the case of NPs with radius 4 nm (diameter twice as large as the membrane thickness), all nucleation events occurred near the NP surface. In the case of 2 and 3 nm nanoparticles, both homogeneous and heterogeneous nucleation events were observed, but with larger probability for heterogeneous nucleation. The statistics of homogeneous and heterogeneous nucleation events are presented in the Supporting Information, section II.

A typical example of heterogeneous nucleation is shown in the left-hand panel of Figure 4. The hole filled by solvent (not shown) is of a quasi-circular shape resembling a 2D bubble residing on the curved NP surface with a certain contact angle at the solid–solvent–lipid points of contact. To consider the heterogeneous nucleation in the spirit of the DG theory, we present the hole as a circle with excluded lens due to the intersection with the NP, as sketched in the right-hand panel of Figure 4. The mechanical equilibrium in this three-phase, two-dimensional model is determined by the balance of the line tensions $\Gamma_{ps}$, $\Gamma_{pl}$, and $\Gamma_{pl}$ of lipid–solvent (blue arc AB$^*A^*$), particle–solvent (red arc AA$^*$), and particle–lipid (black arc) boundaries, respectively. The balance of line tensions defines the effective contact angle, $\theta$, at the three-phase solvent–lipid–solid points of contact (points A and A*) via the Young equation:

$$\cos(\theta) = (\Gamma_{ps} - \Gamma_{pl})/\Gamma_{ls}$$

(5)

This 2D geometrical model allows one to calculate the change of the system free energy, $E_p (r, R_p, \Gamma_{ps}, \Gamma_{ps}, \sigma, \theta)$, due to the formation of a hole of radius $r$ at the NP of radius $R_p$ at the constant tension conditions, which depends on the lipid–solvent line tension, $\Gamma_{ls}$; contact angle, $\theta$; and given tension, $\sigma$:

$$E_p (r, R_p, \Gamma_{ps}, \Gamma_{ps}, \sigma, \theta) = [2\pi r - I_{ABA}]\Gamma_{ls} + I_{ABA}\sigma$$

$$= [2\pi r - I_{ABA} + I_{ABA}\cos(\theta)]\Gamma_{ls} - [2\pi r - I_{ABA}]\sigma$$

(6)

Here, $I_{ABA}$ and $I_{ABA}$ are the lengths of arcs AB$^*$ and AB$^*$A$^*$, respectively, and $I_{ABA}$ is the area of lens AB$^*$A$^*$, all of which depend on $r, R_p, \Gamma_{ps}$, and $\theta$ (detailed equations are given in the Supporting Information, section III). The first term in the left-hand side (LHS) of the first line of eq 6 corresponds to the work of formation of the hole–lipid boundary of length $2\pi r - I_{ABA}$; the second term corresponds to the difference of line energy of solid–lipid and solid–solvent boundaries of length $A_B$; the third term corresponds to the surface energy gained because of formation of the hole of area $2\pi r - I_{ABA}$. The second line of eq 6 utilizes the definition of the contact angle (eq 5).

Equation 6 presents the work of formation of the hole of radius $r$ at the surface of NP of radius $R$ in LB with given $\Gamma_{ps}$, $\Gamma_{ps}$, and $\sigma$. Similar to the DG relationship (eq 1), this dependence is nonmonotonic with a maximum equaled to the energy barrier $E_{pc}$ of the heterogeneous nucleation achieved at the critical hole radius $r_c$, which depends on $R_p$, $\Gamma_{ps}$, $\Gamma_{ps}$, and $\sigma$. An example of such dependence is given in the Supporting Information (Figure S4). The energy barrier (eq 6) determines the rate of heterogeneous nucleation in the same fashion as the DG model does in the case of homogeneous nucleation.

$$k_p = A_p \exp(-E_{pc}/k_BT)$$

(7)

The proposed extension of the DG model to heterogeneous nucleation of holes in LB at the surface of encapsulated NPs is purely macroscopic and can be justified only for large particles of size significantly exceeding the LB thickness. However, as shown below it provides a useful insight on the heterogeneous mechanism of membrane rupture on the same level of accuracy as the original DG model for the homogeneous nucleation.

In the case of NP-loaded membranes, the rupture may be initiated by either heterogeneous or homogeneous nucleation, and the rupture probability $P_r (\tau, \sigma)$ is determined with the sum of the respective rates $k_q$ (eq 3) and $k_l$ (eq 7):

$$P_r (\tau, \sigma) = 1 - \exp(-k_l + k_q)$$

(8)

with the prefactors depending on the system size and NP size. To determine the contact angle $\theta$, we applied the proposed model of heterogeneous nucleation to reproduce the simulation data for a 4 nm NP because in this case no events of homogeneous nucleation were observed. The blue lines in Figure 3 represent the predictions of the expanded DG model for the 4 nm NP systems, calculated according to eqs 6 and 7 with fitted contact angle of $\theta = 61^\circ$ and dynamic prefactors $A_{p,0} = 9.43 \times 10^{12}$ s$^{-1}$ for spherical and $A_{p,0} = 1.24 \times 10^{12}$ s$^{-1}$ for cylindrical NPs. The calculated nucleation barrier for spherical NP equals $E_{pc} = 14.1$ K at respective critical hole radius $r_c = 0.87$ nm at the lysis tension of 33.8 pN/nm. The calculated energy barrier for cylindrical NP is somewhat smaller, $E_{pc} = 12.7$ K, with a critical hole radius of 0.79 nm at the lysis tension of 36.7 pN/nm. Note that we assume the membrane–solvent line tension $\Gamma_{ls} = 38.7$ pN determined for the homogeneous nucleation and ignore its potential dependence on the membrane tension $\sigma$.

