Colloids On Lipid Bilayers: Deformations, Interactions And Migration

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Abstract
In this thesis, I focus on studying interaction between colloidal particles and lipid bilayers. Inspired by proteins that generate membrane curvature, sense the underlying membrane geometry, and migrate driven by curvature gradients, we explore the question: Can colloids, adhered to lipid bilayers, also sense and respond to membrane geometry?

In the first part of the thesis, I report experimental results of homogeneous nanoparticles and microparticles on lipid bilayers. Charged nanoparticles were used to study the dependence on tension of particle wrapping by bilayer membranes. The particle wrapping process is a competition between adhesion energy on the particle/lipid interface, and the energy cost to deform the membrane. I found that when membrane tension was below 0.27 mN/m, the apparent area of an aspirated giant unilamellar vesicle (GUV) decreased during nanoparticles binding, likely due to wrapping of particles by the membrane. This area decrease was eliminated by increasing the membrane tension. I also report results on pair interactions between streptavidin-coated microparticles bound to biotinylated GUVs. A preferred separation distance was found between pairs of particles, and an interaction potential energy on the order of thermal fluctuations was found.

To control the degree of wrapping systematically, I used Janus microparticles with two different surface properties on each of the hemisphere. I report the migration of Janus microparticles adhered to giant unilamellar vesicles elongated to present spatially varying principal curvatures. In experiments, colloids migrated on these vesicles toward sites of high deviatoric curvature. This migration occurred only when the membranes were tense, suggesting that they migrate to minimize membrane area. By determining the energy dissipated along a trajectory, the energy field was inferred to depend linearly on the local deviatoric curvature, like curvature driven capillary migration on interfaces between immiscible fluids. In this latter system, energy gradients were larger, so colloids move deterministically, whereas the paths traced by colloids on vesicles had significant fluctuations. By addressing the role of Brownian motion, I show that the observed migration is analogous to curvature driven capillary migration, with membrane tension playing the role of interfacial tension. Since this motion is mediated by membrane shape, it can be turned on and off by dynamically deforming the vesicle. While particle-particle interactions on lipid membranes have been considered in many contributions, I report here an exciting and previously unexplored modality to actively direct the migration of colloids to desired locations on lipid bilayers.

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COLLOIDS ON LIPID BILAYERS: DEFORMATIONS, INTERACTIONS AND MIGRATION

Ningwei Li

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in

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ABSTRACT

COLLOIDS ON LIPID BILAYERS: DEFORMATIONS, INTERACTIONS AND MIGRATION

Ningwei Li
Kathleen J. Stebe
Tobias Baumgart

In this thesis, I focus on studying interaction between colloidal particles and lipid bilayers. Inspired by proteins that generate membrane curvature, sense the underlying membrane geometry, and migrate driven by curvature gradients, we explore the question: Can colloids, adhered to lipid bilayers, also sense and respond to membrane geometry?

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Chapter 1 Introduction

1.1 Motivation: Curvature sensing activity of proteins on lipid bilayers

Cellular membranes have complex shapes and are highly dynamic. In some biological processes, changes in membrane curvatures occur in the time scale of a few milliseconds\(^1\). The shapes and shape transitions of cellular membrane are essential for numerous cell functions. For example, to transport information between cells or cell compartments, cellular membranes are deformed into highly curved (nanoscale) vesicles or tubules to encapsulate signaling molecules\(^2,3\). In addition, invaginations in cellular membranes can also modulate membrane tension to avoid lysis under osmotic shock\(^4,5\).

In some of these processes, certain peripheral proteins that bind to the cellular membranes play key roles in modulating membrane curvature\(^6\text{-}^8\). Clathrin-mediated endocytosis, a process that enables cells to uptake materials from the surrounding environment, is an important example of protein mediated trafficking in which different types of proteins sense and respond to locations on the cellular membrane with different curvatures.

Several stages in the process of clathrin mediated endocytosis can be distinguished: nucleation, cargo selection, clathrin coat assembly, vesicle scission and coat disassembly\(^6\). The shape of the membrane undergoes dynamic changes in these processes, and numerous proteins play distinct roles in modulating membrane curvature. In the nucleation stage, the membrane curvature is small prior to recruitment of clathrin. One protein that is responsible for the initial formation of a clathrin coated pit is FCHO, a protein containing an F-bar domain which can sense shallow curvature\(^9\). This pit of low curvature further recruits proteins that generate a stronger deformation on the membrane such as epsin, which binds the membrane through the N-terminal homology\(^10\), leading to increased deformation on the membrane. Finally, a small neck is formed connecting the vesicle to the membrane before scission takes place. BAR-domain containing proteins including sorting nexin 9
(SXM9) and amphiphysin are found in this stage of the process\textsuperscript{11}, responsible for further curving the membrane and recruiting dynamin, a protein responsible for scission of the vesicle neck\textsuperscript{12,13}.

What are the mechanisms behind the curvature sensing and generating ability of these proteins? This is a question that interests many biophysicists. The structures and interactions of peripheral proteins reveal their abilities to deform the membrane through scaffolding, helix insertion or oligomerization \textsuperscript{6}. For the case of endophilin and amphiphysin, they both contain an N-BAR domain, a protein domain with a crescent shape after dimerization, and contains an N-terminal amphipathic helix. Upon binding, the N-BAR domain can deform the bilayers by pulling lipids up to the concave binding interface\textsuperscript{14}.

In a number of experimental studies, \textit{in vitro} systems such as giant unilamellar vesicles (GUVs) were used as simple cell membrane models to reveal the physics of these protein-membrane interactions. In general, these studies considered two protein concentration regimes \textsuperscript{15}. In the low concentration regime, where protein-protein interactions and curvature generating abilities are weak, several studies have focused on the segregation of membrane bound proteins according to membrane curvature gradients.

In these studies, a highly curved nanometric tether was pulled from a relatively flat GUV containing proteins bound to lipid bilayers. Proteins such as ENTH\textsuperscript{16} amphiphysin\textsuperscript{17} and endophilin\textsuperscript{18} were found to segregate preferentially to the highly curved tubes. In these studies, fluorescence images of proteins and lipids were recorded and analyzed. The fluorescence intensity of lipid fluorophores decrease with increasing tension due to the decrease in tether radius, while the fluorescence intensity of proteins, and therefore their local density, increases. The protein sorting ratio, $\Phi_t / \Phi_{GUV}$, where $\Phi_t$ is the protein concentration on the tube and $\Phi_{GUV}$ is the protein concentration on the GUV, is found to be dependent on the tether curvature. This dependency can be well fitted by a thermodynamic model, where the bending energy contribution to the membrane’s free energy per
unit area is expressed as $\kappa (C - C_0)^2$, $\kappa$ is the membrane bending rigidity, $C$ is the mean curvature of the membrane, and $C_0$ is the spontaneous curvature induced by protein binding $^{19}$. By fitting this mode to the protein sorting data, the spontaneous curvature of the protein can be found; the inverse of this quantity is on the order of tens of nanometers for endophilin N-BAR$^{18}$. This implies that when these curvature sensing proteins are on the nanometric tether, they stabilize the curvature of the tether and therefore minimize the system’s energy.

In the high protein concentration regime, proteins interact with each other and cooperatively generate deformations of the membrane. In the limit that membrane bound proteins behave like an ideal gas, the relationship between sorting ratio and curvature is predicted to be linear $^{19}$. However, Zhu et al. observed a non-linear sorting relationship for endophilin, indicating that non-ideal protein-protein interactions occur $^{18}$. In the work by Sorre et al., the authors discovered that at high concentrations, the tether radius and tether pulling force can be reduced by bound proteins, indicating their abilities to not only sense, but also generate curvature $^{17}$. When the protein concentration on the membrane reaches a threshold that depends on protein type and the mechanical properties of the membrane such as bending stiffness or membrane tension, the proteins can spontaneously generate nanometric membrane tubes or vesicles from a larger vesicle. This tubulation or vesiculation process has been observed directly by electron microscopy $^{20}$, and indirectly as decrease in total apparent vesicle area $^{21}$. A numerical study by Yin et al. discovered that, when a number of N-BAR domains arrange themselves into a lattice, they can curve a piece of planar bilayers into a cylinder with nanometric radius of curvature $^{22}$. In another numerical study, curvature sensing proteins are approximated as curvature sources with shapes of a Gaussian function on a triangulated membrane with tension and bending energy. When the concentration of these curvature sources is high and membrane tension is low enough, aggregation of the Gaussian deformation formed tubules $^{23}$, recapitulating experimental results from Shi et al. $^{21}$. 
1.2 Brief review: particles on lipid bilayers

Because curvature sensing proteins have complicated structures, many factors including protein molecular properties and concentration, as well as membrane lipid composition can play a role in their function. This complexity makes it challenging to develop a detailed mechanistic understanding of membrane mediated transport of these molecules. Can a simple system, such as colloids with well-defined shapes and surface chemistry, be used as a probe to systematically study aspects of the mechanism of transport on lipid bilayers? Moreover, due to increased applications of colloids in pharmaceutics and consumer products, colloids on membranes are also recent topics that have sparked interest among biophysicists and biologists.

1.2.1 Deformation of lipid bilayers induced by particle attachment

As discussed in section 1.1, proteins migrate to high curvature sites to minimize the deformations they make on the membranes. The deformation field created by membrane bound object is the origin of curvature sensing ability. Here, I will discuss studies that focus on the relationship between membrane bending stiffness/tension and deformation induced by membrane bound colloids.

In the work by Deserno et al., the authors numerically solve the Helfrich equation to calculate the membrane shape for low membrane tension, so that the bending energy of the membrane is dominant. An energy functional that includes the sum of the adhesion energy between the membrane and the colloid, the energy cost to bend the membrane, and the role of weak membrane tension depends on the degree of wrapping of the colloid. The equilibrium degree of wrapping is found by minimizing this energy expression. A phase diagram was generated, where different degrees of wrapping, i.e., free colloid, partially wrapped and fully wrapped colloid, were found for different membrane tensions and adhesion energies. The major finding from the phase diagram is that, the transition from free to partially wrapped states is continuous, while there is an energy barrier between two states that increases with increasing tension. In a later study, the author
generalize this theory to a situation that includes the regime where bending energy is no longer dominant. It is further confirmed that the major contributor to the energy barrier between partial and fully wrapped state is membrane tension. The author hypothesized that the physical meaning behind this observation is that, since the membrane around the particle is greatly distorted in the partially wrapped state, the progress of wrapping is hindered.

A similar numerical study conducted by Raatz et al. reached the same conclusion: in the absence of tension, the transition from free to fully-wrapped state is continuous, and the partially wrapped state is absent. However, they introduced an adhesion energy potential that depends on the distance between the particle and the membrane. When the range of this interaction potential is not negligible compared to the size of the particle, they found that stable partially wrapped states exist even when membrane tension is not playing any role.

Dasgupta et al. studied wrapping of spherical and non-spherical particles under the effect of both membrane tension and bending energy. In this work, the open source code Surface Evolver was used to calculate the energy of a colloid adhered to a membrane, including the roles of adhesion, bending and tension, under different degrees of wrapping. This total energy depends non-monotonically on the degree of wrapping, and an energy minimum indicating a stable partially wrapped state can be found. While the energy barrier between the partially and fully wrapped states for a spherical particle is rather shallow, it increases when the particle is no longer isotropic. For prolate ellipsoids, this barrier increases with increasing aspect ratio, while for oblate ellipsoids, the trend is opposite.

Several experimental studies have also investigated how membranes wrap around adhered particles. van der Wel et al. showed that, when attaching streptavidin coated beads to biotin-containing lipid bilayers, the degree of wrapping depends on membrane tension. The tension of the membrane of lipid vesicles was adjusted using osmotic pressure. Under hypotonic conditions, the membrane was tense and particles attached to the bilayers without deforming the bilayers. On the other hand, when
the outer solution was hypertonic, the membrane tension decreased and particles were completely wrapped. This conclusion is consistent with the reported numerical studies. However, no stable partially wrapped state was found in any of these conditions. In the work by Zhang et al.\textsuperscript{29}, silica particles of different size, hence curvature, were adhered to lipid vesicles. It was found that bigger silica particles were wrapped by the bilayers, while smaller particles attached to the bilayers without making a shape deformation. Instead, the smaller particles drove a local rearrangement in the bilayer; the particles attracted the lipid head groups and ‘froze’ the bilayers, as indicated by a decrease in lipid diffusivity, with concomitant buckling in the bilayers. The authors hypothesized that the adhesion energy sourced from the Van der Waals interaction between lipid head groups and silica is able to overcome the energy cost to wrap around a 200nm particle. On the other hand, when the size of the particle dropped to 18 nm, the bending energy cost to deform the bilayer increased drastically, resulting in failure of wrapping. Instead, the negatively charge silica particles interact with the positively charged portions of the lipid head groups, which results in a phase transition in the lipid bilayers.

Wang et al.\textsuperscript{30} reported similar reconfiguration of lipids upon binding of particles to the bilayer. In this work, they embedded a fluorophore, Laurdan, in the bilayers to detect phase changes. Upon binding of anionic nanoparticles, they found that the fluid lipid bilayers showed a transition to a gel phase.

In summary, the binding of colloidal particles can deform the bilayers in the continuous limit through wrapping, where the bilayers are assumed to be sheets with zero thickness. On the molecular level, binding of particles can also alter the arrangement of lipid molecules in the lipid bilayers, causing a phase transition and therefore change in mechanical properties of the lipid bilayers.

While the colloids studied above have diameters on the order of one micron or smaller, they can make deformations that are orders of magnitude larger than their sizes if they binds to the bilayers.
in a large quantity. Zhang et al.\textsuperscript{29} have shown that, through freezing the lipid bilayers, binding of 18nm particles can lead to micronsize pore formation and crumpling of lipid bilayers on a giant unilamellar vesicle (GUV). In the work by Yu et al.\textsuperscript{31}, they encapsulate 200nm charged polystyrene (PS) particles in GUVs, and observed that the GUVs broke into chains of smaller GUVs when the concentrations of particles on the inside and outside of the GUVs were different. They hypothesized that binding of charged nanoparticles changes the area of the inner leaflet of the bilayers, and such an area mismatching forces a curvature change on the GUVs. In the work by Li et al.\textsuperscript{32}, the authors found binding of cationic nanoparticles (20nm) onto zwitterionic lipids vesicle can cause micronsize bilayers protrusion and pore formation on the GUVs.

1.2.2 Particle-particle interactions on lipid bilayers

Like proteins, the collective interactions of membrane bound particles can create deformations that are up to orders of magnitude larger than their size. Thus, while isolated particle-membrane interactions are indeed of interest, interactions among membrane bound particles also play an important role in particle-induced membrane deformation.

Numerous studies have proposed possible mechanisms for non-specific interactions between membrane inclusions. Note that the term “inclusions” here refer to not only to surface-bound proteins or particles, which I discussed above, but also transmembrane proteins, which span the bilayers. Several studies discussed below aim to understand these transmembrane proteins\textsuperscript{33–35}.

Protein induced lipid phase change has been discussed as a source for interactions between inclusions within the lipid bilayers. Such inclusions can cause change in lipid ordering\textsuperscript{33} and bilayer thickness\textsuperscript{34}, and therefore drive interactions between two inclusions. However, pair interactions between inclusions induced by this mechanism decay rapidly, decreasing exponentially with separation distance between two inclusions. Longer range interactions between inclusions mediated by thermal fluctuations, or membrane properties including membrane tension and bending energy have also been investigated. Recently, numerical studies suggested that adhesion energy between
the inclusions and membrane can also play a role. I will discuss these contributions that induce long range interactions below.

Goulian et al.\textsuperscript{35} proposed an interaction potential for two disks with defined contact angle and tilt angle on fluid bilayers (Figure 1-1 (A)) under low surface tension as a model for interacting cell membrane inclusions. In the limit of low temperature where thermal fluctuations are negligible, they calculated the membrane height profile around a rigid disk according to the Helfrich Hamiltonian in the small slope limit. Subsequently, an interaction potential can be calculated from the height profile. This potential predicts a long-range attraction interaction that decays with $\frac{1}{R^4}$, with $R$ being the separation distance between the two membrane bound disks. They then investigated this system with thermal fluctuations. A partition function was found by considering all possible orientation of a rigid inclusion on a fluctuating bilayers, with the height and tilt of the inclusion as degrees of freedom. An interaction potential was calculated from this partition function, and interestingly, this interaction is attractive for rigid disks and also decays with $\frac{1}{R^2}$.

The study of fluctuation-induced pair interactions was generalized by Lin et al.\textsuperscript{36} by including membrane tension in the Hamiltonian. The authors investigated on the interaction potential in bending dominated, tension dominated regimes and the middle regime where both bending energy and tension played roles. In addition to recovering the $\frac{1}{R^4}$ dependence when bending energy is dominant, they discovered that when tension dominates, the interactions became dependent on $\frac{1}{R^8}$.

In the middle regime, the interaction was found to be dependent on both of these decaying modes.

For the case of a static membrane, the problem of membrane inclusions inducing an equilibrium shape perturbation in the membrane, and subsequently lead to pair interactions to minimize the energy cost was also addressed by Weikl \textsuperscript{37}. The author calculated the membrane shape around an
infinitely long cylinder that binds to the membrane, i.e., he considered a 1-D problem (Figure 1-1 (B)), under the effect of both membrane tension and bending energy in a small slope limit. It was concluded that interaction between these cylinders is repulsive if they adhere on the same side of the membrane, and becomes attractive if the cylinders adhere to opposite sides of the membrane. When adding a harmonic potential to the membrane energy to model membrane near a wall, interactions between the cylinders become attractive under both adhesion scenarios.

While all the analytical studies mentioned are under the assumption of small slope, Müller et al.\(^38\) investigated the non-linear regime by calculating the force acting on a membrane bound particle from the stress tensor around it instead of calculating the shape of the membrane. Using this method, they successfully recovered the solution for interacting membrane bound cylinders by looking at the small slope limit of their solution.

These membrane elasticity-induced interactions between inclusions have also been studied experimentally. Koltover et al.\(^39\) observed attraction between two polystyrene microparticles that bound to GUV via biotin-streptavidin interaction. When trying to use the theory predicted by Goulian et al.\(^35\) to analyze their trajectory, they found that forces induced by membrane fluctuations were too weak and should have been overcome by Brownian fluctuations of the particles. The interactions were short ranged, differing from the long range interaction predicted for fluctuations induced interactions. Although lacking further quantitative analysis, they hypothesized that such an attractive interaction was possibly driven by minimizing membrane deformation.

In a recent study by van der Wel et al.\(^28\), more detailed experiments were performed on interactions between GUV-bound spherical microparticles on membranes with very low tensions (of magnitude nN/m). They observed a long range attraction between particles that are wrapped by the bilayers, while this interaction was absent for particles that were not wrapped. By analyzing the trajectory, they calculated an interaction potential from the transition probability for the particle to move from one separation distance to another. The shape of the interaction potential closely matches the one
generated from numerical simulation of pair interaction driven by bending energy. A similar study has been done by Sarfati et al.\textsuperscript{40}, where long range attractions between GUV bound microparticles were observed. The trajectories of the interacting particles were analyzed by the maximum likelihood analysis\textsuperscript{41}, and they concluded that the trajectories can be fit by a force expression that was developed for capillary driven monopole attractions.

Recently, adhesion energy has been proposed as a driving force for the assembly of particles on lipid bilayers. In a numerical study by Saric et al.\textsuperscript{42}, linear aggregates of particles were found, in spite of the fact that this configuration requires to bend the membrane more than a hexagonally packed configuration. However, when considering the contribution of adhesion energy, they found that the linear configuration actually maximized the adhesion energy between the particles and bilayers, and overcame the cost to bend the bilayers. Another numerical study by Bahrami et al.\textsuperscript{43} also discovered that particles can form tube like protrusions in a vesicle to maximize adhesion energy.

1.2.3 Interactions between particles and cell

Due to their well-controlled shape and surface properties, scientist have been interested in using nano or microparticles as tools to target certain cells. In a study by Veiseh et al.\textsuperscript{44}, they demonstrated that iron oxide nanoparticles can be taken up by glioma cells, a lethal brain tumor, making the cells detectable by both fluorescence microscopy and magnetic resonance imaging.

There are also studies regarding the effect of shape and size of particles on the uptake rate. In the work by Chithrani et al.\textsuperscript{45}, gold nanospheres and nanorods coated with transferrin were used to study their uptake rates by mammalian cells. An optimum size of approximately 50 nm was found for nanospheres for which the largest number of nanoparticles were endocytosed. The authors suggested that particles in this size range required less energy to be endocytosed than smaller particles; this optimum was thought to reflect curvature costs as well as time required for receptors to migrate toward the particles. They also found a decrease in uptake rate with increasing aspect
ratio, and hypothesized that inhomogeneous distribution of transferrin and longer wrapping time of the anisotropic particles lead to this result. A similar study was conducted by Florez et al.\textsuperscript{46}, but instead of iron oxide particles, polystyrene particles, spherical or stretched to ellipsoidal shape, were used. They found similar decreasing trend of uptake rate with increasing aspect ratio. However, uptake selectivity increased with increasing aspect ratio, possibly due to larger surface area of the ellipsoids.

Finally, an interesting targeting method called ‘hitchhiking’ was discovered by Anselmo et al.\textsuperscript{47}. Rod-like particles were attached to the surface of red blood cells to avoid clearance from the immune system. By using this hitchhiking method, targeting rate was significantly improved.

1.2.4  Particles on isotropic fluid interfaces

To conclude this literature review, it is worthwhile to mention that boundary value problems akin to those solved by Goulian et al.\textsuperscript{35} and Weikl\textsuperscript{37} apply to particles interacting on isotropic fluid interfaces. The primary difference between these problems is that, for isotropic fluid interfaces, only surface tension contributes to the energy of the interface. Therefore, particles interact to minimize surface area rather than bending energy.

In a seminal work, Stamou et al.\textsuperscript{48} considered pair interactions of spherical particles on fluid interfaces. Absent body forces or torques, they hypothesized that the deformation field around a particle with random pinned contact line could be described as an expansion of decaying multipoles in polar coordinates, with the quadrupolar mode being the first surviving term. For this mode, the height of the interface around the particle has this form: $h(r,\phi) = h_{qp} \cos 2\phi \frac{a^2}{r^2}$, in which $h_{qp}$ is the magnitude of the quadrupole, $\phi$ is the polar angle, $a$ is the particle radius and $r$ is the distance from the center of mass of the particle. Assuming this deformation, they developed a theory to explain the attraction force between uncharged spheres bound to the interface, and 2-D structures made by spheres on the interface, that is to minimize the deformation and therefore lowering the
surface energy. The predicted interactions obeyed power laws of separation distance versus time to contact in the far field.

In experiment, pair interactions at planar fluid interfaces were reported for ellipsoids \(^{49,50}\), cylinders\(^{51}\) and other shapes\(^{52,53}\). Pair interactions have been simulated for ellipsoids and cylinders\(^{54}\). Experiments and simulations show that homogeneous, non-spherical particles obey the interactions predicted by Stamou in the far field, and deviate from this prediction in the near field, as details of the distortion field around the particles become important. Ellipsoids assemble with preferred orientations, with side-to-side assembly reported for pairs of ellipsoids interacting via capillarity. Cylinders assemble end-to-end to form rigid, bamboo-like chains. Anisotropic Janus particles have also been studied, with deviations from these preferred orientations\(^{55,56}\).

Related phenomena occur for isolated particles on curved interfaces. Minimization of interface area drives particles to sites of high deviatoric curvature. On curved interfaces, migration of cylinders\(^{57}\), disks\(^{58}\) and spheres\(^{59}\) to high curvatures sites were observed. For all cases, energy trajectories were observed from tracking migrating particles, and a good agreement was found between experimental data and theory developed for particles that make quadrupolar deformation on a curve interface\(^{60}\).

Collective interactions are also interesting for this setting. When concentration of particles is high, capillary-induced ordering of particles was found on curved oil-water interface\(^{61}\). It was found that under relatively low density of particles, balance between capillary attraction and electrostatic repulsion drives assembly of particles into square packing lattices. Upon increase in concentration, the repulsion become dominant and changes the packing in to a hexagonal packing lattice.

The studies mentioned above are all considering contact line pinning on the particle. There is another possibility theoretically, where the contact line is not pinned. Instead, the location of the contact line depends on the surface energies of the particle, the water phase and the oil or gas phase.
On a planar interface, these particles make no deformation and therefore no interaction. However, when the interface is curved, the particles make deformations on the interface to satisfy their wetting condition. Theoretical studies have suggested that migration can occur \(^6\), but there is no experimental evidence for particles with equilibrium contact line or their migrations so far.

1.3 Overview of this thesis

The focus of this thesis is to study questions regarding the interaction of particles on lipid bilayers quantitatively with experiments. First, I will discuss investigation of the relationship between mechanical properties of the membrane and wrapping of particles. Despite the fact that many theory investigations have been done on this topic, experimental evidence is still lacking. I used charged nanoparticles that bound to pipette-aspirated GUVs under various values of membrane tension to experimentally study how membrane tension changed the wrapping states of membrane bound particles.

Next, I will discuss the study of pair interactions between membrane bound homogenous microparticles. Inspired by experiment results from Koltover et al.\(^3\), I used streptavidin coated polystyrene particles and on biotinylated GUVs. In the work by Koltover et al., the tension of the bilayers remained unknown. To study the role of membrane tension in membrane mediated pair interactions, the experiments were carried out on aspirated GUVs where membrane tension was accurately controlled.

To have better control on the degree of wrapping, Janus particles that have two hemispheres with different surface properties were used. In this part of the thesis, I will discuss results from membrane bound Janus particles. Strong attraction could be seen when a pair of Janus particles diffuse to the vicinity of each other, indicating strong deformation made by these particles on the bilayer. Can these Janus particles sense membrane curvature to reduce the deformation as the curvature sensing proteins, or as particles on oil-water interface when the membrane is tense? Does membrane tension or membrane bending energy play a more important role? Curvature driven
aggregation has been predicted by numerical studies\textsuperscript{7,63}, as well as in experiment by Koltover et al., but without quantitative analysis. To address these questions, I studied trajectories of migrating Janus particles on a deformed GUV under controlled membrane tension. A boundary value problem was set up and solved to calculate the energy of the system as the particles migrate. Theory and experimental data were compared to reveal the driving force behind this curvature driven migration of Janus particles.

**Figure for chapter 1**

![Figure 1-1 Schematic of interacting disks and infinite cylinders on bilayers](image)

- **(A)** Disk on a bilayer.
- **(B)** Infinite cylinders.

Figure 1-1 *Schematic of interacting disks and infinite cylinders on bilayers*
Chapter 2  Materials and Methods

2.1 Materials
Lipids including 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), 1,2-dioleoyl-3-trimethylammoniumpropane (DOTAP), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-biotinyl(polyethylene glycol)-2000 (DSPE-PEG-biotin) were purchased from Avanti Polar Lipids (Alabaster, AL). Lipid dyes N-(4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacene-3-propionyl)-1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine (BODIPY FL DHPE), triethylammonium salt, and 1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine (Texas Red DHPE), triethylammonium salt, and 1,1'-Dioctadecyl-3,3,3',3'-Tetramethylindocarbocyanine Perchlorate (DiI) were purchased from Invitrogen (Carlsbad, CA). D-sucrose, dextrose (D-glucose) anhydrous, phosphate buffered saline (PBS) and chloroform were purchased from Fisher Scientific (Hampton, NH). Glass pipette with diameter of 1mm and 1.5mm were purchased from World Precision Instrument (Sarasota, FL). Poly-l-lysine 1mM solution and bovine serum albumin (BSA) were purchased from Sigma-Aldrich. 5 Minute Epoxy was purchased from Devcon (Danvers, MA). Indium tin oxide (ITO) coated glass slides were purchased from Delta technologies.

2.2 GUV formation
Lipid and fluorescence dyes were dissolved in chloroform to yield a total concentration of 1 mM with lipid mixtures with desired compositions. 40 µL of the lipids mixture are measured and deposited on each indium tin oxide (ITO) glass slide by Hamilton Syringes. After solvent evaporation in a vacuum chamber for two hours, chambers formed by sandwiching a silicon spacer between two ITO slides were hydrated by 430 µL of 700 mM sucrose solutions and subjected to an alternating current of 0.5 A and 10 Hz for two hours.
2.3 Image acquisition

Confocal fluorescence images were obtained by an inverted microscope (IX81, with an FV300 confocal scan box, Olympus, Japan) with a 60x 1.1 NA water immersion objective (LUMFL, Olympus, Japan). The bright field images were taken by a CCD camera mounted on the same microscope (XC-ST30, Sony, Japan).

EPI fluorescence images were obtained by an inverted microscope (IX7.1, Olympus) with a 60x 1.1 NA water immersion objective (LUMFL, Olympus, Japan), and equipped with a CCD camera (C9100-13, Hamamatsu Photonics, Japan).

2.4 GUV aspiration and tension measurement

GUV aspiration was performed in an observation chamber formed between two microscope coverslips. The cover slips were separated by a 2 mm thick spacer. 300 µL of background solution (mixture of PBS, sucrose and glucose, compositions are different for different experiments) was deposited in each chamber. 10 – 20 µL of vesicle stock solution was added to the background solution. Since solution inside the GUVs (glucose) is usually denser than the background solution, GUVs will sink to the bottom of the chamber (Figure 2-1).

A single GUV was picked up from the bottom of the chamber using a glass micropipette. Glass micropipettes were fabricated from pulling 1mm glass capillaries with a Brown-Flaming micropipette puller (P-77, Sutter Instrument Co., San Francisco, CA). The pulled micropipettes were cut open at desired opening radii (about 2 – 5 µm) by a microforge controller (DMF1000, World Precision Instruments, Sarasota, FL).

Pressure drop across the aspiration pipette was controlled by the height of a water reservoir connecting to the pipette. The height of the water reservoir was monitored by a pressure transducer measuring the hydrostatic pressure of the water reservoir (DP-1520, Validyne, Northridge, CA).
An aspirated GUV can be divided into three parts, a spherical part outside of the aspiration pipette, a cylindrical part inside the aspiration pipette called ‘projection length’, and a spherical cap with radius equal to the inner radius of the pipette at the end of the projection length (Figure 2-2). All of these parameters can be measured from GUV images. There are, in general three types, of GUV images considered in this work: confocal fluorescence, EPI fluorescence, and transmitted light images. For EPI fluorescence and transmitted light images, the GUV radius was tracked manually by the selection and measure functions in ImageJ, or by first roughly locating the lipid signal intensity peaks, followed by fitting a Gaussian function around the intensity peak (see appendix A). For confocal images, a Gaussian ring was fitted to the GUV image, and the radius of the Gaussian ring was assumed to be the radius of the GUV. The fitting was performed in Matlab, and the fitting code written by Dr. Zheng Shi. The radius of the pipette and the projection length were measured manually within ImageJ using the selection and measure functions.

Applying the Young-Laplace equation to the portion of vesicle in the aspirated cap, and to the spherical portion outside yields two equations with two unknowns, the membrane tension $\sigma$, and the pressure inside the GUV. I can therefore calculate the membrane tension from the relationship:

$$\sigma = \frac{P_{\text{out}} - P_{\text{in}}}{2} \frac{R_P}{1 - R_P / R_V}$$

where $\sigma$ is the membrane tension, $P_{\text{in}}$ is the pressure inside the pipette, $P_{\text{out}}$ is the pressure outside of the pipette, $R_P$ is the radius of the pipette and $R_V$ is the radius of the GUV.

All glassware including the aspiration pipettes and coverslips were treated with either 5 mg/ml casein solution or BSA and poly-l-lysine to prevent adhesion between the glass surface and the bilayer. In the case when the vesicle was stuck, the tension was marked as unknown, since it was out of the mechanical equilibrium that this method assumes.
2.5 GUV transfer

When studying particles adhesion to GUVs, two observations chamber described in 2.4 were made, separated by a silicon spacer. One chamber contains GUV suspension as mentioned above, while another chamber contains particle suspension in the same background solution as in the GUV chamber. A single GUV was captured by the aspiration pipette and held under constant tension. A protective sleeve made by a 1.5 mm capillary mounted around the 1 mm aspiration pipette was manually moved into the chamber to cover the GUV. The covered GUV was then removed from the GUV chamber by a motorized controller with degrees of freedom in three orthogonal directions (mini 25, Luigs & Neumann, Germany). The protected GUV then was moved into the particle chamber, and the protective sleeve was manually removed to uncover the GUV. A schematic of the process can be found in Figure 2-3. This way, a single vesicle’s interaction with other particles can be observed in a chamber free of disturbance from other vesicles. When the concentration of particles in the particle chamber was so high that fluorescence imaging and transmitted light imaging was perturbed by scattering from the particles, the aspirated vesicle was transferred using the same procedures to a third chamber containing only background solution.

2.6 Particle tracking

Depending on the quality of the image of the particle, different particle tracking methods were used. Good quality images, usually fluorescence images without significant background noise, or transmitted light images that remained perfectly in focus, were tracked by a Matlab code written by Blair and Dufresne\textsuperscript{66} based on an algorithm developed by Crocker et al.\textsuperscript{67}. A code written to operate the algorithm can be found in appendix B. In brief, this method first roughly locate the particle by finding the pixel with maximum intensity. Then, a 2-D Gaussian is fitted to the intensity profile of the particle to locate the real center of mass.

For bright field images where there were significant background noise and particles kept coming in and out of focus constantly, the particles were located by the Analyze particle function in ImageJ.
First, the image was manually converted to a binary image, so that the particle appeared to be a clear white dot, and the centroid of this white dot selected in the yellow square is located as shown in Figure 2-4.

I measured the systematic error of tracking by tracking particles fixed on a glass slide in similar illumination environments. To mimic the condition of a particle coming in and out of focus, the focus was adjusted manually. Particles imaged using fluorescence and bright field microscopy and tracked by Matlab and ImageJ. The variance of the apparent position of the fixed particles is 0.003 μm$^2$ in both the x and y directions measured by ImageJ in bright field images.

### 2.7 Scanning electron microscopy (SEM)

A droplet of the particle suspension was dried on a cover slip. A thin layer (<10 nm) of a palladium and gold alloy was deposited on the sample by sputtering (Sputter Coater 108, Cressington Scientific, UK). The sputtered sample was imaged by a scanning electron microscope (Quanta 600 ESEM, FEI Corporations, Hillsboro, OR).

### 2.8 Zeta potential measurement

Zeta potential measurements for all particles used in this study were obtained using DelsaNano C analyzer, (Beckman Coulter, Inc., Brea, CA). Particle suspensions were diluted roughly 100 fold from a stock concentration of 1% solid to be sufficiently dilute for detection of particle displacement under the applied field within the analyzer.

### 2.9 Atomic force microscopy (AFM)

AFM was performed on particles both dry and hydrated, and dry functionalized surfaces, such as cover slips and silica wafers. For particles, a drop of the suspension of particles was dried on a piece of flat PDMS, and imaged in the tapping mode (Dimension Icon AFM, Bruker, Billerica, MA). For dry surfaces, the same method was used. For AFM measurements in a water environment, dry particles were deposited on a thin SU8 layer around 500 nm thick, and the SU8 was cured by
UV light. The particles thus fixed in the SU8 film were rehydrated by DI water, and imaged in contact mode in water (Asylum Research, Santa Barbara, CA). AFM images were processed using the open source software Gwyddion. First, a polynomial was fitted and subtracted to the background to eliminate surface tilt and curvature. For particles, a quadratic function was fitted and subtracted from the particle surface, and the root mean square (RMS) of the height fluctuations of the flattened particle surface was measured.

**Figures for Chapter 2**

![Schematic of a GUV aspirated in the observation chamber](image)

Figure 2-1 *Schematic of a GUV aspirated in the observation chamber*

Noted that it is not drawn to scale.
Figure 2-2 Confocal fluorescence image of an aspirated GUV

Scale bar=20 μm.

Figure 2-3 GUV transfer schematic
Figure 2-4 Example of converting a grey scale image to a binary image

(A) Bright field image of a particle (B) A converted to binary. Yellow box indicates the tracked particle. Scale bar=5 µm
Chapter 3  Homogeneous particles

In this chapter, changes in bilayers induced by homogeneous particle binding, and pair interactions of membrane bound homogenous particles are studied. Particles with size in two different length scales and surface properties were used: 200 nm diameter nanoparticles functionalized with amino groups (Amino nanoparticles, ANPs) were studied in interaction with oppositely charged GUVs; 200 nm diameter nanoparticles functionalized with Neutravidin (Protein coated nanoparticles, PNPs) were studied in interaction with biotinylated GUVs and compared to the ANPs; and 1 µm particles functionalized with streptavidin (Protein coated microparticles, PMPs) were used for pair interactions. There is interesting prior work in the literature on these issues. For example, Yu et al.\(^\text{31}\) and Li et al.\(^\text{32}\) have observed interesting deformation induced by charged nanoparticles, and Koltover et al.\(^\text{39}\) have observed interactions between streptavidin-coated particles. However, these studies lack quantitative analysis on the mechanisms behind the observed deformations or interactions. In a recent study by van der Wel et al.,\(^\text{28}\) the transitions between non-wrapped and fully wrapped states were observed for Neutravidin coated particles that bound to tense (> 0.001 mN/m) and floppy vesicles (< 10 nN/m), and interactions attributed to be bending energy mediated between two wrapped particles were observed. However, membrane tension in this case was not precisely controlled. Here, I am interested in studying particle induced membrane deformation and membrane mediated interaction under controlled tension.

3.1 Nanoparticles on GUVs

3.1.1 Comparison between SNPs and ANPs

I used the techniques of GUV aspiration and GUV transfer to study binding of nanoparticles on GUVs. This experimental approach has several advantages. It allows the study of particle binding on a single GUV without disruption from lipids in the background solution at fixed tension. Furthermore, this method also allows the GUV to be suddenly exposed to a particle suspension.
Thus, the particle-GUV interactions can be observed from the very first moment when they come into contact. Finally, the aspirated fraction of the GUV within the micropipette acts as an area reservoir. Changes in this aspirated fraction reveal the area supplied from the reservoir as particles bind to maintain constant tension. These changes are tracked by measuring the projection length of the aspirated fraction in the micropipette.

Here, I compared how nanoparticles bind to bilayers via electrostatic and ligand receptor interactions. I discovered that the number of particles that bind to the GUVs depends on their surface properties. In these experiments, pipette aspirated GUVs were first transferred under constant tension to a chamber containing a dense particle suspension for a period of time defined as the incubation time. Thereafter, they were further transferred to a chamber containing only background solution. For ANPs, after a short incubation time, particles started bind to GUVs made with zwitter-ionic lipids DOPC (99.5%) and trace amount of negatively charged Texas Red (0.5%) in a solution containing 10 mM PBS and 300 mM glucose (Figure 3-1). A decrease in projection length or even disappearance of the projection length can be observed at long incubation times (Figure 3-2). On the other hand, PNPs bind in a much smaller quantity to biotinylated vesicles (100-0.5-x % DOPC, 0.5% Texas Red DHPE, 0.5 % PEG biotin DSPE); only sparse population of particles adhere even after residence time of 5 min. Increasing the concentration of biotin in the lipids up to 10% did not significantly change the number of bound particles (Figure 3-3). For biotin connected to a PEG polymer chain with around 30 monomers, the onset of attractive interaction between the biotin and streptavidin measured by surface force apparatus was found to be at separation distance of around 10 nm. I hypothesized that due to the short range (~10 nm) of the biotin-streptavidin interactions, the PNPs can move away from the GUVs via diffusion or convection before the particles bind. On close inspection, a significant number of bound PNPs appear to be located towards the outside of the vesicle (Figure 3-4), and no change in projection length was observed during the transfer process. Together, these data suggest that few if any PNPs
were wrapped by the bilayer. This is surprising. In spite of the fact that the net adhesion energy from biotin-streptavidin interactions between total available binding sites on the particle and lipids should be sufficient to bend the bilayer (Appendix C), I could not confirm wrapping of either PNPs or PMPs in our experiments. However, the biotin was linked to the DSPE lipid via a PEG polymer chain containing roughly 30 monomers. This polymer linkage can play a significant role. Prior experiments using the Surface Force Apparatus have demonstrated that the binding to streptavidin of biotin linked to a polymer chain likely initiates when the polymer linker is in an extended state \(^{68}\). Therefore, the energy cost to stretch the polymer into an extended configuration plays a role in the adhesion process, lowering the effective interaction below that characterizing free biotin binding to free streptavidin. Numerical studies also have shown a lack of particle wrapping for particles bound to bilayers via ligand-receptor interactions when the membrane tension was high enough to damp visible fluctuations \(^{28,69}\). For a GUV aspirated by the pipette, the lowest tension I measured was around 0.005 mN/m, significantly higher than the bilayers of a GUV with visible fluctuations (<10 nN/m) \(^{28}\). Thus, the observed lack of PNP wrapping is consistent with these simulations.

In summary, ANPs bind to GUVs in large quantity and are wrapped by the membrane, resulting in detectable change in the projection length. I therefore selected ANPs as a tool to study deformation of GUVs upon particle binding under controlled membrane tension.

3.1.2 Wrapping of ANPs as a function of membrane tension

In this section, I will discuss results on deformation induced by ANPs binding under different membrane tension. First, I calculated the area and volume change of the transferred GUVs, to ensure that the decrease in project lengths was indeed due to change in apparent membrane area. Thereafter, results from experiments performed in low (<0.28 mN/m) and high (>0.28 mN/m) membrane tension regimes were discussed. Theoretically, the particle wrapping process is a competition between bending energy and membrane tension cost during the wrapping process, and
the adhesion energy gain between the membranes and the particles. I studied this competition experimentally by varying membrane tension, while keeping the membrane bending stiffness and adhesion energy constant.

There can be two possible causes for decrease in projection length of a GUV aspirated under constant tension: volume increase due to osmosis, or apparent area decrease due to bilayer deformations. Particle wrapping, as discussed, can be a source of apparent area consumption. When the bilayer wraps around a bound particle as indicated in Figure 3-5, the particle pull points on the membrane closer together. Due to the limit of the optical resolution, this configuration cannot be clearly resolved in a microscopy image. Instead, the area of the vesicle appear to shrink, since the wrapping of particles bring points on the membrane closer to each other. For a wrapped particle, I define a parameter, degree of wrapping, $\theta$, where $\theta=0^\circ$ for a non-wrapped particle, and $\theta=180^\circ$ for a completely wrapped particle (Figure 3-9). The apparent area change for one particle is the difference between the adhered spherical cap and the circular ‘hole’ made by the particle-membrane contact line:

$$
\Delta A_p = 4 \pi a^2 \left( 1 - \cos \theta - \frac{\sin^2 \theta}{2} \right) \tag{3.1}
$$

I was able to distinguish the contributions from volume increase and area decrease through experimental design and analysis. Experimentally, the osmolarity of the particle chamber was adjusted to be 50 mM larger than that of the vesicle chamber. Upon transfer, the volume of the GUVs would decrease, which lead to an increase in projection length under constant aspiration pressure. Therefore, any decrease in projection length under this condition can be attributed to decrease in apparent area. In addition, I analyzed confocal fluorescence images of the aspirated GUVs during particle binding to calculate the apparent area and volume of the vesicle:

$$
A_{GUV} = 4 \pi R_v^2 + 2 \pi R_p^2 L + 2 \pi R_p^2 \tag{3.2}
$$
\[ V_{GUV} = \frac{4}{3} \pi R_v^3 + \pi R_p^2 L + \frac{2}{3} \pi R_p^3 \]  

3.3

Where \( R_v \) is the radius of the GUV, \( R_p \) is the radius of the aspiration pipette, and \( L \) is the projection length as indicated in Figure 3-2.