In the case of 2 and 3 nm NPs, we have to account for both mechanisms of homogeneous and heterogeneous nucleation according to eq 8. The respective theoretical predictions are shown by solid lines in Figure 3. The calculations are performed without using any adjustable parameters using the values of line tension and contact angle determined above. However, the dynamic prefactors are adjusted to account for the system geometry. The prefactor $A_p$ of the rate of the heterogeneous nucleation is scaled with respect to the NP radius $R_p$ as

$$A_p = A_{p,0}R_p/R_{p,0}$$

(9)

where $A_{p,0}$ is the prefactor for the NP of radius $R_{p,0}$ that is taken as the reference (here, $R_{p,0} = 4$ nm). In the case of spherical NPs, the NP radius is additionally adjusted to account for the NP curvature, as described in the Supporting Information, section V. The rate of homogeneous nucleation prefactor, $A_{h,0}$, is adjusted by subtracting from the membrane area the area occupied by NP where the homogeneous nucleation cannot be initiated

$$A_h = A_{h,0}(S - \pi R_p^2)/S$$

(10)

where $S$ is the total surface area of the membrane and $A_{h,0}$ is the prefactor for the unloaded membrane (eq 3). The calculated energy barriers, lysis tension, and critical radii for spherical and cylindrical NPs are presented in the Supporting Information, section IV.

Overall, the agreement with the simulation data shown in Figure 3 confirms that the proposed model, despite its
simplistic 2D representation of the NP–LB interface, captures the specifics of the heterogeneous hole nucleation and the mechanisms of rupture of NP-loaded membranes. The introduced contact angle provides a rationale for quantitative assessments of NP–LB interactions. It can be estimated from the data of in silico or in vitro experiments on tension-driven membrane rupture based on the extended DG model presented here. It is worth noting that in order to use the simulation results to predict experiments, it is necessary to account for the difference by many decades in the system size and the observation time. Because of this difference, as well as an elevated level of environmental fluctuations, the lysis tension observed in experiments is significantly smaller than in simulations. The reported data for the lysis tension in different types of unloaded pure experimental lipid membranes varies in the range of 5–10 pN/nm, and the respective range of calculated line tension lies within 4–28 pN.\(^{11,12,25−27}\) A simple estimate can be done based on eq 4 by adjusting the parameters determined in simulation taking into account the experimental membrane size and observation time. Assuming that the prefactor in eq 3 is proportional to the membrane area, \(A_{\text{exp}} = A_{\text{sim}} S_{\text{exp}} / S_{\text{sim}}\) and using the characteristic membrane area \(S_{\text{exp}} = 380 \mu m^2\) (compared with simulated \(S_{\text{sim}} = 500 nm^2\)) and the experimental observation time of \(t_{\text{exp}} = 360 sec\) (compared with simulated \(t_{\text{sim}} = 176 ns\)), as reported in ref 11, the predicted experimental lysis tension is \(\sigma^* = 17.7 pN/nm\), which is significantly lower than the value \(\sigma^* = 41 pN/nm\) determined in simulations yet still higher than the experimental result. Another factor affecting the reduction of the lysis tension is the decrease of the line tension with the decrease of the surface tension.\(^{12,19}\) and further improvement of the proposed model requires a relationship between the line and surface tensions. For example, if we assume a smaller value of the line tension of 24.5 pN (instead of 38.7 pN determined in simulation), the lysis tension reduces to \(\sigma^* = 7.0 pN/nm\), which is within the experimentally observed range.

Dissipative particle dynamics is used to simulate the lipid bilayer system under tension. All systems are run in a box size of \(30 \times 60 \times 25 R_c\) (20 \(\times\) 39 \(\times\) 16 nm), bead density of 3 beads/\(R_c^3\). A time step of 0.01\(\tau\) with \(\tau = 88\) ps. The velocity Verlet algorithm is implemented in an NVT-ensemble, setting \(T = 298 K\). A 3-water-per-bead coarse graining with the bead size \(R_c = 0.65\) nm is implemented. Calculations are performed using DL-MESO software\(^{48}\) run on the Extreme Science and Engineering Discovery Environment (XSEDE).\(^{49}\) To create the initial configuration, 1170 lipid molecules are placed between the frame bars in the bath of 120 000 water molecules, and the system is equilibrated under zero surface tension for one million steps (880 ns) to form a stable homogeneous LB. The LB surface tension is increased in increments of 5 pN/nm at a rate of 0.0228 pN/nm/ns, to a point where preliminary scanning suggests rippling may occur. At this point, the increments are decreased to 1 pN/ns at a rate of 0.0057 pN/nm/ns. All data-gathering points are run for 200 000 steps (176 ns). The DPD model and further simulation details are presented in the Supporting Information, section 1. System images are visualized using Visual Molecular Dynamics.\(^{50}\)
The Journal of Physical Chemistry Letters

Letter