When the GUVs were transferred to the ANPs suspension under tension smaller than around 0.2 mN/m, a decrease in projection length can be observed. The apparent GUV area indeed decreased, and typical trends of area and volume are shown in Figure 3-6 for a vesicle aspirated under low tension (0.09 mN/m), and in Figure 3-7 for a vesicle aspirated under high tension (0.54 mN/m). As expected, the volume of the vesicle decreased for both cases, due to osmotically induced loss of water from inside of the vesicles. However, the apparent area of the low tension vesicle decreased to about 85% of its original area after 12.5 s, while the apparent area of the high tension vesicle had a 5% increase. When a vesicle was transferred to a chamber that contained no particles at moderate tension (0.16 mN/m), the change in area was negligible as shown in Figure 3-8, where the standard deviation of the area was about 0.08%. Clearly, the changes in apparent GUV area with the presence of particles were much larger than area fluctuations that would cause inaccuracies in area determination.

Why does particle binding lower the apparent area of a vesicle under low membrane tension, and increasing the membrane tension eliminate the apparent area decrease? In the following, I will discuss theory describing how degree of particle wrapping by membranes depends on membrane tension, membrane bending energy, and adhesion energy. First, I will introduce a simple case, where the membrane has zero membrane tension, and the wrapping process is a competition between bending energy cost and adhesion energy gain. Then I will include membrane tension, and show how membrane tension can change the wrapping process.
3.1.2.1 Particle wrapping by membrane with zero membrane tension

Considering a membrane with bending energy at zero tension, the total energy of the system in which a particle adhered to this membrane can be expressed:

\[
E_b = E_2 - E_1 = \int_{\mathcal{M}-\mathcal{P}} 2\kappa H^2 dA + \int_{\mathcal{P}} \left(2\kappa H^2 + f^*_{ad}\right) dA \tag{3.4}
\]

where \(E_1\) is the energy of a base state where a particle is completely submerged in the fluid, \(E_2\) is the energy of the state where the particle binds to the membrane, \(\mathcal{M}\) denotes the entire membrane, \(\mathcal{P}\) denotes the membrane in contact with the particle, \(a\) is the radius of the particle, and \(dA\) is an area element on the interface. \(f^*_{ad}\) is the adhesion energy between the membrane and the surface of the particle, containing the contributions from the attractive interactions between the lipid head groups and the functional groups on the particle, as well as the contribution from the difference between the surface energies of the particle-liquid interface and the particle-lipid interface. The membrane shape outside of the adhesion region, i.e., in the \(\mathcal{M}-\mathcal{P}\) domain, adopts shapes with zero mean curvature such as a catenoid, in order to minimize bending energy\(^\text{24}\). Therefore, the integral over the \(\mathcal{M}-\mathcal{P}\) domain goes to zero in equation 3.4. For the integral within the \(\mathcal{P}\) domain, assuming that the particle is a perfect sphere, and that the membrane conforms to the particle in perfect contact, equation 3.4 becomes:

\[
E_b = 2\pi a^2 \left(2\frac{\kappa}{a^2} + f^*_{ad}\right) \left(1 - \cos\theta_c\right) \tag{3.5}
\]

where \(\theta_c\), \(0^\circ < \theta_c < 180^\circ\), is the degree of wrapping as defined in the schematic in Figure 3-5. Equation 3.5 indicates that if \(2\frac{\kappa}{a^2} + f^*_{ad} < 0\), the energy of the system \(E_b\) decreases monotonically with increasing \(\theta_c\), and vice versa if \(2\frac{\kappa}{a^2} + f^*_{ad} > 0\). Therefore, the transition from non-wrapped (\(\theta_c = 0^\circ\)) to completely wrapped (\(\theta_c = 180^\circ\)) is continuous, without a stable intermediate state\(^\text{24}\).
In summary, absent membrane tension, there are two stable states in this case. When the adhesion energy per unit area \( f_{ad} \) is not strong enough to overcome the bending cost \( \frac{2K}{a^2} \), the particle cannot be wrapped by the membranes. However, once the adhesion energy becomes strong enough, the particle becomes fully wrapped.

3.1.2.2 Particle wrapping by membranes with finite membrane tension

For a membrane with finite membrane tension, the energy change upon particle adhesion to the membrane \( E_{BT} \) is:

\[
E_{BT} = \int_{\mathcal{M-P}} \left( 2\kappa H^2 + \sigma \right) dA + 2\pi a^2 \left[ \left( \frac{2K}{a^2} + f_{ad} + \sigma \right) \left(1 - \cos \theta_c\right) - \sigma \frac{\sin^2 \theta_c}{2} \right] \tag{3.6}
\]

where \( f_{ad} \) includes the contribution from \( f_{ad}' \), as well as the membrane tension change before and after particle adhesion. The third term in Equation 3.6 is the work require to pull on a tense membrane to wrap the particle and cause a change in apparent area. Using membrane tension and particle radius to cast equation 3.6 in dimensionless form, i.e. defining \( \tilde{E}_{BT} = \frac{E_{BT}}{\sigma a^2} \), this balance becomes:

\[
\tilde{E}_{BT} = \tilde{E}_{BT}^{\mathcal{M-P}} + \tilde{E}_{BT}^{\mathcal{P}} = \tilde{E}_{BT}^{\mathcal{M-P}} + 2\pi \left[ \left( \frac{2K}{a^2} + f_{ad} + \sigma \right) \left(1 - \cos \theta_c\right) - \sigma \frac{\sin^2 \theta_c}{2} \right] \tag{3.7}
\]

The term \( \tilde{E}_{BT}^{\mathcal{M-P}} \) is energy cost owing to deformations made by the particle in the surrounding free membrane, which depends on the choice of boundary conditions at the three phase contact line, the degree of wrapping, and the properties of the membranes. The contributions of membrane tension to the \( \tilde{E}_{BT}^{\mathcal{P}} \) term include the work against membrane tension to recruit membrane area to form the wrapped cap, given by the area of the wrapped cap minus its projected area.
I defer discussion of $\tilde{E}_{\alpha \gamma}$ and first examine the $\tilde{E}_p$ term for local extrema by considering the first and second order $\theta_c$ derivative of $\tilde{E}_p$:

$$\frac{\partial \tilde{E}_p}{\partial \theta_c} = 2\pi \left( \frac{2}{a} \frac{\kappa}{\sigma} + \frac{f_{ad}}{\sigma} + 1 \right) \sin \theta_c - \sin \theta_c \cos \theta_c = 0$$ \hspace{1cm} (3.8)

Since $0 \leq \theta_c \leq 180^\circ$, $-1 \leq \cos \theta_c \leq 1$, and $\cos \theta_c \neq 0$, there exists a local extremum if

$$\cos \theta_c = 2 \frac{\kappa}{a^2 \sigma} + \frac{f_{ad}}{\sigma} + 1 = C_1,$$

where, for admissible values of the wrapping angle, $-1 \leq C_1 \leq 1$. The second order derivative of $E_{\alpha \gamma}$ evaluated at $\cos \theta_c = C_1$ is:

$$\frac{\partial^2 \tilde{E}_p}{\partial \theta_c^2} = 2\pi \left( C_1 \cos \theta_c - 2 \cos^2 \theta_c + 1 \right) = 2\pi \left( 1 - C_1^2 \right)$$ \hspace{1cm} (3.9)

For $0^\circ < \theta_c < 180^\circ$, the second derivative is positive, and this extremum is a local minimum.

Considering only $\tilde{E}_p$, there are three cases for particle-membrane wrapping. Case 1: when $C_1 > 1$,

i.e., $2 \frac{\kappa}{a^2} + \frac{f_{ad}}{\sigma} > 0$, $\tilde{E}_p$ increases monotonically with increasing $\theta_c$. The only stable state is the non-wrapped state; this discussion is identical to that of the tensionless membrane, i.e. when the adhesion energy is not strong enough to overcome the bending energy cost, the particle will not be wrapped. This criterion is independent of membrane tension, therefore the transition from no wrapping to partially wrapped states cannot occur as a function of tension. Case 2: when $-1 < C_1 < 1$,

i.e., $-2 \frac{\kappa}{a^2 \sigma} + \frac{f_{ad}}{\sigma} < 0$, a minimum exist for $\tilde{E}_p$ at $\theta_c = \arccos(C_1)$, indicating that the stable state is a partial wrapping state. The tension-related energy cost of membrane work to recruit area to wrap the particle is a non-monotonic function of wrapping angle. Within the values of bending energy, membrane tension and adhesion energy considered in this case, this non-monotonic dependence can define an energy minimum that defines a stable partially wrapped state. Case 3:
when $C_i < -1$, i.e., $2 \frac{k^2}{a^2 \sigma} + \frac{f_{ad}}{\sigma} < -2$, the energy decreases monotonically with increasing $\theta_c$, resulting in a stable fully wrapped state. This case is similar to the fully wrapped state for a tensionless membrane, in that, for sufficiently strong adhesion energies, the particle becomes fully wrapped. However, with finite membrane tension, the threshold value for complete wrapping is tension dependent. The energy profiles under different values of $C_i$ and the wrapping diagram are shown in Figure 3-10. When appropriately rescaled, these criteria as well as the phase diagram correspond to those predicted in the literature by Deserno $^{24}$.

To account for the contribution from the free membrane ($E_{BTP}$), the shape of the free membrane has been solved numerically in the regime where $\lambda \gg a$, with $\lambda = \sqrt{k/\sigma}$, and in the regime where $\lambda = a$. For certain values of membrane tension and adhesion energy, by taking the deformation in the $M-P$ domain into consideration, these studies report a coexistence of the partially wrapped and fully wrapped state$^{24,25,27}$. The total energy of the system has two minima, one corresponding to a partially wrapped state, the other to a fully wrapped state, with a local maximum in between, i.e., the membrane deformation is associated with an energy barrier. In my experiments, I observed a transition from apparent area decrease at low tension to apparent area increase at high tension. The arguments presented above indicate that the transition between a non-wrapped to wrapped state is independent of tension, while the transition from a coexistence of partially to fully wrapped is tension dependent. It is possible that the transition I observed was a transition from partially to fully wrapped state. The predicted partially wrapped states have small wrapping angle ($\theta_c < 36^\circ$)$^{25}$, therefore will lead to small changes in apparent area. Other contributions such as elimination of membrane fluctuations under high tension can counteract the change of apparent area due to partial particle wrapping. To study the tension dependence and to find the transition tension, I systematically varied the aspiration pressure in these transfer experiments, and recorded the change of area for each tension value. The percentage change in area
was plotted against tension in Figure 3-11, and an abrupt change of sign can be seen between $\sigma=0.28$ mN/m and $\sigma=0.30$ mN/m. Using a transition tension of 0.28 mN/m, a bending energy of $10 \ k_B T$, and a particle radius of 100 nm, I can estimate an adhesion energy by using criteria for case 3, where the particle will transition to a fully wrapped state: $2\kappa^2 + f_{ad}a^2 = -2\sigma a^2$. The adhesion energy that gives rise to a transition at 0.28 mN/m is calculated to be 0.13 $k_B T$/nm$^2$, which is very similar to adhesion energy between zwitterionic lipid head groups and a silica surface\cite{70}. Similar result has been observed for wrapping 1 µm particles by bilayers, where fully wrapped particle was observed for membrane under low tension, and non-wrapped particle was observed for membrane under high tension\cite{28}. However, the tension in these experiments were not precisely control, and therefore no quantitative analysis was done. It is possible that the non-wrapped state observed for the microparticles was a weakly wrapped state, which was not resolved due to optical resolution limit.

So far, I have discussed membrane wrapping of particles under the assumption of an equilibrium contact line. However, the contact line can become kinetically trapped by pinning sites on the particle surface. This scenario is common for particles at the oil-water interface\cite{71,72}. This is an open issue worthy of continued study. If pinning sites are significant, tension could play a role in overcoming pinning energies, thereby unwrapping the membrane from the particle. Partially wrapped states have been reported for larger particles, such as streptavidin-coated PS particles with radius of 2 µm\cite{40}. Furthermore, I have observed partially wrapped states for streptavidin functionalized PS particles with radius of 6 µm (Figure 3-12) with wrapping angle larger than 90°. However, I have not observed partially wrapped states for smaller homogeneous particles, such as particles with radius of 500 nm. This might be attributed to longer contact lines on the larger particles, which may have a higher probability of encountering pinning sites on the particles. To probe this issue in detail, experiments similar to those performed at fluid-fluid interfaces would have to be designed to determine the approach of colloids to equilibrium states over time.
3.2 Interactions between homogeneous particles

How do these homogeneous particles with uncontrolled membrane wrapping interact on lipid bilayers? To study this phenomenon, I designed experiments to study pair interactions of membrane bound microparticles. There are several reasons that I selected PMPs of diameter 500 nm for this study. First, the position of these relatively large particles can be tracked with small errors. By using protein binding to adhere the particles, I could study pair interactions with significant electrostatic interactions. Finally, I found that these PMPs bind to biotinylated GUVs in a very small quantity. Each GUVs usually contain under five bound particles, allowing pair interactions to be studied without complications from collective behaviors from other particles. However, there is little chance that such sparse particles will diffuse close enough to each other to study pair interactions. Therefore, to increase the probability for observing interaction, I used an optical trap to bring a pair particles together. The optical tweezer is a home built device, and detail design can be found in the work by Heinrich et al. After bringing the particles to contact, the tweezer was turned off and the pair of particles started to diffuse away from each other (Figure 3-13). Their trajectories were recorded, and the center to center distance between the pair, $r_{12}$, was calculated.

Considering that the particles moved on the surface of the spherical vesicle, the observed projected center-to-center distance was converted to the orthodromic distance on a sphere. First, the difference between the location of the particle and the location of the GUV center is calculated:

$$x_s = x_p - x_c \tag{3.10}$$

$$y_s = y_p - y_c \tag{3.11}$$

where $(x_p, y_p)$ is the location of the particle, and $(x_c, y_c)$ is the location of the center of the GUV. By assuming that the GUV is a perfect sphere, and that the particle’s relative distance to the membrane surface does not change, the $z$ location of the particle can be found:
For two particles, their center to center distance in 3-D space, \( r_{12}' \), can be easily calculated from their 3-D position. The orthodromic distance on the sphere is related to \( r_{12}' \) by:

\[
    r_{12} = 2 \arcsin \left( \frac{r_{12}'}{2R} \right) R
\]  

A typical trajectory of \( r_{12} \) is plotted in Figure 3-14. When the particles were brought together by the trap, \( r_{12} \) fluctuated at around 1 µm, meaning that the particles were in contact. After the trap was turned off, the particles diffused away, and an increase in \( r_{12} \) was seen.

There are cases where \( r_{12} \) clearly fluctuates at preferred distances, implying a preferred separation between the pair of particles, possibly driven by competition between weak attractive and repulsive interactions. The probability density of a particle diffusing under a potential energy field can be related to the interaction potential by assuming a Boltzmann distribution. A detailed derivation can be found in the work by Crocker et al.\(^{67}\). In brief, the probability density for \( r_{12} \) at a certain distance can be calculated as:

\[
    \rho_{i}^{eq} = \sum_{j} P_{ij} \rho_{j}^{eq}
\]

where \( \rho_{i}^{eq} \) is the equilibrium probability density of \( r_{12} \) to be in the \( i^{th} \) bin, \( j \) is \( r_{12} \) in the video frame before \( i \), and \( P_{ij} \) is the probability for \( r_{12} \) to change from \( j \) to \( i \) in one movie frame. The \( r_{12} \) is binned into bins with size of 100 nm. For example, I found all the frames containing \( r_{12} \) in the 2\(^{nd} \) bin, then I looked at the frames before them. I found that the \( r_{12} \) in those frames belong to \( N \) bins, and there are \( n_{j} \) occurrence in each bin. For the \( j^{th} \) bin, there are in total \( N_{j} \) occurrence throughout the entire video. The probability density for \( j \) can be calculated as:
\[ \rho_{eq}^j = \frac{N_j N_{bin}}{N_f} \]  

3.15

where \( N_j \) is the total number of frames in the video and \( N_{bin} \) is the number of bins. The transition probability can be calculated as:

\[ P_j = \frac{n_j / \rho_j}{\sum n_j / \rho_j} \]  

3.16

It is weighted by \( 1/\rho_j \) due to the inhomogeneous distribution of \( j \). Equation 3.15 and equation 3.16 can then be plugged into equation 3.14 to calculate \( \rho_i \), and finally the interaction potential can be found:

\[ \frac{U_i}{k_B T} = -\log(\rho_{eq}^i) \]  

3.17

For some pairs of particle, an attractive well in the interaction potential at some preferred separation distance can be found at 5 particle radii, and the depth of the well is around \( k_B T \) (Error! Reference source not found.). However, for the same exact pair of particles, if re-aspirated by the laser trap and released again, the trajectory and the interaction potential can look very different (Figure 3-16). This might be due to a change in the particle/membrane contact line during laser trapping. The interactions does not seem to be dependent on membrane tension (Figure 3-17). Incubation of particles with GUVs confirmed that the particles were barely wrapped by the bilayers (Figure 3-18). In this regime, the weak interactions due to weak distortions are expected. The variability of the interactions strength and preferred separation may be attributed to the uncontrolled state of wrapping and the particles’ reconfigurations induced by the optical trap.
3.3 Conclusion

In observing nanoparticles binding onto membrane under different tension, I found a transition from fully wrapped to weakly wrapped states for membrane bound particles. Assuming equilibrium contact line, this transition can be explained by the competition between membrane tension, membrane bending energy cost and adhesion energy gain. However, whether contact line pinning play a role in particle wrapping remains open for further investigation.

For homogeneous particles with uncontrolled wrapping states, I found weak interactions on the order of thermal fluctuations between two membrane bound microparticles. The interaction strength and range varies between pairs. Due to such variation, it is difficult to identify the role of the underlying membrane in these interactions. I am unable to conclude a mechanism for this interactions.

In order to observe stronger and deterministic interactions between membrane bound particles, I need to seek particles where I can systematically control the degree of wrapping by bilayers, and induce large deformation. In the next chapter, I will discuss results from Janus particles that show very different wrapping behavior from homogeneous particles.

Figures for chapter 3
Figure 3-1 ANPs on an aspirated biotinylated GUV

Left panel: focus is on the equator; right panel focus is at the bottom of the vesicle. Scale bar: 15 µm

Figure 3-2 Change in projection length with residence time after transferred to ANPs suspension

Scale bar=20 µm
Figure 3-3 SNPs on an aspirated biotinylated GUV

Left panel: focus is on the equator; right panel focus is at the bottom of the vesicle. Scale bar: 15 µm

Figure 3-4 SNPs on an aspirated GUV

Lipid channel (red, averaged over 20 frames) overlapping with particle channel (green). Scale bar: 15 µm. Inset: zoom in of the region within the yellow square.
Figure 3-5 **Schematic of a particle completely wrapped by lipid bilayers**

Particle: green. Lipids: red.

Figure 3-6 **Apparent area change of a vesicle during particle binding under low membrane tension.**

Right panel: trace of area and volume after GUV transfer. Left panel: snap shot of GUV at two different time points. Scale bar=20 µm. σ=0.09 mN/m.

Figure 3-7 **Apparent area change of a vesicle during particle binding under high membrane tension**

Right panel: trace of area and volume after GUV transfer. Left panel: snap shot of GUV at two different time points. Scale bar=20 µm. σ=0.54 mN/m.
Figure 3-8 Area and volume change of a vesicle after being transferred to a chamber containing no particles

\( \sigma = 0.17 \text{ mN/m}. \)

Figure 3-9 Schematic for degree of wrapping
Figure 3-10 **Energy of the membrane vs. degree of wrapping and wrapping phase diagram**

(A) $\tilde{E}_{BT}^p$ vs. $\theta_c$ for different values of $C_1$. (B) Wrapping phase diagram with varying membrane tension and adhesion energy. When rescaled, the boundaries where $C_1 = 1$ and $C_1 = 0$ are identical to those in Figure 3 in Ref 24, and the boundary where $C_1 = -1$ approximate the spinodal in Ref 24 where the energy boundary between partial and fully wrapped state disappears.

Figure 3-11 **Area change summary plotted against membrane tension**
Figure 3-12 6 µm in radius streptavidin coated particles on an aspirated GUV
Scale bar=15 µm.

Figure 3-13 Time stamped image of a pair of PMPs.
Blue: positions of particle 1. Green: positions of particle 2. Time between position: 0.3 s.
Figure 3-14 **Center-to-center distance normalized by particle radius of a typical pair aspirated by then released from optical trap plotted against frame number**

Figure 3-15 **Center-to-center distance and potential energy of a pair of interacting particles**

Left panel: center to center distance of a pair of microparticles. Right panel: interaction potential energy vs. center-to-center distance. $\sigma=0.25$ mN/m.
Figure 3-16 Center-to-center distance and interaction potential for same pair of particles as in Figure 3-15 aspirated and released for 5 times by an optical trap.

Figure 3-17 Well location and depth of the interaction potential plotted against tension.
Figure 3-18 **PMPs incubated with GUV**

Lipid composition: 98.7% DOPC, 1% DSPE-PEG-biotin, 0.3% Texas Red. Lipids: red. Particles: green. Scale bar=15 µm.
Chapter 4  Janus particles*

In the last chapter, I discussed interactions between homogeneous particles, which are weak and highly variable for different pairs. I hypothesize that the homogeneous particles create weak and uncontrolled deformations on the membrane due to the low degree of wrapping. Owing to this lack of reproducibility, and the uncertain nature of these interactions, it was difficult to identify the mechanism based on analyzing the particle’s trajectory. To control the degree of wrapping, and therefore possibly to induce larger deformation on the bilayers, I collaborated with Dr. Daeyeon Lee and Dr. Fuquan Tu, who fabricated Janus microparticles. These Janus microparticles have two hemispheres with different surface chemistry, one primarily comprising polystyrene (PS), the other polyacrylic acid (PAA).

Isolated colloids adhered to vesicles have been reported to accumulate at curved sites; their preferred localization was attributed to minimization of bending energy. Similarly, trajectories for interacting particle pairs were reported39. What role could membrane tension play? I explore this question here. For bilayers with finite bending rigidity $\kappa$ and membrane tension $\sigma$, the natural length scale $\lambda = \sqrt{\kappa / \sigma}$ determines whether minimization of bending energy or of excess area dictate system behavior 74. For colloids of radius $a$, tension and excess area minimization would determine system response only if $\lambda << a$. By making membranes tense, membrane tension-mediated migration could be assessed.

In this chapter, results for interactions between Janus particles, and Janus particles migration on tense bilayers (defined as $\lambda << a$) with curvature gradients will be discussed.

* Adapted with permission from Curvature-Driven Migration of Colloids on Tense Lipid Bilayers, Ningwei Li, Nima Sharifi-Mood, Fuquan Tu, Daeyeon Lee, Ravi Radhakrishnan, Tobias Baumgart, and Kathleen J. Stebe Langmuir 2017 33 (2), 600-610 DOI: 10.1021/acs.langmuir.6b03406 Copyright 2017 American Chemical Society 92
4.1 Fabrication of Janus particles

Janus particles were fabricated via seeded emulsion polymerization followed by hydrolysis. First, monodispersed linear polystyrene (LPS) seeds with diameter of 700 nm were made by dispersion polymerization with ammonium persulfate as an initiator. Then, the LPS particles, mixed with a monomer emulsion consisting of styrene, tert-butyl acrylate (tBA, 98 %), 1 vol % divinylbenzene (DVB, 55 %), and 0.5 wt % initiator 2,2′-azobis(2,4- dimethylvaleronitrile), were tumbled in an oil bath to complete the seeded emulsion polymerization. Finally, the tBA was hydrolyzed by stirring the particles in 80 vol % trifluoroacetic acid (TFAA, 99 %) and 20 vol % formic acid (FA, ≥ 95 %). In Tu et al.\textsuperscript{75}, a more detailed protocol is described.

Upon seeded polymerization, the PS rich hemisphere was covered with sulfate groups and a small concentration of carboxylic acid groups and forms a rough and negatively charged surface. On the other hand, the other hemisphere was completely covered with carboxylic acids, forming a smooth surface that is negatively charge at PH 7 (Figure 4-1). The root mean square roughness measured by AFM was 1.9 ± 0.8 nm for the smooth PAA side, 13.3 ± 3.1 nm for the rough PS side, and 21.9 nm for on the border between the two sides. The electrophoretic mobility was $-3.03 \pm 0.18 \text{ cm}^2/\text{Vs}$ for these Janus particles.

Some of the Janus particles were labeled with Nile Red by infusing the dye into the particles polymer network for confocal imaging.

Figures for section 4.1
**Figure 4-1  Scanning electron microscope image of Janus particles**

The PAA side is smooth and the PS side with PAA domains is rough. Scale bar: 1 µm.

### 4.2 Wrapping of Janus particles

Does the Janus feature of the particles successfully control their degree of wrapping? To answer this question, I measured the degree of wrapping of Janus particles, and compared them to charged homogeneous particles. I used confocal microscopy and bright field microscopy to image GUVs and GUV bound particles simultaneously for degree of wrapping analysis. Spherical GUVs were used for these experiments. Since particles diffused slowly (rather than migrating rapidly) on spherical GUVs, I could accurately track the position of the particles. While the degree of wrapping may change when the GUVs were stretched, the aim of these experiments was to compare the degree of wrapping between homogenous and Janus particles on spherical vesicles under high tension (0.2-0.6 mN/m). Since the particles were negatively charged, I used GUVs containing 40% of positively charged DOTAP, 59.5% DOPC and 0.5% Bodipy DHPE. The Janus particles were labeled with Nile Red for particle tracking purpose.

I analyzed images for which the particles are located near the equator of the GUVs, as shown in Figure 4-2 (A). The location of the lipid membrane of a spherical vesicle is found by fitting a Gaussian ring to the intensity of the fluorescence image where the aspirated portion is discarded.
and I only fit the circular portion of the vesicle. A typical fit is shown as the white dashed line overlain on the vesicle image shown in Figure 4-2 (A). I obtained the vesicle center, \( x_G \) and \( y_G \), and the vesicle radius, \( R_G \) from the fitting results. I then tracked the center of mass of the particle, \( x_P \) and \( y_G \). Assuming that the vesicle radius is large compared to the particle radius, I define a degree of wrapping, \( \theta_c \), as shown in the schematic in Figure 4-2 (B). The value of \( \theta_c \) can be calculated by:

\[
\theta_c = \arccos \left( \frac{R_G - r_{gp}}{a} \right).
\]

where \( r_{gp} = \sqrt{(x_P - x_G)^2 + (y_P - y_G)^2} \) is the distance between the center of mass of the particle and the GUV center, and \( a \) is the particle radius. When \( R_G - r_{gp} > a \), the particle is located completely on the inside of the GUV, and the degree of wrapping is considered to be 180.

The homogeneous particles used for comparison here were carboxyl functionalized PS particles (Invitrogen, electrophoresis mobility: \(-4.61 \pm 0.15 \text{ cm}^2/\text{Vs}\)). I measured the degree of wrapping for seven homogeneous particles and seven Janus particles. The results are presented in Figure 4-3. The error bars in are standard error of the mean. I performed a Student’s t-test on the two data sets to see if they are significantly different.

Indeed, there is a clear difference of degree of wrapping comparing the Janus particles and homogeneous particles. As expected, the Janus particles were roughly half-wrapped. Since the fabrication process introduced negative charge on both hemispheres, I hypothesize that the limited degree of wrapping may be attributable to electrostatic interactions of the PAA face to oppositely charged lipid bilayers or pinning at the rough surface where the two faces meet.

**Figure for section 4.2**
Figure 4-2 Degree of wrapping calculation for membrane bound particles

(A) Image of an aspirated vesicle (green) with an adhered particle (red). The white dashed line is the tracking result of the membrane's position, and the red cross indicates the location of the particle. Scale bar: 15 µm

(B) Schematic of the wrapping angle of the membrane on the particle
4.3 Janus particles interactions

With these partially wrapped Janus particles, I observed a strong attraction interaction when a pair of particles were within 10 particle radii from each other (Figure 4-4). Unlike the homogeneous PMPs, the particles moved towards each other in a deterministic manner, and remained trapped together afterwards. Such behavior indicates that the attraction force between particles was significantly larger than random Brownian force. Similar pair interactions have been observed for particles on isotropic fluid interfaces. For spherical particles on fluid interfaces, the three-phase contact line can be kinetically trapped by random pinning sites on a particle, creating an undulated contact line. Such a contact line can be decomposed into Fourier modes, and in the absence of body forces and torques, the first surviving mode is the quadrupolar mode. Microspheres and disks with pinned contact lines were reported to migrate on curved interfaces. I hypothesize that in the case of a Janus particle adhered to bilayers, the bilayers adhered and trapped at random sites on
the rough surface of the particle, creating an undulated contact line (Figure 4-5), which can be decomposed into:

\[ h(r = r_0, \phi) = h_{0\phi} \cos 2\phi + H.O.T \]

where \( h \) is the membrane shape around the particle in Monge representation (Figure 4-6), \( r \) and \( \phi \) are axes of a polar coordinate originates at the center of the particle, \( r_0 \) is the location of the contact line, and H.O.T. indicates higher order terms. The deformation field made by a particle with pinned contact line on a flat interface is illustrated in Figure 4-8.

I tracked the position of the interacting pairs, and the interaction trajectory is plotted in Figure 4-7. The observed trajectory does not obey a power law consistent with interacting quadrupolar modes\(^{48,49,77}\), presumably owing to other, near field details in the deformation fields around the particles.

**Figures for section 4.3**

![Snapshots of an interacting pair of Janus particles on the vesicle](image)

**Figure 4-4 Snapshots of an interacting pair of Janus particles on the vesicle**

Elapsed time is labeled in seconds in the images. The focus was re-adjusted in the fourth panel. Scale bar: 5 \( \mu \text{m} \).
Figure 4-5 Schematic of a particle partially wrapped by a lipid bilayer with undulated contact line

Figure 4-6 Schematic of membrane shape in Monge representation

Figure 4-7 Trajectory of center-to-center distance between two interacting particles in log-log scale

Blue dotted line: power law predicted by interacting quadrupoles.
4.4 Curvature driven migration

Can Janus particles that have pronounced pair interactions sense membrane curvature? In this section, results from migration of Janus particle on curved tense membrane are reported.

4.4.1 GUV elongation

I used GUV transfer to bring an aspirated GUV into contact with Janus particles. Once the GUV was introduced into the chamber, particles began to adhere to the membrane. Concomitantly, the GUV was deformed using a large, clean, glass bead of radius 10-30 µm glued to a second micropipette. Using micro-manipulators, the bead was tapped gently against the GUV to promote adhesion to the membrane, and gently retracted to elongate the GUV into a lemon-like shape (Figure 4-9 (A)). The entire arrangement was then held fixed. The micropipettes were configured so that the elongated vesicle shape is axisymmetric. A GUV aspirated by the pipette, shown in Figure 4-9 (B), consists of two parts: a part outside of the pipette that is elongated by pulling, and a part inside the pipette with a shape that can be roughly approximated by a cylinder with a spherical cap of radius $R_p$ at its end.
The contour of the elongated part of the GUV was tracked from both confocal and bright field images using ImageJ and Matlab. A typical contour imaged via confocal fluorescence microscopy is shown in Figure 4-9 (B). I defined a coordinate \((R,Z)\) with \(Z=0\) located at the large bead, and \(R=0\) on the axis of symmetry. The meridional arc length \(s\) along the contour is measured from the bead, as well. After elongation, I observed that the GUV has constant mean curvature \(H\). By minimizing an objective function characterizing the difference between the experimental contour (black line in Figure 4-9 (B)) and numerically generated curves, the location for the axis of symmetry (blue line in Figure 4-9 (B)), values for \(H\) and values the principle curvatures of the membrane along the contour were determined. The fitted profile (yellow dashed line, Figure 4-9 (B)) agrees well with the experimental contour. The axis of symmetry is a line that is found to be located at equal distance from both sides of the GUV contour.

We used a fitting algorithm developed by Ms. Liana Vaccari, and is available in the appendix E. Here, I will introduce the procedure briefly. In the coordinate system shown in Figure 4-9 (B), the shape of an axisymmetric surface with constant mean curvature can be described by a set of differential equations:

\[
\frac{d\psi}{ds} = -\frac{\sin\psi}{R} + H, \quad 4.3
\]

\[
\frac{dR}{ds} = \cos\psi, \quad 4.4
\]

\[
\frac{dZ}{ds} = \sin\psi, \quad 4.5
\]

where \(\psi\) is the turning angle, \(Z\) is the axis of symmetry, \(R\) is the distance between \(Z\)-axis and the surface, and \(s\) is meridional arc length. The shape of the surface can be found by solving simultaneously this set of equations numerically with initial conditions \(Z_0, \psi_0\), where \(Z_0\) is defined to be 0, and \(Z_i, \psi_0\) and \(H\) are fitting parameters. \(Z_i\) is defined as the \(Z\) location of the first point of
the experimental data set. I minimized the objective function calculating the difference between the experimental obtained GUV contour and the numerical solution. From this analysis, I found the mean curvature $H$ of the GUV, as well as the principal curvatures in the parallel direction $c_p$ and in the meridional direction, $c_m$:

\[ c_p = \frac{\sin \psi}{R}. \]  \hspace{1cm} 4.6

\[ c_m = \frac{d\psi}{ds}. \]  \hspace{1cm} 4.7

Deviatoric curvature, defined as the difference between the two principal curvatures, $\Delta c = |c_m - c_p|$, is plotted against the $Z$-axis in Figure 4-9 (C). $\Delta c$ reaches maximum at the aspiration pipette and the larger glass bead, and is at its minimum near the center of the elongated vesicle.

To access the error in fitting, I first found the uncertainty in localizing the membrane by tracking the contour of an elongated vesicle over several frames in a video. The standard deviation of the position $\sigma_z$ was calculated. The averaged position was fitted to a shape with constant mean curvature; the fit and residual is plotted in Figure 4-10 (A). Since the fit result was a discrete sequence, the residual was defined as the minimum distance between an experimental data point and the fitting result. The sign of the residual was negative if $Z_{\text{experiment}} < Z_{\text{fit}}$.

I varied one of the three fitting parameters while keeping the other two constant at their optimum values and calculated a goodness-of-fit parameter $\chi^2 = \sum \frac{1}{\sigma^2} \left[ (r - r_f)^2 + (z - z_f)^2 \right]$. The $\chi^2$ was plotted against the varying fitting parameter, and a parabola was fitted to it. The uncertainty of the fitting parameter can then be calculated by $\sigma_i^2 = 2 \left( \frac{\partial^2 \chi^2}{\partial a_i^2} \right)^{-1}$. The best fit values and uncertainties I found are: $H = 0.208 \pm 0.00004 \mu m^{-1}$, $\psi_0 = 0.6637 \pm 0.0034$ and $Z_i = 0.75 \pm 0.04 \mu m$. Using these values, I integrated to find the uncertainty of the GUV shape and calculate the
standard deviation of the deviatoric curvature (Figure 4-10 (B)). The error in deviatoric curvature is around two orders of magnitude smaller than its value.

Now that the shape of an elongated GUV was known, I used the same method as for spherical GUV as described in the method section (equation 2.1) to calculate the membrane tension by replacing $R_{GUV}$ with the inverse of the mean curvature $1/H$.

Figures for 4.4.1

Figure 4-9 Shape of an elongated GUV

(A) Schematic of an aspirated GUV being elongated by two micro-manipulated micropipettes in a liquid chamber with suspended particles. One micropipette holds the aspirated area reservoir. A bead, affixed to the other micropipette, adheres to the GUV and is used to impose elongation. (B) Bright field microscopy image of an elongated GUV. The yellow dashed lines are the contours with constant $H$; black lines underneath the dashed lines are the contour found by tracking the shape of the membrane; the blue line is the calculated axis of symmetry. Scale bar: 10 µm (C) Deviatoric curvature (upper panel) and gradient of deviatoric curvature with respect to arc length (lower panel) are plotted against arc length, $s$. 

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4.4.2 Experimental observation of Janus particles migration

I compared the behavior of two types of particles that adhere electrostatically to this membrane, specifically, isotropic, carboxy-functionalized PS microparticles and the Janus PS-PAA microparticles, both of radius $a=0.5 \ \mu m$. Note that on elongated vesicles, a particle’s degree of wrapping can be different than on a spherical vesicle. However, a clear difference in wrapping state could still be seen for homogeneous particles and Janus particle (Figure 4-11). On elongated GUVs, over lag times of tens of seconds, the PS microparticles moved with linear mean square displacement (MSD) in arc length $s$ versus lag time (Figure 4-12); this indicates that their migration was diffusion-dominated, and that the motion of the wrapped particle was not strongly coupled to the membrane geometry. The lack of directed motion of the homogeneous particles also indicates that lipid flow in the elongated vesicle, if present, was not sufficient to drive particle migration.
The dynamics differed strongly for the partially wrapped Janus microparticles. Once attached to a spherical GUV, the lateral particle movement on the membrane could be described by a linear MSD, i.e., they moved diffusively (Figure 4-13 (A)). On elongated GUVs, however, the particles moved super-diffusively (Figure 4-13 (A), (B)), with motions that could approach deterministic limits, over significant distances to sites of high deviatoric curvature. In some cases, particles traversed distances in excess of 30 particle radii, corresponding to arc length ranging from 15 to 20 µm within 10 to 15 s. The associated $s$-directed migration velocity $U$ was large enough in regions of steepest curvature gradient that particles moved with only weak fluctuations, implying that the driving force far exceeded forces owing to Brownian fluctuations (Figure 4-13 (C)). This migration was not related to the weak drift velocities in the fluid, as verified by using particles in suspension around the vesicle as tracer particles. In an experiment where a Janus particle was migrating towards the pipette, I tracked the migrating particle as well as the tracers in the background (Figure 4-14 (A)) to calculate the velocity in the $s$-direction of all particles (Figure 4-14 (B)). No correlation was found between the bulk convection and the migration direction of the particle on the GUV.

There are two potential sources of energy to drive the observed migration: energies associated with changes in particle adhesion and energy stored in the membrane shape. For particles with pinned contact lines, only the latter would play a role. Detailed studies of the contact line and its potential rearrangement remain an open issue worthy of detailed study. In this work I focused on the role of membrane shape, assuming pinned contact lines.

Both bending energy and membrane tension are associated with membrane shape. In section 4.4.3 below, I investigate the contributions from both contributions by calculating the energy change during particle migration. In experiment, the particle trajectories was analyzed to reveal the dependence of the energy field driving this migration on membrane geometry. The particles moved with negligible inertia, i.e., the Reynolds number $Re = \rho_p Ua / \mu << 1$, where $\rho_p$ is the particle density, and $\mu$ is the solution viscosity. In this limit, energy dissipated along a particle trajectory
is balanced by the work performed on the particle by forces driving its motion. Given the noisy trajectory, it is clear that random Brownian forces played a role. The noisiness is most significant in the regions of a weak deviatoric curvature gradient. I show below that I could divide regions of the trajectory into Brownian-dominated and near-deterministic regions. In the latter regions, by fitting a polynomial to $s$ vs. time $t$ and differentiating with respect to time (Figure 4-13 (C)), the velocity $U$ can be determined. Neglecting Brownian contributions in this region, the energy balance on the particle implies:

$$\Delta E = \frac{k_B T}{D} \int_{s_i}^{s_f} U ds,$$

where $D$ is the particle diffusivity on the membrane and the resistance for an isolated particle is given by the Stokes-Einstein relationship. By integrating equation 4.8, $\Delta E$, the energy dissipated in moving from an initial position $s_i$ to a final position $s_f$, can be inferred for each trajectory. In the Stokes’ flow limit, neglecting the contribution from Brownian migration, this energy difference is equal to the energy driving the particle migration. I initiated this integration at an arc length $s_i$ within 20 particle radii from the boundaries; at distances greater than this value, the motion was diffusive. I also terminated this integration at an arc length $s_f$ at least 5 radii from boundaries, i.e., either the bead or the micropipette, to avoid artifacts associated with hydrodynamic interactions with the bounding surfaces.

I characterized the diffusivity of Janus particles on elongated GUVs. In five cases, particles adhered initially to regions of the membrane with weak curvature gradients, and moved diffusively, allowing the diffusion coefficient to be determined from their respective MSD with a value of $D = 0.12 \pm 0.05 \, \mu m^2/s$ (Figure 4-15). For two of these cases, the particles then diffused to regions of steep curvature, where they then migrated at rates far in excess of the diffusion-dominated displacement. Thus, I was able to measure the particle's diffusivity before migration, and subsequently, for the same particle, characterize energy dissipated during the particle's migration.
associated with the membrane geometry. The particle diffusivity was \( D = 0.07 \, \mu m^2/s \) for one trajectory, and \( D = 0.09 \, \mu m^2/s \) for another (Figure 4-15). For these two cases, I report \( \Delta E \) in units of \( k_B T \) (Figure 4-16); \( |\Delta E| > 100 \, k_B T \) were found. For all other cases in which I observed particle migration, particles adhered initially to high curvature gradient sites where \( U \) was large, precluding measurement of \( D \) for those specific trajectories. The product \( \Delta ED / k_B T \) is reported for those cases (Figure 4-16, inset). Assuming \( D = 0.12 \, \mu m^2/s \) for these trajectories, the associated energy dissipated along these trajectories ranges from \( 50 \, k_B T < |\Delta E| < 350 \, k_B T \). For all cases, \( \Delta E \) depends linearly on the difference in the deviatoric curvature \( \Delta C = \Delta C(s) - \Delta C(s_i) \), the difference in deviatoric curvatures along a particle path from between its instantaneous position \( s \) and its initial position, with coefficient of linear regression \( R^2 \approx 0.99 \). For cases reported in the inset, the slopes varied significantly from trajectory to trajectory and no systematic dependence on tension was observed in the energy dissipated along trajectories (Figure 4-17). This may be attributable to differences in adhesion state from particle to particle and associated differences in the magnitudes of particle-sourced distortions and in the particle drag coefficients (or diffusivities). I hypothesize that these random aspects of the adhesion process obscure any dependence on membrane tension.

Such a linear dependence of energy dissipated on deviatoric curvature has been reported previously for particles migrating along curvature gradients on interfaces between immiscible fluids. In that case, analysis for the associated change in curvature capillary energy, the product of the interfacial tension and the difference in excess area (created through particle-interface interaction) as it migrates, can be expressed:

\[
\Delta E = -\sigma \pi r_0^2 \frac{h_{qp} \Delta C}{2}. \tag{4.9}
\]

In this expression, for fluid interfaces, \( \sigma \) denotes the interfacial tension, \( h_{qp} \) is the magnitude of the quadrupolar mode of the distortion made by the particle in the interface owing to its undulated
contact line, and $\Delta C$ is the change in deviatoric curvature along the particle path. Could the migration of colloids on the tense vesicles be analogous to curvature driven capillary migration on interfaces between immiscible fluids?

Below, I develop the analogy between the observed migration and that observed at isotropic fluid interfaces, and argue that the particles migrate to reduce excess area in the membrane associated with the distortions made by the particle. In both of these systems, surface tension (corresponding to lateral tension of the membrane) is constant. There is an important distinction between these systems, however, since for liquid/liquid interfaces the number of interfacial molecules is not fixed. Vesicular bilayer membranes, however, will not re-adjust their total number of lipid molecules in response to changes in membrane geometry. How can a particle change the area of such a membrane at constant tension? At constant tension, the area of the vesicle outside of the pipette can change, since the pipette-aspirated vesicle fraction acts as an area reservoir. By migrating to sites of high deviatoric curvature, the decrease in area of the particle-sourced distortion can return to this reservoir. To maintain constant tension, the vesicle fraction in the pipette rearranges, lowering the free energy of the system. In this context, I define excess area as the amount of membrane area that is extracted from the area reservoir (represented by the micropipette) at mechanical equilibrium in response to particle binding. In equations 4.8 and 4.9, $\Delta E$ represents the change in total internal energy of the system as the particle moves from $s_i$ to $s_f$. Since this is a state function, it does not depend on the path of the particle connecting initial and final points on the trajectory.

Figure for section 4.4.2
Figure 4-11  **Confocal fluorescence microscopy image of a Janus particle and a homogenous particle attached to a lipid bilayer**

Janus particle is on the left, and homogeneous particle is on the right. Scale bar: 5 µm. Membrane shape is traced out by white dashed lines, and locations of particles are indicated by blue crosses. In both cases, the membranes curve outward. These images show that the homogeneous particle is completely wrapped, while the Janus particle is roughly half-wrapped.

Figure 4-12  **Trajectory of the homogeneous particle on an elongated vesicle**

Inset: mean square displacement calculated from this trajectory.
Figure 4-13 **Curvature migration of colloids on an elongated GUV**

(A) Black open circles: MSD of Janus particle trajectory on a spherical GUV. Blue closed circles: MSD of the trajectory of a migrating particle shown in (C). Red line: straight line with slope of 1 as reference. The axes are in log scale. (B) Paths traced by two independently migrating colloids moving along the curvature gradient on an elongated GUV held at fixed tension $\sigma = 0.4$ mN/m. Particle positions are reported at time intervals of 0.3 s. Scale bar: 10 $\mu$m. (C) Trajectory of a particle migrating on a GUV held at fixed tension $\sigma = 0.5$ mN/m. Black circles: distance of particle from contact with the glass bead in the $s$-direction. $s(t)$ is plotted against $t^*-t$ where $t^*$ is the time that the particle is 5 $a$ from contact with the bead. Green dashed line: cubic fit of the data in the region where 5 $a < s < 20$ $a$; red solid line: migration trajectory predicted by imposing a capillary force on a particle with $h_0=150$ nm; solid gray lines: migration trajectories predicted by integration of Langevin equation for $D=0.09$ $\mu$m$^2$/s and $T=298$ K. Inset: Peclét number of the migrating particle plotted against the arc length.

Figure 4-14 **The migration of a Janus particle on an elongated GUV has no correlation to bulk flow**

(A) Path of a migrating Janus particle (red), and tracers (black and white). (B) Velocity in the $s$ direction for the migrating particle (red) and average of the tracers (black).
Figure 4-15 Determination of diffusivities of Janus particles adhered to elongated GUVs, and diffusive trajectory of a Janus particle on a spherical GUV

(A) MSDs vs. lag time of Janus particles adhered to the elongated GUVs at regions with a weak curvature gradient. (B) Diffusivity of Janus particles on elongated GUVs plotted against membrane tension $\sigma$.

Figure 4-16 Energy dissipated along the colloids' trajectories.

The energy dissipated along a trajectory plotted against deviatoric curvature at each location along particle path. These profiles correspond to the two cases for which particle diffusivity were measured prior to particle migration. Inset: the energy dissipated, normalized by diffusivity, plotted against deviatoric curvature for all 6 cases for which trajectories were analyzed. $\sigma = 0.24, 0.50, 0.54, 0.33, 0.69, 0.56$ mN/m, for red, blue, yellow, purple, magenta, and green lines (slope from shallow to steep) respectively.
4.4.3 Energy analysis

This theory study was developed with the help from Dr. Nima Sharifi-Mood. In this section, we will solve for the energy change of the bilayers and the migrating particle with the Helfrich’s model. In Helfrich's model, membranes with bending rigidity $\kappa$ and tension $\sigma$ define a natural length scale $\lambda = \sqrt{\frac{\kappa}{\sigma}}$. Typically, for microscale particles, the ratio $\varepsilon = \lambda / r_0 \ll 1$. For example, in my experiments, for the range of tension studied, $\varepsilon$ varies from 0.02 to 0.04. To develop an energy expression within the Helfrich model assuming $\varepsilon = \lambda / r_0 \ll 1$, several issues must be addressed. First, the Helfrich energy functional must be minimized to find the Euler-Lagrange equations for the membrane shape $h$ and boundary conditions that apply to the contact line in this limit. Thereafter, these equations must be solved to find the shape of the membrane $h$ around the microparticle. This analysis differs significantly from prior analyses in the literature, as the equations are singular, and require analysis in the context of matched asymptotic expansions. Finally, the energy field as a function of membrane curvature can be evaluated. We will show that, assuming $|\nabla h| \ll 1$, the

Figure 4-17 Normalized $h_{qp}$ and slope of the energy dissipation trajectories vs. membrane tension

(A) There is no obvious correlation between the magnitude of the quadrupole deformation and the membrane tension. (B) There is no obvious systematic dependence of the slopes of the energy dissipation trajectories on membrane tension.
membrane shape is determined by a linearized Young-Laplace equation, with bending energy playing a role only in a small region adjacent to the particle of radial extent similar to $\lambda$. The distortion field made by the particle, and the corresponding energy field, reduces, to leading order, to forms reported previously for particles at fluid interfaces. Derivation of Euler-Lagrange equations and associated boundary conditions

We first derive the differential equation governing the membrane shape and the boundary conditions at the contact line for an arbitrary membrane fluctuation. We then focus on the case of pinned contact lines.

**Membrane energy contribution**

The particle-free membrane has energy described by a Helfrich Hamiltonian:

$$E = \int_{\mathcal{M}} 2\kappa H^2 + \sigma dA.$$  \hspace{1cm} 4.10

where $\mathcal{M}$ denotes the entire membrane. When a particle adheres to the membrane, the membrane shape changes. Adopting the Monge parametrization, the area element can be written:

$$dA = \sqrt{1 + (\nabla h)^2} \, dS$$  \hspace{1cm} 4.11

where $h$ is the membrane height above the reference plane tangent to the unperturbed membrane, and $dS$ is an area element of the reference plane. Under the small slope assumption, i.e. $|\nabla h| \ll 1$, $\sqrt{1 + (\nabla h)^2}$ can be expanded:

$$\sqrt{1 + (\nabla h)^2} = 1 + \frac{(\nabla h)^2}{2} - \frac{(\nabla h)^4}{4} + ...$$  \hspace{1cm} 4.12

The mean curvature of the membrane can be approximated as $\nabla^2 h / 2$. The energy of the membrane outside of the particle under small slope assumption becomes:
where $\mathcal{P}$ denotes the domain beneath the particle. Under the assumptions of small slopes, and assuming that curvatures do not diverge, the term $\kappa(\nabla^2 h)^2 (\nabla h)^2 / 4$ can be neglected as a higher order term, so that the energy associated with the shape of the membrane becomes:

$$E = E_0 + \frac{1}{2} \iiint_{\mathcal{P}} \left[ \kappa(\nabla^2 h)^2 + \sigma(\nabla h)^2 \right] dS,$$

where $E_0$ is a reference energy. Imagine that there is an arbitrary variation to the height of the membrane that decays far from the particle:

$$\delta h = h_f - h_i.$$

The perturbation in membrane energy is:

$$\delta E = E(h + \delta h) - E(h),$$

$$= \frac{1}{2} \iiint_{\mathcal{P}} \left[ \kappa(\nabla^2 h + \nabla^2 \delta h)^2 + \sigma(\nabla h + \nabla \delta h)^2 \right] dS - \frac{1}{2} \iiint_{\mathcal{P}} \left[ \kappa(\nabla^2 h)^2 + \sigma(\nabla h)^2 \right] dS,$$

Neglecting terms quadratic in the membrane shape variation since they are of higher order:

$$\delta E = \iiint_{\mathcal{P}} \left[ \kappa \nabla^2 \nabla^2 \delta h + \gamma \nabla h \nabla \delta h \right] dS.$$

The following useful relationships can be derived from the properties of the derivatives:

$$\nabla^2 \nabla \delta h = \nabla \cdot (\nabla^2 h \nabla \delta h) - \left[ \nabla (\nabla^2 h) \right] \cdot \nabla \delta h.$$
\[ \nabla h \cdot \nabla \delta h = \nabla \cdot (\nabla h \delta h) - \nabla^2 h \delta h. \quad 4.20 \]

Using these properties and applying the divergence theorem, the variation in the free energy can be written as:

\[ \delta E = \frac{1}{2} \int_{\mathcal{V}} \left[ \kappa \nabla^4 h - \sigma \nabla^2 h \right] \delta h dS + \left\{ \frac{\partial}{\partial r} \left( \kappa \nabla^2 h \right) \delta h + \left[ \nabla h \sigma - \kappa \nabla (\nabla^2 h) \right] \delta h \right\} \bigg|_{r=r_0} \mathbf{e}_r d \phi \quad 4.21 \]

where \( \mathbf{e}_r \) is the outward pointing normal vector in the plane of the interface normal to the contact line.

**Adhesion energy contribution**

The adhesion energy between the membrane and the particle is given by:

\[ E_p = 4\pi a^2 \gamma_1 + \int_0^{2\pi} \left( \frac{2}{a^2} + f_{ad} - \gamma_1 \right) a^2 \sin \theta d\theta d\phi, \quad 4.22 \]

where \( f_{ad} \) \((<0)\) is the adhesion energy per unit area between the membrane and the particle, \( \gamma_1 \) is the surface energy of the particle-liquid interface, and where \( \theta \) is the degree of wrapping of the membrane on the spherical cap. The first term under the integral in equation 4.22 is the bending cost to wrap the membrane around a particle with radius \( a \). If, owing to the variation in membrane shape, the contact line is displaced by an amount \( \delta h \), the degree of wrapping will be changed by:

\[ \cos \theta = \cos \theta + \frac{\delta h}{a}, \quad 4.23 \]

Integrating over \( \theta \), the variation in the adhesion energy is:

\[ E_p(h + \delta h) = 4\pi a^2 \gamma_1 + \int_0^{2\pi} \left( \frac{2}{a^2} + f_{ad} - \gamma_1 \right) a^2 \left( 1 - \cos \theta \right) d\phi, \]

\[ = 4\pi a^2 \gamma_1 + \int_0^{2\pi} \left( \frac{2}{a^2} + f_{ad} - \gamma_1 \right) a^2 \left( 1 - \cos \theta + \frac{\delta h}{a} \right) d\phi. \quad 4.24 \]
The resulting variation in the adhesion energy is:

\[ \delta E_p = E_p(h + \delta h) - E_p(h) = \int_0^{2\pi} \left\{ \frac{K}{a^2} + f_{ad} - \gamma_s \right\} a \delta h d\phi, \]

Contributions and constraints from surrounding fluids

The variation \( \delta h \) also displaces the surrounding fluid. The resulting energy change \( \delta E_{\text{bulk}} \) is:

\[ \delta E_{\text{bulk}} = \int_{V_\alpha} \rho_{\alpha} \mathbf{g} \cdot \delta \mathbf{u}^\alpha \Delta t dV + \int_{V_\beta} \rho_{\beta} \mathbf{g} \cdot \delta \mathbf{u}^\beta \Delta t dV, \]

where \( \alpha \) and \( \beta \) denote bulk liquids on both sides of the membrane, \( \delta \mathbf{u}^\alpha \) is the perturbative velocity field owing \( \delta h \), and \( \delta \mathbf{u}^\alpha \Delta t \) is the displacement of fluid in time \( \Delta t \). Assuming that the fluids are incompressible, we append the constraints:

\[ C_{\text{bulk}} = \int_{V_\alpha} P^\alpha \Delta t (\nabla \cdot \delta \mathbf{u}^\alpha) dV + \int_{V_\beta} P^\beta \Delta t (\nabla \cdot \delta \mathbf{u}^\beta) dV, \]

where \( \Delta P^\alpha \) and \( \Delta P^\beta \) are Lagrange multipliers. After manipulation, these terms can be written:

\[ \delta E_{\text{bulk}} + C_{\text{bulk}} = \int_{V_\alpha} (\rho_{\alpha} \mathbf{g} - \nabla P^\alpha) \cdot \delta \mathbf{u}^\alpha \Delta t dV + \int_{V_\beta} (\rho_{\beta} \mathbf{g} - \nabla P^\beta) \cdot \delta \mathbf{u}^\beta \Delta t dV \]

\[ + \int_{A_\alpha} P^\alpha \mathbf{u}^\alpha \cdot \mathbf{n}^\alpha \Delta t dS + \int_{A_\beta} P^\beta \mathbf{u}^\beta \cdot \mathbf{n}^\beta \Delta t dS, \]

where \( \mathbf{n}^\alpha \) and \( \mathbf{n}^\beta \) are the normal pointing towards the liquids \( \alpha \) and \( \beta \), respectively; \( \mathbf{n}^\alpha = -\mathbf{n}^\beta \). The kinematic condition for the membrane allows \( \delta \mathbf{u}^\alpha \cdot \mathbf{n}^\alpha \Delta t dS \) to be expressed as \( \delta h dS \), i.e., the volume element swept out by the membrane variation.

Total variation in energy of the system

Combining all energy contributions and constraints, the variation of the total energy owing to \( \delta h \) is:

70
\[ \delta E_{\text{total}} = \delta E + \delta E_{\rho} + \delta E_{\text{bulk}} + C_{\text{bulk}}, \]
\[ = \frac{1}{2} \oint \left[ \left( \kappa \nabla^4 h - \sigma \nabla^2 h + P^\alpha - P^\beta \right) \right] \delta h dS + \]
\[ \int_0^{2\pi} \left[ \left( \kappa \nabla^2 h \nabla \delta h \right)_{\theta} \right] e_r r_0 d\phi + \int_0^{2\pi} \left\{ \left( \nabla h \sigma - \kappa \nabla (\nabla^2 h) \right) e_r r_0 + \left( 2 \frac{K}{a^2} + f_{ad} - \gamma_1 \right) a \right\} \delta h \bigg|_{\theta=0} d\phi \]
\[ + \int_{\Omega_\alpha} \left[ \rho_{\alpha} g - \nabla P^\alpha \right] : \delta \mathbf{u}^\alpha \Delta t dV + \int_{\Omega_\beta} \left[ \rho_{\beta} g - \nabla P^\beta \right] : \delta \mathbf{u}^\beta \Delta t dV, \]
\[ 4.29 \]

To minimize the energy \( \delta E_{\text{total}} = 0 \); this requires each term in square brackets in equation 4.29 to be zero.

**Euler Lagrange equation and associated boundary conditions**

The first term in equation 4.29 yields the Helfrich equation:

\[-\kappa \nabla^4 h + \sigma \nabla^2 h = \Delta P, \quad 4.30\]

where \( \Delta P = P^\beta - P^\alpha \). This differential equation governs the membrane shape.

The two integrals over \( \Phi \) in equation 4.29 yield sets of conditions at the contact line:

**Contact line boundary condition, set 1:**

\[ (\kappa \nabla^2 h) \nabla \delta h \bigg|_{\theta=0} = 0 \quad 4.31 \]

**Contact line boundary condition, set 2:**

\[ \left( \nabla h \sigma - \kappa \nabla (\nabla^2 h) \right) e_r r_0 + \left( 2 \frac{K}{a^2} + f_{ad} - \gamma_1 \right) a \right\} \delta h \bigg|_{\theta=0} = 0 \quad 4.32 \]

These conditions are given as products in equations 4.31 and 4.32. At the contact line, one term in each product must be zero. In our work, we assume \( \delta h = 0 \) (pinned contact line), thereby satisfying
boundary condition set 2. Boundary condition set 1 then imposes two possible cases, i.e. either 
\[ \nabla^2 h \big|_{r = h} = 0 \] or \[ \nabla \delta h \big|_{r = h} = 0 \]. We will consider both cases in the following membrane shape 
calculations, and show that, to leading order, they yield the same result. Finally, the last square 
brackets in equation 4.29 give the hydrostatic equations for the bulk fluids.

4.4.3.1 The membrane shape around an adsorbed particle

We study a particle of radius \( a \) adhered to a vesicle of radius much larger than \( a \). In the absence of 
the particle, the membrane has a shape \( h_0 \). Upon adhesion, the particle forms a pinned, undulated 
contact line of radius \( r_0 \) whose shape can be described in terms of Fourier modes with amplitudes 
small compared to \( r_0 \). This undulated contact line makes a distortion field around the particle that 
decays over distances comparable to \( r_0 \). The particle is assumed to be far from boundaries, so the 
disturbance can be analyzed in an unbounded domain, and the membrane shape far from the particle 
is given by \( h_0 \). We define the small parameter \( \epsilon = \lambda / r_0 \) and analyze equation 4.30 in the high 
tension limit, i.e. \( \epsilon \ll 1 \). We adopt a polar coordinate system \( (r, \phi) \) with origin and the center of 
mass of the adhered particle.

A small parameter multiplies highest order derivative

Since the distortion decays over distances comparable to \( r_0 \), we normalize equation 4.30 by this 
length scale to find:

\[ -\epsilon^3 \hat{\nabla}^4 \hat{h} + \hat{\nabla}^2 \hat{h} = \Delta \hat{P} \quad 4.33 \]

where \( \hat{\nabla} = r_0 \nabla \), \( \hat{h} = h r_0^{-1} \) and \( \Delta \hat{P} = \Delta P r_0 / \sigma \). The highest order derivative in equation 4.33 is 
multiplied by a small parameter. This is characteristic of a boundary layer problem; if one neglects 
the first term, (i.e., the term associated with bending rigidity), the boundary conditions associated 
with bending cannot be satisfied. This implies that the effects of bending rigidity decay rapidly, 
and are important only in a small region (of radial distance from the contact line \( \sim \lambda \) ) immediately
adjacent to the particle. In this limit, the membrane must be divided into separate domains, as shown in the schematic in Figure 4-19 (A). In the BT domain, immediately adjacent to the contact line, both bending rigidity and tension influence the membrane shape. To find the membrane shape in this domain, and to satisfy the boundary conditions associated with bending rigidity, equation 4.30 will be rescaled with characteristic length scale $\lambda$. Outside of this region, tension alone influences the membrane shape according to equation 4.33. We define two tension dominated domains; the $T_{in}$ domain, near the particle where the particle distortion is finite, and the $T_{out}$ domain, far from the particle where the host membrane is not perturbed. The membrane shape $h^{T_{in}}$ in the $T_{in}$ domain matches to that in the BT domain near the particle, and to the unperturbed membrane shape in the $T_{out}$ domain. The analysis of the $T_{in}$ and $T_{out}$ domains to leading order in $\varepsilon$ corresponds to analysis performed in prior work. We summarize the main points of the solution for those domains here. We summarize the solution for the membrane shape $h^{BT}$ in the BT domain, and the matching between the BT and $T_{in}$ domains.

**Analysis of the tension dominated domains**

We define the membrane height (scaled by $r_0$) as $\hat{h}^{T_{in}}$ in the $T_{in}$ domain, and $\hat{h}^{T_{out}}$ in the $T_{out}$ domain. In each domain, the membrane shape can be expanded in powers of $\varepsilon$, e.g.

$$\hat{h}^{T_{in}} = \hat{h}^{T_{in}}_{(0)} + \varepsilon \hat{h}^{T_{in}}_{(1)} + \varepsilon^2 \hat{h}^{T_{in}}_{(2)} + \ldots$$

We begin by discussing only the leading order solutions and omit the subscript “(0)” for conciseness. In the $T_{out}$ domain, the undisturbed host membrane shape can be expanded in a Taylor series around the origin of the polar coordinate and decomposed into the sum of two terms; a term weighted by the difference in principle radii $\Delta c_0$, which is the antisymmetric part of the curvature tensor evaluated at the origin, and a term weighted by $H_0$, the mean curvature or symmetric part of the curvature tensor of the membrane evaluated at the origin:

$$\hat{h}^{T_{out}}_{(0)} = \frac{\Delta c_0 r_0}{4} \rho^2 \cos 2\phi + \frac{H_0 r_0}{2} \rho^2.$$  

4.34
In the $T_{in}$ domain, to leading order in $\varepsilon$, equation 4.34 reduces to the Young-Laplace equation:

$$\nabla^2 \hat{h}^T = 2Hr_0,$$  \hspace{1cm} 4.35

The undulated contact line imposes a quadrupolar mode to leading order with magnitude $\hat{h}^{qp}$:

$$\hat{h}^T (r = 1, \phi) = \hat{h}^{qp} \cos 2\phi + \hat{\omega},$$  \hspace{1cm} 4.36

where $\omega$ is a shift in the center of mass of the particle. Far from the particle:

$$\lim_{r \to \infty} \hat{h}^T = \hat{h}^{T_{in}}.$$  \hspace{1cm} 4.37

The general solution for equation 4.35 in the polar coordinates has the form:

$$\hat{h}^T (r, \phi) = A \ln r + B + \frac{Hr_0 \hat{\omega}^2}{2}$$

$$+ \sum_{n=1}^{\infty} \left[ \hat{r}^{(n)} \left( A_n \sin n\phi + B_n \cos n\phi \right) + \hat{r}^{(-n)} \left( -C_n \sin n\phi + D_n \cos n\phi \right) \right],$$  \hspace{1cm} 4.38

Using these two boundary conditions, all the unknown constants can be found, and $\hat{h}^T$ is:

$$\hat{h}^T = \frac{\Delta cr_0}{4} \cos 2\phi \left( r^2 - \frac{1}{r^2} \right) + \frac{\hat{h}^{qp}}{r^2} \cos 2\phi + \frac{Hr_0 \hat{\omega}^2}{2},$$  \hspace{1cm} 4.39

where $\hat{\omega} = \frac{Hr_0}{2}$. This solution was derived previously for particles at fluid interfaces$^{58,59,77}$.

Physically, this term indicates that, in order for the particle to fit its contact line in the mean curvature field, the location of the particle needs to be shifted by $\omega$(Figure 4-18).

**Analysis of the BT domain**

To complete the analysis, the membrane shape $h^{BT}$ in the $BT$ domain must be resolved. Any changes in $h^{BT}$ occur over distances comparable to $\lambda$. The rescaled governing equation is:

$$-\tilde{\nabla}^4 \tilde{h}^{BT} + \tilde{\nabla}^2 \tilde{h}^{BT} = \varepsilon^2 \Delta \hat{P},$$  \hspace{1cm} 4.40
where \( \vec{\nabla} = \nabla / \lambda \) and \( \hat{h}^{BT} = h^{BT} / r_0 \).

We define a stretched local coordinate \( \xi = (\hat{r} - 1) / \epsilon = (r - r_0) / \lambda \) originating from the particle surface, shown schematically in Figure 4-19 (B). Recasting equation 4.40 in this local coordinate will yield:

\[
\vec{\nabla}^2 \hat{h}^{BT} = \frac{1}{(1 + \epsilon \xi)} \frac{\partial \hat{h}^{BT}}{\partial \xi} \left[ (1 + \epsilon \xi) \frac{\partial \hat{h}^{BT}}{\partial \xi} \right] + \frac{\epsilon^2}{(1 + \epsilon \xi)^2} \frac{\partial^2 \hat{h}^{BT}}{\partial \phi^2} \\
= \frac{\partial^2 \hat{h}^{BT}}{\partial \xi^2} + \epsilon \frac{\partial \hat{h}^{BT}}{\partial \xi} + O(\epsilon^2),
\]

and

\[
\vec{\nabla}^4 \hat{h}^{BT} = \frac{\partial^4 \hat{h}^{BT}}{\partial \xi^4} + 2\epsilon \frac{\partial^3 \hat{h}^{BT}}{\partial \xi^3} + O(\epsilon^2),
\]

We expand the membrane shape in powers of \( \epsilon \), i.e. \( \hat{h}^{BT} = \hat{h}^{BT}_{(0)} + \epsilon \hat{h}^{BT}_{(1)} + O(\epsilon^2) \), and to leading order, the governing equation becomes:

\[
- \frac{\partial^4 \hat{h}^{BT}_{(0)}}{\partial \xi^4} + \frac{\partial^2 \hat{h}^{BT}_{(0)}}{\partial \xi^2} = 0,
\]

The boundary conditions on this region include the Van Dyke matching condition:

\[
\lim_{\xi \to -\infty} \hat{h}^{BT} = \lim_{\xi \to \xi_{\text{fixed}}} \hat{h}^{BT} = \hat{h}^{BT}_{(0)} + \epsilon \hat{h}^{BT}_{(1)} + O(\epsilon^2), \quad 4.44
\]

and boundary conditions at the contact line. We consider the two cases for boundary conditions for a pinned contact lines. In Case 1, the zero mean curvature boundary condition becomes:

\[
\frac{\partial^2 \hat{h}^{BT}_{(0)} (\xi = 0)}{\partial \xi^2} = 0. \quad 4.45
\]
In Case 2, the slopes between the adhered membrane on the particle and the free membrane at the contact line must be continuous. For a wrapping angle $\theta_0 << 1$ in the small slope limit this requires:

$$\frac{\partial \hat{h}^{BT}(\vec{\xi} = 0)}{\partial \vec{\xi}} = \epsilon \left( \theta_0 + \frac{\hat{h}_\text{wp}}{r_0} \cos 2\phi \right),$$

4.46

To leading order:

$$\frac{\partial \hat{h}^{BT}(\vec{\xi} = 0)}{\partial \vec{\xi}} = 0,$$

4.47

The general solution for equation 4.43 is:

$$\hat{h}_{(0)}^{BT} = f_1 \exp(\vec{\xi}) + f_2 \exp(-\vec{\xi}) + f_3 \vec{\xi} + f_4,$$

4.48

where the constants $f_1$, $f_2$, $f_3$ and $f_4$ are functions of $\phi$. Solving for unknown constants with boundary conditions, I find the leading order solution for either case to be:

$$\hat{h}_{(0)}^{BT} = \hat{h}_\text{wp} \cos 2\phi + \frac{H\hat{h}_0}{2}.$$  

4.49

Thus, the two cases for boundary conditions for pinned contact lines in the $BT$ domain both yield constant membrane shape over the $BT$ region to leading order. Details including higher order terms and uniformly valid solutions are given section 4.4.3.3.

4.4.3.2 The energy landscape around an adhered particle

Absent a colloid, the internal energy of the system is:

$$E_1 = \frac{\kappa}{2} \int_{\mathbb{R}} (\nabla^2 h_0)^2 dS + \sigma \int_{\mathbb{R}} (1 + \frac{\nabla h_0 \cdot \nabla h_0}{2}) dS - \Delta P \| h_0 dS$$

4.50

The last term arises from the $PV$ work done by the pressure drop across the interface $\Delta P = \frac{\sigma}{2H}$.

When a particle attaches, its adhesion changes energy over the domain $\mathcal{P}$ attached to the particle.
The particle-sourced distortion field changes the energy over the domain $\mathcal{M} - \mathcal{P}$ exterior to the contact line.

\[
E_2 = \frac{k}{2} \left[ \int_{\mathcal{M} - \mathcal{P}} (\nabla^2 h)^2 dS + \sigma \int_{\mathcal{M} - \mathcal{P}} \left( 1 + \frac{\nabla h \cdot \nabla h}{2} \right) dS - \Delta P \int_{\mathcal{M}} h dS + E_0 \right],
\]

where $E_0$ is with terms associated with adhesion, wrapping, and transfer of area from the area reservoir (the micropipette aspirated fraction). The change in internal energy owing to the particle adhesion is $E = E_2 - E_i$. We evaluate this energy change to leading order here and first order corrections in section 4.4.3.3. The leading order solution is:

\[
E = E_2 - E_i = \left\{ E_0 + \sigma \pi h_{qp}^2 - \sigma \pi r_0^4 \frac{3H_0^2}{4} \right\} - \sigma \pi r_0^2 \frac{h_{qp} \Delta c_0}{2}. \tag{4.52}
\]

In equation 4.52, the terms in curly brackets are constant for a particle with pinned contact lines on a surface of constant mean curvature. The term proportional to $h_{qp} \Delta c_0$ is the curvature capillary energy driving migration; in this expression, the deviatoric curvature $\Delta c_0$ is evaluated at the particle center of mass. If a particle moves from one position $s_i$ to another position $s_f$, the change in energy $\Delta E$ is given by equation 4.9. Thus, gradients in $\Delta c$ along a particle path drive colloids to sites of high deviatoric curvature to minimize the excess membrane area.

Notice that there is also contribution from mean curvatures in equation 4.52, coming from the area eliminated by the particle and the PV work done to relocate the curved interface, as shown by the dotted parabolic in Figure 4-18. This contribution has also been predicted for particles at the oil-water interface. However, in those studies, interfaces with negligible mean curvature were considered. One could design experiments in which mean curvature gradients play a pronounced role for GUVs. To realize such scenarios, one could use a lipid tether pulled from a flat bilayer, where the mean curvature varies from the flat bilayer to the tether. Alternatively, a lighter or a
heavier solution can be encapsulated in a vesicle in order benefit from gravity to create a vesicle shape similar to that of a pendant drop, thereby introducing a mean curvature gradient. Using such methods, in future studies, deformed lipid bilayers could provide a platform for the study of mean curvature contributions to the migration of colloids on membranes.

4.4.3.3 Leading order correction to membrane shape around the particle and membrane energy

Above, we report the leading order form for the shape of the interface around the particle and for $E$, the change in system energy associated with particle adhesion on a curved tense membrane. Here we find the first order corrections for an asymptotic expansion in powers of $\varepsilon$ for the membrane shape in the $BT$ region and the corrections in $E$.

The order $\varepsilon$, the governing equation of membrane shape in the $BT$ domain, derived from equations 4.41 and 4.42, is:

$$-2\frac{\partial^3 h_{(i)}^{BT}}{\partial \xi^3} + \frac{\partial h_{(i)}^{BT}}{\partial \xi} = 0,$$

With associated general solution:

$$h_{(i)}^{BT} = a_1 + a_2 \exp(\frac{\xi}{\sqrt{2}}) + a_3 \exp(-\frac{\xi}{\sqrt{2}}),$$

Matching to the $T_{in}$ domain, which has no correction of order $\varepsilon$, requires:

$$h_{(i)}^{BT}(\xi \to \infty) = 0,$$

Case 1: Zero mean curvature at the particle surface

The zero mean curvature boundary conditions requires $a_1 = a_2 = a_3 = 0$, so $h_{(i)}^{BT} = 0$. Under this boundary condition, the leading order correction to the membrane shape is zero.

Case 2: Continuous slopes at the contact line

To order $\varepsilon$, continuity of slopes at the contact line requires:

$$\frac{\partial h_{(i)}^{BT}(\xi = 0)}{\partial \xi} = \theta + \frac{h_{m}}{r_0} \cos 2\phi,$$
with associated solution:

\[ h_{BT}^{(1)} = -\sqrt{2} \left( \theta_c + \frac{h_{rp}}{r_0} \cos 2\phi \right) \exp \left( -\frac{\xi}{\sqrt{2}} \right), \tag{4.57} \]

In combination with the leading order solution as shown in equation 4.49, the solution to the membrane shape in the \( BT \) region becomes:

\[ h_{BT} = h_{wp} \cos 2\phi + \frac{Hr_0}{2} - \sqrt{2} \varepsilon \left( \theta_c + \frac{h_{rp}}{r_0} \cos 2\phi \right) \exp \left( -\frac{\xi}{\sqrt{2}} \right) + O(\varepsilon^2), \tag{4.58} \]

To calculate the correction to the membrane energy, we obtain a universally valid solution for both \( BT \) domain and \( T_{sp} \) domain:

\[
\begin{aligned}
 h_{UV} &= r_0 h_{T_{sp}} + r_0 h_{BT} - r_0 \lim_{\xi \to 0} \frac{h_{T_{sp}} n}{\xi} \\
 &= \frac{\Delta \varepsilon}{4} \left( r^2 - \frac{r_0^2}{r^2} \right) \cos 2\phi + \frac{h_{wp} r_0^2}{r^2} \cos 2\phi + \frac{Hr_0^2}{2} - r_0 \sqrt{2} \delta \left( \theta_c + \frac{h_{rp}}{r_0} \cos 2\phi \right) \exp \left( -\frac{\xi}{\sqrt{2}} \right)
\end{aligned}
\tag{4.59}
\]

Substituting \( h_{UV} \) into equations 4.51 and 4.52, we found finite corrections on the order of \( \varepsilon \) for the continuous slope boundary condition, so the membrane free energy becomes:

\[ E = E^{(0)} + \varepsilon E^{(1)}, \tag{4.60} \]

where \( E^{(0)} \) is identical to equation 4.52, and the first order correction is:

\[ E^{(1)} = \sigma \pi r_0^2 \sqrt{2} \Delta \varepsilon h_{wp} + \sigma \pi r_0^3 \frac{3\sqrt{2}}{4} H \theta_c - \sigma \pi \frac{3\sqrt{2}}{4} h_{wp}^2 + \sigma \pi r_0^2 \theta_c^2. \tag{4.61} \]

Figure for section 4.4.3
Figure 4-18 Schematic of a particle in a mean curvature field

Black parabolic: shape of the membrane considering the contribution from mean curvature; black dotted parabolic: the part of the curved interface eliminated by the particle; red dotted line: location of the contact line.

Figure 4-19 Domain layer schematics

(A) Top view of the domain layers: $\mathcal{P}$ indicates the domain beneath the adhered particle; the $BT$ (bending and tension) domain is the region where both bending rigidity and membrane tension play roles to determine membrane shape; the $T_{in}$ domain is the tension dominated domain in which the particle changes the membrane shape; the $T_{out}$ domain is the domain far away from the particle, where the membrane is unperturbed. Domain $\mathcal{M}$ includes all the domains described.
4.4.4 Brownian dynamics

This section was in collaboration with Dr. Nima Sharifi-Mood. A force balance on the particle allows the role of Brownian motion to be discussed and the concepts of weak and strong curvature gradient to be made quantitative. In the $s$ direction, a Langevin equation includes a capillary force, given by the negative gradient of the energy expression in equation 4.9 and a random force owing to thermal fluctuations:

$$\frac{k_B T}{D} \frac{ds}{dt} = -\frac{d\Delta E}{ds} + \sqrt{\frac{2(k_B T)^2}{D}} R(t),$$

where $R(t)$ is a random number that has the following characteristics: $\langle R(t) \rangle = 0$ and $\langle R(t)R(t-\tau) \rangle = \delta(\tau)$. We integrated equation 4.62 for a typical value of $h_{qp} = 150$ nm. Details of the numerical integration can be found in the appendix F. Because of the Brownian term, each integration was different; 15 simulated trajectories, depicted as grey solid lines in Figure 4-13 (C), show distinct zones of behavior. Qualitatively, where curvature gradients were weakest, the simulated trajectories were noisy. In this region, MSD vs. lag time is linear for both experiment and simulation, indicating that this zone is thermally-dominated. Where the curvature gradients were steepest, the predicted and experimental trajectories converged, and agreed with the polynomial fit to this region. We defined a local Péclet number $Pe = U(s) h_{qp} / D$ along a trajectory, defined in terms of the migration velocity of the particle $U(s)$; domains with $Pe >> 1$ indicates that curvature gradients there are strong enough for curvature force to drive the particle motion; $4 < Pe < 30$ over the domain of arc lengths where the realizations converge in Figure 4-13 (C). This corresponds to the domain of arc lengths over which $\Delta E$ was estimated in Figure 4-16.

Figures for section 4.4.4 can be found in section 4.4.3
4.4.5 *Janus particle on membrane with low tension*

Particles failed to migrate to sites of high curvature for the lowest tension that I studied (\(\sigma=0.05\) mN/m) over the course of an experiment; our experiments were limited to around one minute owing to vesicle rupture. This tension value corresponds to \(\varepsilon = 0.06\), for which analysis suggests that weak energy gradients could drive colloids to migrate given long enough times to do so. Integration of equation 4.62 for the vesicle shapes like those in experiments for \(\sigma=0.05\) mN/m indeed predicted that adhered particles would move under weak potential gradients of order \(k_B T\) over the half-length of the vesicle within a five minute interval (Figure 4-20, Figure 4-21). Thus, the failure to migrate at this tension represents a weakening of the capillary force to values too weak to drive migration over the typical time course of an experiment (Figure 4-21).

For tensions an order of magnitude lower, however, \(\varepsilon = 1\), and our analysis does not apply. Both bending and tension would determine the energy landscape. This remains an open regime for study.

**Figures for section 4.4.5**
Figure 4-20 Simulated trajectory of a Janus particles adhered to an elongated GUV at low membrane tension

(A) Trajectory in s-direction for one experiment (black circles) and 5 simulated realizations (gray lines) for simulation time of 60 s. $\sigma=0.05$ mN/m, $h_{qp}=150$ nm, $D=0.09$ $\mu$m$^2$/s. (B) MSD in s-direction calculated from trajectories in (A). Black circles: MSD from the experimental trajectory. Gray open circles: Averaged MSD from the simulated trajectories. (C) Gray lines: trajectories in s-directions for simulations run over 5 min. Parameters are the same as those in (A). Red line: migration under only capillary force with the same conditions. Black circles: high tension experimental data for $\sigma=0.50$ mN/m, $h_{qp}=150$ nm, $D=0.08$ $\mu$m$^2$/s. Inset: Pe number calculated from the capillary migration plotted against migration trajectory in s-direction.
Figure 4-21 **Normalized capillary force vs. s-position**

Force, calculated by differentiation of equation 4.9, for various tension values. GUV shapes and initial particle positions were taken from experiment. The magnitude of the particle-sourced distortion, $h_{qp}$, is assumed to be 150 nm for all cases.

### 4.5 Conclusion

In this chapter, I have presented experiments that explore Janus particles interactions on lipid bilayers, including Janus particles that sense and respond to bilayers curvature. Due to the different chemistries of the two hemispheres of the Janus particles, as well as surface roughness on the particles, I hypothesize that the Janus particles was partially wrapped with a kinetically trapped contact line. Indeed, microscopy images showed that Janus particles are roughly half wrapped, while homogeneous particles are almost fully wrapped.

These Janus particles interacted with each other strongly on bilayers. They also migrated to sites with large deviatoric curvature on tense lipid bilayers. This mode of interaction opens exciting possibilities for active control of particle assembly. Since the force is inherently coupled to vesicle
curvature, the motion can be modulated by changes in the membrane shape. I demonstrate this ability by following trajectories of a particle on a vesicle whose shape is dynamically tuned. When the vesicle is elongated, the particle moves. When the elongation is removed, the particle stops (Figure 4-22).

By calculating of energy dissipated along the migration trajectories, I found that the energy scaled linearly with deviatoric curvature. This is a characteristic of a quadrupolar deformation field coupling with the interface deviatoric curvature to lower the total interfacial area, and has been observed for particles on isotropic fluid interfaces. The fact that migration of particles on lipid bilayers is analogous to particle migration on isotropic fluid interfaces is quite interesting, as these are distinct physicochemical systems. For fluid interfaces, molecules freely enter and leave the interface from the neighboring phases, and the interfacial tension is an intrinsic equilibrium thermodynamic property. This differs significantly from lipid bilayers; tension in these membranes arises from strained packing of the lipids from a preferred configuration as the membrane is set under tension.

In addition to surface tension, a lipid bilayer also has bending rigidity. However, under the tension at which the experiments were performed, analysis of the energy indicated that the role of bending rigidity was higher order. Studying the behavior of these Janus particles under low membrane tension, where bending rigidity and surface tension are equality important, is an interesting direction for future studies.

Moreover, entropic interactions induced by the particle’s suppression of the bilayer’s fluctuations were neglected in the discussions in this chapter. For a tensionless membrane, it has been predicted that fluctuation induced interactions between two rigid disks has the form $E_f = -9k_B T \left( \frac{a}{R_{12}} \right)^8$, where $a$ is the radius of the disks, and $R_{12}$ is the separation distance$^{36}$. According to this expression, the strength of the entropically induced interaction decays to below $k_B T$ upon
separation distance of $1.3 \alpha$. In our system, the particles migrated over distances more than ten times the particle radii with energy dissipated on the order of $100 k_B T$. I therefore concluded that contribution from bilayer fluctuations is negligible in the limit I have studied.

Finally, the relation between this study and curvature sensing activities in cells will likely be subject of interesting future studies. The membrane tension of certain cells can bring $\lambda$ to as low as a few nanometers. For large membrane inclusions such as lipid raft\textsuperscript{82}, viruses\textsuperscript{83} and large protein oligomers\textsuperscript{84}, $\varepsilon = \lambda / r_0$ can be small. Therefore, the membrane curvature sensing mechanism for these inclusions can be dominated by membrane tension, similar to a micron size Janus particle on curved bilayers. However, for smaller molecules such as individual curvature sensing proteins, $\varepsilon$ can be greater or equal to 1, depending on the membrane tension. The membrane curvature sensing mechanism for these small inclusions can be dominated by bending energy, or have mixed contributions from both membrane bending energy and membrane tension. This leaves significant room for interesting future studies.

**Figures for section 4.5**
Figure 4-22 Dynamic tuning of particle migration

The position of the glass bead to a reference point in the center of the vesicle in inset one (red line), and the position of a Janus particle attached to the vesicle (black dots), are plotted against time. The insets show the shape of the vesicle. When the bead is moved further away from the aspiration pipette, the vesicle has a more elongated shape. Simultaneously, the black dots form a steeper slope, indicating that the particle is migrating faster.
Chapter 5  Conclusions and outlook

In the preceding chapters, results on the deformation of lipid bilayers induced by particle binding, interactions between spherical homogeneous and Janus particles, and membrane curvature sensing and migration of Janus particles were discussed. I discovered that by changing membrane tension, the particle wrapping process by the membrane can be controlled. When increasing membrane tension, transitions from weakly wrapped to fully wrapped states were observed. For homogeneous microparticles where the degree of wrapping cannot be precisely controlled, the interactions between pairs of particles were on the order of $k_BT$, and varied between different pairs. To precisely control degree of wrapping, I used Janus particles with hemispheres adhesive to the bilayers. These Janus particles migrated to minimize membrane tension on curved lipid bilayers. By analytically studying the energy of the Janus particles during migration, it was found that the contribution from bending energy was in higher order.

In this chapter, I will introduce a few projects inspired by these studies.

5.1 Anisotropic particles

Anisotropic particles such as ellipsoids, cylinders and dumbbells interact on planar interfaces and assume preferred orientations that depend on their center to center distances and the details of the particle shapes. On curved interfaces, these particles can align along principal axes on the interface. The anisotropic shapes of the particles allow the formation of directional bonds between particles, making them building blocks for hierarchical structures. In this section, preliminary data on lipid bilayers in which anisotropic particles aligned in a curvature field, and in which pairs of particles assumed preferred orientations are reported.

In the first example, a dumbbell shaped aggregate was formed by two Janus particles localized on a vesicle. When the vesicle was elongated, the dumbbell rotated and migrated to sites of high deviatoric curvature while maintaining this orientation (Figure 5-1). I calculated an angle of
alignment, $\alpha$, defined as the angle between the line passing through the centers of the two particles forming the dumbbell, and the axis of symmetry of the elongated vesicle (Figure 5-2). The angle $\alpha$ is plotted against time in Figure 5-3. When the vesicle was elongated, $\alpha$ decreased, indicating that the particle was rotating to align with the axis of symmetry of the vesicle. Such rotation and alignment has been observed for cylinders on isotropic interfaces. It is evidence of alignment of the quadrupolar deformation made by the anisotropic particle aligning with the deviatoric curvature of the interface. In this experiment, the membrane tension was 0.3 mN/m, rendering an $\epsilon = 0.01$. Such alignment of the dumbbell to the curvature field of the membrane was likely driven by membrane tension.

To systematically fabricate anisotropic particles, I have collaborated with Dr. Samantha Wilner on stretching spherical polymeric particles into ellipsoids. We used a published protocol to deform spherical polystyrene particles into ellipsoids. In brief, polystyrene particles with various surface functional groups were suspended in 5 weight % poly (vinyl alcohol) (PVA, molecular weight: 124000, 80% hydrolyzed, Sigma Aldrich) solution in water and 10 % (v/v) isopropanol. The suspension was dried on petri dishes to form solid PVA films. The films were cut into 1 cm wide, 3-6 cm long strips, and clamped into a mechanical stretcher. The films were heated to around 90°C and simultaneously stretched to twice of their original lengths. The films were then dissolved in water with 10% isopropanol, and centrifuged in 15 ml glass tubes at 3000 rpm for 1 hour. Once a pellet of particles could be seen, the supernatant was removed and replaced with water. The particles were re-suspended and sonicated, and centrifuged at 3000 rpm for 20 min. We repeated this process at least three times to remove as much PVA as possible. SEM images of stretched particles are shown in Figure 5-4. Since the PVA film was deformed from a perfect rectangle during stretching when taking particles from an entire PVA film, with wider sides and narrower middle section, the aspect ratio of the particles was observed to be poly-disperse. It is possible to improve
the control over the aspect ratio by selecting only the center portion of the PVA film, but this obviously lowers the yield of the particles.

In preliminary experiments, we stretched particles with radius of 500 nm functionalized with amine groups (Invirogen). The zeta potential indicated that the particles were slightly negatively charged before and after stretching (-10 mV), likely due to sulfate groups on the particles. We therefore attached these ellipsoids electrostatically to positively charged GUVs (20 % DOTAP, 79.5% DOPC and 0.5% Texas Red). We found that a pair of particles aligned preferably in a tip-to-tip orientation on an aspirated GUV (Figure 5-5). The membrane tension was 0.2 mN/m, a value for which we have seen strong analogies between particles on lipid bilayers and particles on isotropic fluid interfaces. Such orientation has been observed and simulated for charged ellipsoids on the oil-water interfaces, due to the interplay between capillary attraction and electrostatic repulsion. It is still unclear in this case if the mechanism of alignment observed here is analogous to that of charged ellipsoids on the oil-water interface. However, since this experiment was performed for values of the membrane tension for which the Euler Lagrange equation for the Helfrich model reduces to the Young-Laplace equation (i.e. $\epsilon \ll 1$), it is unlikely that the alignment is due to effects from membrane bending rigidity or fluctuations. To fully understand this phenomenon, further investigations using uncharged ellipsoids coated with proteins are to be carried out in order to eliminate effect from electrostatic interactions.

**Figures for section 5.1**
Figure 5-1 A Dumbbell particle on an elongated GUV

First 3 panels: snapshots of a pair of Janus beads that form a dumbbell shaped dimer. The dumbbell rotates at roughly fixed position to align along the meridional direction. Elapsed time is labeled in seconds in the image. Fourth panel: time-stamped images reporting the location of the dumbbell after this rotation. Red curve: path traced by dumbbell, which migrates while maintaining its orientation. Time is labeled in seconds adjacent to the image of the dumbbell. Scale bar= 15 µm.

Figure 5-2 Illustration of angle of alignment

Scale bar= 15 µm.
Figure 5-3 **Angle of alignment, $\alpha$, vs. elapse time**

The red line indicate the starting point of vesicle elongation.

Figure 5-4 **SEM images of stretched PS particles**

Left panel: particles from several entire PVA films. Scale bar=10 $\mu$m. Right panel: particles from the center portions of a few PVA films. Scale bar=40 $\mu$m.
Figure 5-5  **Time elapse images of interacting ellipsoids on an aspirated vesicle**

$\sigma=0.2$ mN/m. Time is indicated in seconds in each image. A pair of ellipsoids (indicated by red arrow heads) switched from a zig-zag alignment to a tip-to-tip orientation and remained in such orientation. Scale bar=15 $\mu$m.

### 5.2 Surface pinned GUVs

In chapter 4, I discussed migration of Janus particles on tense bilayers driven by curvature gradients and membrane tension. In that chapter, the limit $\epsilon = \frac{\lambda}{a} \ll 1$ was studied, where bending stiffness played a negligible role. In that study, I used an aspiration pipette and an aspirated glass bead to induce curvature gradients on a GUV. Aspirated GUVs usually do not show visible membrane undulations, and the lowest membrane tension of an aspirated GUV is typically on the order of 0.001 mN/m, yielding $\epsilon = 0.5$ for particles with radius of 500 nm and membrane bending stiffness of $10 k_B T$. In order to study curvature driven interaction in the limit where $\epsilon \geq 1$, a method to deform GUVs under low membrane tension is needed. In this section, I will introduce a way to deform GUVs with low tension by pinning them on a chemically patterned surface.

To fabricate the surface, I started by functionalizing glass cover slips with a layer that prevented lipid bilayer adhesion. The glass cover slips were first cleaned by immersing in 98% sulfuric acid with 1.3 weight % Nochromix crystals (Sigma Aldrich). The cleaned coverslips were subsequently dried with a stream of nitrogen and heated at 200°C for 2 hours to remove any trace of water. The dried cover slips were then immersed in an anhydrous toluene solution with 10 mM of 2-
[methoxy(polyethylenoxy)6-9propyl]trimethoxysilane (PEG-silane, Gelest) for 12 hours. The silanized cover slips were rinsed with 200 proof ethanol for at least 2 min on each side, and subsequently dried by blowing the samples with dry nitrogen. The silanized cover slips showed changes in hydrophilicity: after acid cleaning, the contact angle was zero; after silanization, the contact angle increased to around 50 degrees. AFM data also showed changes in surface roughness: after acid cleaning, the root mean square roughness of the surface was around 8 nm; after silanization, the roughness decreased to 0.8 nm, with islands that were around 20 nm tall (Figure 5-6). I hypothesized that the islands were aggregates of PEG chains on the surface, but the change in surface hydrophility and surface roughness indicated that the silanization was successful.

Afterwards, the silanized cover slips were patterned by photolithography. A layer of positive photoresist S1813 (Dow Chemical Company) with thickness of around 1 µm was spin-coated onto the cover slip at 3000 rpm for 1 min (Model CZ-650, Laurell). The cover slips with photoresist were then soft baked at 115°C for 5 min. After the cover slips were cooled to room temperature, they were exposed to UV light on a mask aligner (Hybralign Series 200 UV mask aligner, QAI) to reach an energy per unit area of 230 mJ/cm² through photomasks with desired transparent patterns (30 µm by 30 µm squares or triangles with sides of 30 µm, CAD/Art services, Inc.). The power per unit area of the UV lamp was measured from the sample stage before the exposure, and the exposure time was calculated by dividing the energy per unit area by the power per unit area. The exposed cover slips were developed in MF-26A developer (Dow Chemical Company) for 1 min with agitation. Since the UV exposed positive photoresist can be dissolved away, holes were formed on the layer of photoresist. The cover slips were then etched by oxygen plasma at radio frequency of 95 MHz for 2 mins. The oxygen plasma destroyed the PEG coating on the regions on the cover slips that were not protected by the photoresist. After washing away the photoresist with acetone, cover slips were left with a layer of PEG with holes. A schematic of the process and AFM image
can be found in Figure 5-7. AFM image showed clear height difference between the holes and the surrounding silane layer.

GUV in suspension were deposited on these patterned cover slips; they adhered to the PEG-free regions to form pinned GUVs. GUVs containing 0.1% of DSPE-PEG-biotin, 99.4% DOPC and 0.5% DiI were successfully pinned on the patterns in 300 mM PBS buffer (Figure 5-8). These pinned GUVs had interesting shapes. At the bottom of the coverslip, they reveal complex shapes near the substrate, and gradually changed to a spherical shape towards the top of the GUV. The corners can potentially act as docking sites to attract particles, as has been observed for particles in a curvature field near a square micropost on the oil-water interface. The surface tension of these pinned vesicle can be adjusted by varying the osmotic pressure. Under hypertonic conditions, the membrane tension of these GUVs can reach the limit of $\epsilon \geq 1$.

In summary, I have introduced a method to pin GUVs onto patterned surface and mold them into interesting shapes. The pinned GUVs can be used as an interface to study particle migration under low membrane tension, or particle migration in complex curvature fields. Unfortunately, the application of this method has been challenged by the following limitation. This specific surface chemistry is only effective for specific GUV compositions: so far, I have successfully used this technique with DOPC GUVs with very small amount of DSPE-PEG-Biotin (< 0.5%), and with DOPC GUVs with 5% of positively charged DOTAP lipids, since the lipids need to be attractive to clean glass surfaces in order to induce pinning, and simultaneously be repulsive to the PEG layer to confine the pinning only to the patterns. To maintain this balance, the salt concentration of the background solution also needs to be above 150 mM. In future investigations, using PEG with different charges to make surfaces with more complex compositions can potentially allow GUVs with more general composition to be studied.

**Figures for section 5.2**
Figure 5-6 AFM images of acid cleaned and silanized cover slips

The acid cleaned cover slip is on the left, and the silanized cover slip is on the right. There is an obvious difference in surface roughness.
Figure 5-7 Schematic of the fabrication process for chemically patterned surfaces

(A) Schematic of the silanization process. (B) Schematic of the lithography process, and bright field microscopy image of the patterned photoresist. Scale bar=30 μm. (C) Schematic of the plasma etching process, and AFM image of the patterned silane layer.
Figure 5-8 **Confocal z-stack images of a pinned GUV**

The z-location measured from the bottom of the cover slip is labeled in µm in the images. Scale bar=10 µm.

### 5.3 Membrane interferometer

Resolution of optical microscopy in the x-y plane is usually around 200 nm, and is even lower in the z-direction. Membrane protrusions and changes in fluctuations generated by curvature sensing and generating proteins are usually too small and dynamic to be resolved by optical microscopy\(^{19,21}\). Here, I will introduce a membrane interferometer, where height fluctuations of a bilayers can be detected as fluorescence intensity changes.

Fluorescence interference contrast microscopy is a technique developed to detect the distance between a fluorophore and a reflective surface. Lights rays travelling to or emitted from fluorophores interfere with rays reflected off from the surface; the resulting fluorescence intensity depends on the distance between the fluorophore-containing entity (such as the lipid bilayer) and
the surface \cite{86}. Using this technique, the distance between the fluorophores contained in the membrane and a surface, (i.e., the membrane height above the surface), can be determined. This technique has been used to observe out-of-plane fluctuations of membranes modulated by osmotic stress\cite{87}, and shape changes in black lipid membranes\cite{88}. In a collaboration with Dr. Katarzyna Jankowska, we fabricated a device to detect membrane height changes upon binding of curvature sensing and generating proteins.

The principal experimental approach is to suspend a lipid bilayer over a reflective surface, similar to the set up for black lipid bilayers in the work by Ganesan et al.\cite{88}. We used the techniques of photolithography and metal deposition to fabricate arrays of 100 nm deep holes on an Au coated film, where the bottom of the holes were reflective surface of a silica wafer.

A thin layer of LOR photoresist (Dow Chemical Company) was deposited onto the silicon wafer by a 2-step spin coat process (CZ-650, Laurell). After approximately 5 ml of photoresist was deposited on the bare silicon wafer, the wafer was spun at 500 rpm for 5 s at 100 rpsms, then at 3000 rpm for 30 s at 300 rmps. The wafer with LOR photoresist was baked at 180°C for 3 min on a hot plate. SPR photoresist (Dow Chemical Company) was spun on the LOR photoresist covered silicon wafer using the same 2-step spin coat process with the same speeds. The silicon wafer covered by two layers of photoresist was baked at 115°C for 2 min.

After depositing the photoresists, the wafer was exposed to UV light (Hybralign Series 200 UV mask aligner, QAI) with a photomask of desired features (dark circles with diameters of 10 µm and 7 µm, Applied Image Inc.). The exposure power was 230 mJ/cm². The lamp power was measured before exposure, and exposure time was calculated. Subsequent to exposure, the wafer was immersed in MF-26A developer (Dow Chemical Company) for 1 min with agitation to remove the non-crosslinked photoresists.
The patterned silicon wafer was sputtered with Chromium at 100 W for 2 min using a home built sputtering chamber. For functionalization purposes, the wafer was then sputtered with a thin layer of gold with thickness of 10-15 nm (Sputter Coater 108, Cressington Scientific). The LOR and SPR photo resists were removed by washing with acetone, and immersing in PG remover (Microchem) at 80°C for 40 min, followed by sonication in isopropanol for 10 min. The metal covered substrates were functionalized by hydroxyl thiols and used no later than 2 weeks after metal deposition (Figure 5-9).

The thiol reaction was carried out in 1.5 ml centrifuge tubes. 150 µL of 200 proof ethanol was added to the centrifuge tube using a Hamilton syringe. 5 µl of 6-Mercapto-1-Hexanol (hydroxyl thiols, 99 %, Sigma Aldrich) was added to the ethanol using another Hamilton syringe. The metal covered wafer was placed into the tube using a pair of clean tweezers, and the tube was purged with nitrogen and sealed with Parafilm. The tube with the substrate inside was sonicated for 1 min to dissolve the thiols. The substrate was incubated in thiols solution for at least 24 hrs. After incubation, the substrate was taken out of the thiol solution and placed in a centrifuge tube with fresh 200 proof ethanol, and sonicated for 1 min to remove excess thiols. The substrate was washed 3 times with DI water and PBS, respectively, by solvent exchange within the same centrifuge tube to avoid exposure to air. Successfully functionalized substrate is hydrophilic due to the hydroxyl groups on the thiols. A drop of water spreads quickly upon dropping on to the substrate.

To introduce lipid bilayers, 5-10 µL of vesicles made in 300 mM sucrose solution was added to a functionalized substrate covered with approximately 30 µL of buffer (20mM HEPES, pH=7.4, 1mM DTT, 1mM EDTA, 150 mM NaCl). After 20 min incubation, the substrate was subsequently washed with fresh buffer by solvent exchange: 10 µL of solution was taken from the substrate with a pipette, then 10 µL of fresh buffer was added back to the substrate. This process was repeated 7-8 times to ensure there were few free vesicles were left and there was no strong fluorescence background. We hypothesized that after this process, GUVs adhered and ruptured on the gold
surface but did not adhere to the bottom of the holes, forming bilayers suspended over the holes. It is important to place the pipette on the edge of the substrate, to avoid breaking the bilayers that were suspended over the holes. Since the substrate was opaque and imaging was carried out on an inverted EPI fluorescence microscope, the substrate was glued onto a cover slip, and was positioned so that the face with the holes and GUVs was facing the objective. A typical image and schematic of a membrane-covered hole is shown in Figure 5-10. Concentric circles can be seen in the fluorescence image of the hole-spanning bilayers. We hypothesized that this was due to the shape of the bilayers that was curving away from the bottom of the holes due to a slight pressure difference across the bilayer.

To introduce protein onto these hole-spanning bilayers, we experimented with two different methods: pipette injection, and fluid channels. In these experiments, epsin, a protein containing a curvature sensing and generating domain (the ENTH domain), and sorting nexin 9 (SNX9), containing a BAR domain, were used. The proteins were purified by Dr. Katarzyna Jankowska. In protein injection experiments, SNX9 solutions with concentration of 4 μM were injected by a pipette near a hole covered with a bilayer. Upon protein injection, a membrane tube with radius on the micrometer scale was formed (Figure 5-11). However, data from these protein injection experiments were difficult to interpret, due to the non-constant protein density on the bilayers. To improve the system, we designed a fluidic channel that allowed exposure of hole-spanning bilayers to a protein solution with protein constant concentration.

A design of the fluidic channel is shown in Figure 5-12. As mentioned, the substrates needed to be mounted so that the side with the patterns were facing the objective. To achieve this, a channel with dimensions as indicated in the schematic was made out of Polydimethylsiloxane (PDMS, SYLGARD® 184 silicone elastomer kit, Dow Chemical Company). A substrate was glued to a window that was cut out on ceiling of the channel using silica grease. The channel and its ceiling needed to be thinner than 2 mm combined, so that the hole-spanning bilayers were within the
working distance of the objective. To fabricate the channel, two layers of electrical tape were cut out to desired width and length, and adhered to the bottom of a 2 inch petri dish to fabricate a channel mold. 2 grams of PDMS and crosslink agent mixture was deposited into the petri dish. The PDMS and the mold were placed in vacuum for at least 30 min to eliminate bubbles trapped in the PDMS. The PDMS was then cured by heating at 80°C on a hot plate for 30 min. Afterwards, the channel was peeled off from the mold, and a square hole was crafted on the channel ceiling where the sample was placed.

In experiments using the fluidic channels, the GUVs were incubated with the substrate for 20 min in the channel. Excess GUVs were washed away by injecting 1 ml of fresh buffer into the channel at a rate of 800 µL/min. Then 400 µL ENTH solution with concentration of 800 nM was injected into the channel at a rate of 800 µL/min. In some cases, we observed drastic topography change of the bilayers. To quantify the changes, standard deviation of the images of the hole-spanning membrane was calculated for every 30 frames. The images of the hole-spanning membrane with changing topography and the standard deviation is shown in Figure 5-13. An increase in the fluorescence intensity fluctuations could be seen, suggesting that the height fluctuations of the membrane changed drastically during protein binding. Note that the intensity changes occur after the flow of the proteins was stopped. Therefore, it is unlikely that such fluctuation changes were caused by flow. We hypothesized that, when curvature sensing and generating proteins bind to the membranes, they induced instability in membrane fluctuations, resulting in fluctuations with amplitudes that increased with time 19.

In summary, hole-spanning bilayers can be a robust tool to study membrane height changes. This system is advantageous over supported bilayers and black lipid membranes, since the membrane can freely fluctuates, which is a closer resemblance to biological conditions compared to supported bilayers and black lipid membranes, whose fluctuations are suppressed. However, there are still a few open issues to be addressed. Membrane shape changes, or changes in membrane undulation
patterns induced by protein binding were inconsistent among different holes. In the ENTH injection experiments, we observed 7 membrane covered holes in total. 4 holes showed formation of micronsized tubes, 2 holes showed drastic topography changes, 1 hole showed membrane rupture, and no changes in membrane shape could be seen in 1 hole. We hypothesized that membrane tensions throughout these hole-spanning membranes are different. Membrane tension can be determined from the out-of-plane fluctuations of the membrane calculated if the exact height fluctuations of the membranes can be measured. However, our setup allowed illumination only with incident light that showed significantly varying incidence angles. This resulted in deviation in the experimentally observed dependence of membrane fluorescence intensity on membrane height from theoretical predicted values. Through modification of the illumination light path, illumination with controlled incidence angles can be achieved in future studies.

**Figures for section 5.3**

![Schematic of the fabrication process for holes on a metal layer](image)

Figure 5-9 *Schematic of the fabrication process for holes on a metal layer*
Figure 5-10 **Images of hole-spanning bilayers**

(A) A schematic of the side view of a hole-spanning bilayer (B) Fluorescent confocal microscopy image of a hole-spanning bilayer (C) Scanning electron microscopy image of a hole. Scale bars: 10 µm.

Figure 5-11 **Hole-spanning bilayers before and after injection of SNX9.**

Before injection is on the left, and after injection is on the right. Scale bar=15 µm.
Figure 5-12 **Schematic of the fluidic channel designed for hole-spanning bilayers**

Figure 5-13 **Fluctuation change of a hole-spanning bilayer after GFP-ENTH injection**

(A) Time elapse images of the hole-spanning membrane. Protein injection time: 28s. Scale bars: 10 µm. (B) Standard deviation of the images’ gray scale values calculated for 30 frames around each time point.
Appendix

A. Matlab code for tracking the membrane location in EPI fluorescence images

clear all
close all
clc
% loading the tif image
name='redaverage';
F=1;
for t=1:F
I=imread([name '.tif'],t);
N=size(I,1);
M=size(I,2);
% click on center
imagesc(I);
[xc,yc]=ginput(1);
% click on start edge
[xe1, ye1]=ginput(1);
[xe2, ye2]=ginput(1);
x1=xe1-xc;
y1=ye1-yc;
x2=xe2-xc;
y2=ye2-yc;
x=x=zeros(10,1);
y=x;
R=round(mean([sqrt(x1^2+y1^2);sqrt(x2^2+y2^2)]));
theta1=asin(-x1/R);
theta2=2*pi-asin(x2/R);
nangle=20;
for i=1:nangle
theta=theta1+i*(theta2-theta1)/(nangle+1);
x(i)=-sin(theta)*R;
if 0.5*pi<theta && theta<1.5*pi
y(i)=-sqrt(R^2-x(i)^2);
else
y(i)=+sqrt(R^2-x(i)^2);
end
% find maximum
nn=10;
for j=-nn/2:nn/2
xm(j+nn/2+1)=x(i)+j;
ym(j+nn/2+1)=xm(j+nn/2+1)*y(i)/x(i);
Im(j+nn/2+1)=I(round(ym(j+nn/2+1)+yc),round(xm(j+nn/2+1)+xc));
end
id=find(Im==max(Im));
xmax(i)=xm(id);
ymax(i)=ym(id);
end
figure
imagesc(I); colormap gray
hold on
xr=xmax+xc;
yr=ymax+yc;
plot(xr,yr,'rx')

% fit to circle
   cfit=@(p)sum(((xr-p(1)).^2+(yr-p(2)).^2-p(3).^2).^2);
   [results,fval1]=fminsearch(cfit,[xc,yc,R]);
xf=results(1);
yf=results(2);
Rf=results(3);

thetac=0:0.1:(2*pi-0.1);
xcp=xf+Rf*sin(thetac);
ycp=yf+Rf*cos(thetac);
plot(xcp,ycp,'bx');
plot(xf,yf,'ro');
save([name '_results.mat'],'results');

end
B. Matlab code to operate the tracking codes written by Blair and Dufresne\textsuperscript{66}

The Matlab files “pkfnd”, “cntrd”, and “bpass” can be found in Ref. \textsuperscript{66}.

```matlab
clc;
clear all;
name='Data4_0826';
savedata=1;
NNP=2;
aa=double(imread([name '.tif'],1));%importing the tif image
N=size(aa,1);
M=size(aa,2);
a=zeros(N,M);
range=0;
colormap('gray'),imagesc(aa);
if range==1
    center=ginput(1);
    side=ginput(1);
    R_range=sqrt((center(1)-side(1))^2+(center(2)-side(2))^2);
else
    R_range=inf;
    center=[0,0];
end

time=0;
% A=137;
% R=sqrt(A*0.5/pi);
% centerx=9.5;
% centery=9.5;

NNF=1057;
xx=zeros(1,NNF);
yy=xx;
stf=1;
for t=stf:1:NNF
    time=time+1;
a=double(imread([name '.tif'],t));
for x=1:N
    for y=1:M
        atotal(x,y,time)=a(x,y);
    end
end
dia=7;
    b=bpass(a,0,dia);
%%
%local max intensity
maxI=max(max(b))*0.6;
pk=pkfnd(b,maxI,dia);
cnt=cntrd(b,pk,dia);
s=size(cnt);
smax(time)=s(1);
x=zeros(smax(time),1);
y=zeros(smax(time),1);
number(time)=0;
```
for i=1:smax(time)
    dtoc=sqrt((cnt(i,1)-center(1))^2+(cnt(i,2)-center(2))^2);
    if dtoc<R_range
        number(time)=number(time)+1;
        distance(i,time)=dtoc;
        x(i)=cnt(i,1);
        y(i)=cnt(i,2);
        xx(i,time)=cnt(i,1);
        yy(i,time)=cnt(i,2);
        for k=1:4
            POS(i,k,time)=cnt(i,k);
        end
    end
end
if time==1;
    aaa=imread([name '.tif'],t);
    colormap('gray'),imagesc(aaa);
    hold on;
    plot(x,y,'rx')
    if range==1
        viscircles(center,R_range);
        plot(center(1),center(2),'bx')
    end
end
end

%lost=time*(1-mean(smax))
dpix=135/(512*1.6);

% for NF=1:size(xx,2)
%     if xx(1,NF)~=0 & & xx(2,NF)~=0
%         DP(NF)=sqrt((xx(1,NF)-xx(2,NF)).^2+(yy(1,NF)-yy(2,NF)).^2);
%     else
%         DP(NF)=nan;
%     end
% end
% xe=91.341;
% ye=80.222;
% R=sqrt(10580/pi);
% xs=xx(1,:)-xe;
% ys=yy(1,:)-ye;
% zs=sqrt(R^2-xs.^2-ys.^2);
% position=[xs',ys',zs'];
if savedata==1
    position=zeros(2,(NNF-stf+1),NNP);
    for iii=1:NNP
        position(1,:,iii)=xx(iii,:);
        position(2,:,iii)=yy(iii,:);
    end
    save(['position_' name ],'position');
end
C. Biotin binding sites measurement and adhesion energy calculation:

Biotin powder was purchased from Sigma Aldrich, and dissolved in PBS buffer. The exact concentration of the biotin solution was measured by Pierce™ Biotin Quantitation Kit (Thermo Fisher). The absorbance of HABA/avidin at the wavelength of 500 nm was measured by UV-vis spectroscopy before and after addition of biotin. The biotin concentration can be calculated from the absorbance value. The concentration of biotin was found to be $c_{\text{biotin \ before}} = 6.89 \times 10^{-6}$ mmole/ml before addition of PNPs. Thereafter, 5 μL of PMPs suspension was added to 300 μL of the biotin solutions. The concentration of biotin was measured again after a 20 min incubation time, and was found to be $c_{\text{biotin \ after}} = 4.43 \times 10^{-6}$ mmol/ml. Particle concentration (from Invitrogen) was $\rho_{\text{particle}} = 1.4 \times 10^{10}$ particles/ml. The number of binding sites per particle and therefore be calculated:

$$N_{\text{biotin \ consumed}} = V_{\text{before}}c_{\text{before}} - V_{\text{after}}c_{\text{after}} = 300 \times 6.89 - 305 \times 4.43 = 14.3 \text{ nmole/ml} \quad A1$$

$$n_{\text{biotin}} = \frac{N_{\text{biotin \ consumed}} \times N_A}{\rho_{\text{particle}}} = \frac{14.3 \times 10^{-9} \times 6.02 \times 10^{23}}{1.4 \times 10^{10}} = 615314 \text{ biotin/particle} \quad A2$$

The area of a particle, $A_{\text{particle}}$, is 3,141,592 nm². The bond strength of a streptavidin-biotin bond, $f_{\text{biotin–streptavidin}}$, is approximately $32 k_B T$. The adhesion energy therefore can be approximated:

$$E_{\text{adhesion}} = \frac{c_{\text{biotin}}}{A_{\text{particle}}} \times f_{\text{biotin–streptavidin}} = 6.23 k_B T / \text{nm}^2 \quad A3$$

For a membrane with a bending stiffness of $10 k_B T$ and membrane tension of 0.2 mN/m, the energy cost per area to wrap a PNP is:

$$\frac{K}{a^2} + \sigma = 0.05 k_B T / \text{nm}^2 \quad E_{\text{adhesion}} \quad A4$$
D. Probability density calculation Matlab code

clear id
clear P
clear Pf
NF=size(DP,1);
NFreal=NF-sum(isnan(DP));

% for n=2:NF
%     if DP(n)>6 && DP(n-1)<6
%         DP(n)=12-DP(n);
%     end
% end
%Find initial position’s density
bin=0.1;
[dt,Ht]=histbinsize(bin,DP,1);
Nbin=size(dt,1);

for j=1:Nbin
    count=0;
    for i=2:NF
        if DP(i)>(min(DP)+(j-1)*bin-0.00000001)&& DP(i) <(min(DP)+bin*(j))
            count=count+1;
            id (count)=i;
        end
    end
    DP0=DP(id-1);
    %Find initial positions
    binid=round((DP0-min(DP))/bin)+1;
    Nt=size(binid,1);
    rouij=zeros(Nbin,1);
P=zeros(Nbin,1);
    number=P;
nw=P;
    for l=1:Nbin
        number(l)=size(find(binid==l),1);
        if number(l)~=0
            nw(l)=number(l)/Ht(l);
        end
    end
end
tw=sum(nw);
for ll=1:Nbin
    pw(ll)=nw(ll)/tw;
    rouij(ll)=pw(ll)*Ht(ll)*Nbin/NF;
end
%calculate the probability density
Pf(j)=nansum(rouij);

% P(j)=sum(Ht(j)/(NF/(Nbin)^2)*H);
end

Histogram calculation code:

function [d,H]=hist(binsize,Data,Dim)
N=size(Data,1);
binn=round((max(Data)-min(Data))/binsize);
H=zeros(binn+1,Dim);
for bb=1:N
    for ii=1:Dim
        if isnan(Data(bb,ii))==0
            id(ii)=round((Data(bb,ii)-min(Data(:,ii )))/binsize(ii))+1;
            H(id,ii)=H(id,ii)+1;
        end
    end
end
mind=min(Data);
for bbb=1:binn+1
    for iii=1:Dim
        d(bbb,iii)=mind(iii)+binsize(iii)*(bbb-1);
    end
end

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E. GUV shape fitting code

Matlab code for locating the membrane in a binary image

clear all
clc
I=imread('data1_2015_07_22_error_bw_r.tif',6);
I=transpose(I);
imagesc(I);colormap gray;
xl=1;
[xc,yc]=ginput(1);
[M,N]=size(I);
upper=0;
lower=1;
pipetteside=1;

count=0;

for i=round(xl):N

    if lower==1
        Iy=I(round(yc):M,i);
        if sum(Iy)~=0
            count=count+1;
xmax(count)=i;
        maxI=mean(find(Iy==255));
        ymax(count)=-1*(maxI-min(maxI))/size(maxI,1)+min(maxI)+round(yc);
        end
    end

    if upper==1
        Iy=I(1:round(yc),i);
        if sum(Iy)~=0
            count=count+1;
xmax(count)=i;
        maxI=mean(find(Iy==255));
        ymax(count)=-1*(maxI-min(maxI))/size(maxI,1)+min(maxI);
    end
end

if pipetteside==1
    xmax=xmax(end:-1:1);
ymax=ymax(end:-1:1);
end
hold on 
plot(xmax,ymax,'rx');

resultr6=[ymax',xmax'];

Finding the axis of symmetry of the GUV

%finding axis of symmetry
%left
xl=xl(5:end);
yl=yl(5:end);
xr=xr(5:end);
yr=yr(5:end);
xp=0;
yp=0;

% pl=polyfit((1:size(xl,2)),xl,2);
% idl=abs(round(pl(2)/(2*pl(1))));
% pr=polyfit((1:size(xr,2)),xr,2);
% idr=abs(round(pr(2)/(2*pr(1))));
plot(xl,yl,xr,yr);
idl=1;
idr=1;
hold on
plot(xl(idl),yl(idl),'rx',xr(idr),yr(idr),'rx')

N=300;
for i=1:N
    id(i)=i;
    mid(i,1)=xl(idl+id(i))-(xl(idl+id(i))-xr(idr+id(i)))/2;
    mid(i,2)=yl(idl+id(i))-(yl(idl+id(i))-yr(idr+id(i)))/2;
end

plot(mid(:,1),mid(:,2),'x')

%p=polyfit(mid(:,1),mid(:,2),1);
x=150:0.01:155;
x2=150:480;
%p=polyfit(x,pl(1)*x+pl(2),'r');
a=-1/(p(1));

%finding the real mid point to rotate
Nl=max(size(xl));Nr=max(size(xr));Nf=max(size(xp));
ynl=zeros(1,Nl);
xnl=ynl;
ynr=zeros(1,Nr);
xnr=ynr;
xn=zeros(1,Nf);
yn=xn;
for i=1:max([NL, NR, NF])
  if i<NL+1
    b(i)=yl(i)-a*xl(i);
   VML(i)=(b(i)*p(1)-p(2)*a)/(p(1)-a);
    XML(i)=-sqrt((XL(i)-XML(i))^2+(YL(i)-YML(i))^2);
    if i>1
      Y1L(i)=Y1L(1)+sqrt((XML(i)-XML(1))^2+(YML(i)-YML(1))^2)*sign(-YML(i)+YML(1));
    end
  end
  if i<NR+1
    b2(i)=yr(i)-a*xr(i);
    YMR(i)=(b2(i)*p(1)-p(2)*a)/(p(1)-a);
    XMR(i)=sqrt((XR(i)-XMR(i))^2+(YR(i)-YMR(i))^2);
    YNR(i)=sqrt((XMR(i)-XML(1))^2+(YMR(i)-YML(1))^2)*sign(-YMR(i)+YML(1));
  end
  if i<NF+1
    b2(i)=yp(i)-a*xp(i);
    YM(i)=(b2(i)*p(1)-p(2)*a)/(p(1)-a);
    XM(i)=sqrt((XP(i)-XM(i))^2+(YP(i)-YM(i))^2);
    YN(i)=sqrt((XM(i)-XML(1))^2+(YM(i)-YML(1))^2)*sign(-YM(i)+YML(1));
  end
end
ynr=-ynr;
ynl=-ynl;

Fitting the shape to a shape with constant mean curvature, written by Liana Vaccari.

clc
clear all

%---------------------------------------------%
% Files needed to run this code %
%---------------------------------------------%

% edge_detection.m
% ylp_full_grad_hess_ode45_min.m
% ylp_full_grad_hess.m

%---------------------------------------------%
% Parameters to change %
%----------------------%

% global needle_mm

% Choose file to be analyzed
% I = imread('OD080at 610nm 22hr robustness_1.tif');

% Threshold for edge detection - see figure 1 to compare smoothness -
% the
% algorithm used can be changed in the edge detection function
threshold = 0.3;

% Number of pixels to cut off from the top of the image. This is
% assuming
% the needle is at the top of the image - if it isn't, either invert the
% image or go into the next level of nested function.
cutoff = 70;

% Outer diameter of needle
needle_ID = 4.;

% Difference in density between the fluids in question.
drho = 223. ; %kg/m^3

% Gravitational constant
gravity = 9.81 ; %m/s^2

%----------------%
% Edge detection %
%----------------%

% global Z_edge_iso X_l_edge_iso X_r_edge_iso ROI_iso
% % Isotropic drop
load error_result_rotated
dpix=0.11;
xl=(xnr)*dpix;
yl=(ynr)*dpix;

% [ Z_edge_iso, X_l_edge_iso, X_r_edge_iso, ROI_iso, BWI] =
edge_detection( I, threshold, cutoff, needle_mm );
Z_edge_iso=yl;
X_l_edge_iso=-xl;
X_r_edge_iso=xl;
ROI_iso=size(X_r_edge_iso,2);
figure(1)
imshow(BWI);

%----------------------%
% Optimization routine %
%----------------------%
for i=50:50
% Initial guesses for Xo Zo Ro and bond
y0 = [-0.0000 -0.71 9.5991 0.];
%lower bounds of variables
lb = [-0.0 -1 -5 0.0];
%upper bounds of variables
ub = [0.0001 5 30 0.01];

%Using fmincon function with tweakable parameters
options = optimoptions('fmincon', 'Algorithm', 'trust-region-reflective', 'GradObj', 'on', 'Hessian', 'user-supplied', 'TolX', 1e-15, 'TolFun', 1e-20, 'TolCon', 1e-40, 'MaxIter', 1, 'MaxFunEvals', 0, 'FinDiffType', 'central');
options.Display = 'iter-detailed';
[y, fval, exitflag, output, Volume, Area, Y, so_needle] = ylp_full_grad_hess_ode45_min_old(Z_edge_iso, X_l_edge_iso, X_r_edge_iso, ROI_iso, needle_ID, y0, lb, ub, options);

%---------%
% Results %
%---------%

%Calculation of surface tension from resulting radius of curvature at the
%apex and bond number.
Ro = y(3); %mm
Y;
bond = y(4);
ST = drho*gravity*Ro^2/(bond*1000); %mN/m
end
save('error_4','Y','y','Ro');

The function “ylp_full_grad_hess_ode45_min_old”

function [ y, fval, exitflag, output, Volume, Area, Y, so_needle ] = ylp_full_grad_hess_ode45_min(Z_edge_iso, X_l_edge_iso, X_r_edge_iso, ROI_iso, needle_ID, y0, lb, ub, options)

    global bond

    options.Display = 'iter';
    [y, fval, exitflag, output] = fmincon(@ylp_full_grad_hess_ode45_min_nested, y0,[],[],[],[],lb,ub,[],options);

    function [ e, g, h ] = ylp_full_grad_hess_ode45_min_nested(y)
        q1 = y(1); %Xo
        q2 = y(2); %Zo
        q3 = y(3); %Ro
        bond = 0; %Bo

        %Experimental drop
\[ Z = Z_{\text{edge_iso}}; \]
\[ X_{\text{l}} = X_{\text{l_edge_iso}}; \]
\[ X_{\text{r}} = X_{\text{r_edge_iso}}; \]

% span of arc length over which to integrate
\[ \text{arc_span} = [0.0 \ 6.0]; \]
\% y1(1)=\( \psi \), y1(2)=r, y1(3)=z,
\% y1(4)=d\( \psi \)/dbond, y1(5)=dr/dbond, y1(6)=dz/dbond,
\% y1(7)=d2\( \psi \)/dbond2, y1(8)=d2r/dbond2, y1(9)=d2z/dbond2
\% y1(10)=V, y1(11)=A, y1(12)=s
\%
\text{slope}
\[ p = \text{polyfit}(X_{\text{l}}(15:25),Z(15:25),1) \ast 0.68 + 0.0034; \]
\[ y1 = [-p(1) -2*\text{mean}(X_{\text{l}}(1)) / 22.5 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0]; \]

% integrate YLP
\text{options} = \text{odeset}('Refine',100);
\[ [S,Y] = \text{ode45}(@ylp_full_grad_hess, \text{arc_span}, y1, \text{options}); \]

% find the closest point on the calculated curve to the
experimental one
\[ \text{S_right_min} = \text{ones(ROI_iso,1)}; \]
\[ \text{S_left_min} = \text{ones(ROI_iso,1)}; \]
\[ x = \text{zeros(length(S),1)}; \]
\[ z = \text{zeros(length(S),1)}; \]
\text{for exp = 1:1:ROI_iso,}
\text{min_right = inf;}
\text{min_left = inf;}
\text{i = 1;}
\text{while (Y(i,3) < 1.5*Z(ROI_iso)) \&\& (i < length(S)),}
\text{calculated curve}
\text{x(i) = Y(i,2);}
\text{z(i) = Y(i,3);}
\text{dist_right = sqrt( ( q3*x(i) + (q1-X_{\text{r}}(exp)) )^2 +}
\text{ ( q3*z(i) + q2-Z(exp) )^2 );}
\text{if dist_right < min_right,}
\text{min_right = dist_right;}
\text{S_right_min(exp) = i;}
\text{end}
\text{dist_left = sqrt( ( q3*x(i) - (q1-X_{\text{l}}(exp)) )^2 +}
\text{ ( q3*z(i) + q2-Z(exp) )^2 );}
\text{if dist_left < min_left,}
\text{min_left = dist_left;}
\text{S_left_min(exp) = i;}
\text{end}
\text{i = i + 1;}
\text{end}

% objective function
\[ e = 0.0; \]
% gradient
\[ g = \text{zeros}(4,1); \]
% hessian
\[ h = \text{zeros}(4); \]
psi_r = zeros(ROI_iso,1);
dpsi_rds = zeros(ROI_iso,1);
dpsi_rdB = zeros(ROI_iso,1);
psi_l = zeros(ROI_iso,1);
dpsi_lds = zeros(ROI_iso,1);
dpsi_ldB = zeros(ROI_iso,1);

x_r = zeros(ROI_iso,1);
dx_rds = zeros(ROI_iso,1);
d2x_rdB2 = zeros(ROI_iso,1);
dx_rds = zeros(ROI_iso,1);
d2x_rds2 = zeros(ROI_iso,1);
d2x_rdsdB = zeros(ROI_iso,1);

x_l = zeros(ROI_iso,1);
dx_ldB = zeros(ROI_iso,1);
d2x_ldB2 = zeros(ROI_iso,1);
dx_lds = zeros(ROI_iso,1);
d2x_lds2 = zeros(ROI_iso,1);
d2x_ldsdB = zeros(ROI_iso,1);

z_r = zeros(ROI_iso,1);
dz_rdB = zeros(ROI_iso,1);
d2z_rdB2 = zeros(ROI_iso,1);
dz_rds = zeros(ROI_iso,1);
d2z_rds2 = zeros(ROI_iso,1);
d2z_rdsdB = zeros(ROI_iso,1);

z_l = zeros(ROI_iso,1);
dz_ldB = zeros(ROI_iso,1);
d2z_ldB2 = zeros(ROI_iso,1);
dz_lds = zeros(ROI_iso,1);
d2z_lds2 = zeros(ROI_iso,1);
d2z_ldsdB = zeros(ROI_iso,1);

compare = zeros(ROI_iso,4);

for i = 1:1:ROI_iso,
    psi_r(i) = Y(S_right_min(i),1);
x_r(i) = Y(S_right_min(i),2);
z_r(i) = Y(S_right_min(i),3);
dpsi_rdB(i) = Y(S_right_min(i),4);
dx_rdB(i) = Y(S_right_min(i),5);
dz_rdB(i) = Y(S_right_min(i),6);
d2x_rdB2(i) = Y(S_right_min(i),8);
d2z_rdB2(i) = Y(S_right_min(i),9);

psi_l(i) = Y(S_left_min(i),1);
x_l(i) = Y(S_left_min(i),2);
z_l(i) = Y(S_left_min(i),3);
dpsi_ldB(i) = Y(S_left_min(i),4);
dx_ldB(i) = Y(S_left_min(i),5);
dz_ldB(i) = Y(S_left_min(i),6);
d2x_ldB2(i) = Y(S_left_min(i),8);
d2z_ldB2(i) = Y(S_left_min(i),9);

if x_r(i) == 0,
    dpsi_rds(i) = 1;
else
    dpsi_rds(i) = 2 - bond*z_r(i) - sin(psi_r(i))/x_r(i); %
/+ before bond says sessile/pendant
end
dx_rds(i) = cos(psi_r(i));
d2x_rds2(i) = - dpsi_rds(i) * sin(psi_r(i));
d2x_rdsdB(i) = - dpsi_rdB(i) * sin(psi_r(i));
dz_rds(i) = sin(psi_r(i));
d2z_rds2(i) = dpsi_rds(i) * cos(psi_r(i));
d2z_rdsdB(i) = dpsi_rdB(i) * cos(psi_r(i));

if x_l(i) == 0,
    dpsi_lds(i) = 1;
else
    dpsi_lds(i) = 2 - bond*z_l(i) - sin(psi_l(i))/x_l(i); %
/+ before bond says sessile/pendant
end
dx_lds(i) = cos(psi_l(i));
d2x_lds2(i) = - dpsi_lds(i) * sin(psi_l(i));
d2x_ldsdB(i) = - dpsi_ldB(i) * sin(psi_l(i));
dz_lds(i) = sin(psi_l(i));
d2z_lds2(i) = dpsi_lds(i) * cos(psi_l(i));
d2z_ldsdB(i) = dpsi_ldB(i) * cos(psi_l(i));

% looking at which points are being compared
compare(i,1) = x_r(i)*q3;
compare(i,2) = z_r(i)*q3;
compare(i,3) = X_r(i)-q1;
compare(i,4) = Z(i)-q2;

x_right = q3*x_r(i) + (q1-X_r(i));
x_left = q3*x_l(i) - (q1-X_l(i));
z_right = q3*z_r(i) + q2-Z(i);
z_left = q3*z_l(i) + q2-Z(i);

% partial derivatives

d2eds2 = q3*( x_right*d2x_rds2(i) + z_right*d2z_rds2(i) +
                   x_left*d2x_lds2(i) + z_left*d2z_lds2(i) ) + 2*q3^2;

d2edsdq1 = q3*( dx_rds(i) - dx_lds(i) );
d2edsdq2 = q3*( dz_rds(i) + dz_lds(i) );
d2edsdq3 = x_right*dx_rds(i) + z_right*dz_rds(i) +
                   x_left*dx_lds(i) + z_left*dz_lds(i) + q3*( x_r(i)*dx_rds(i) +
                   z_r(i)*dz_rds(i) + x_l(i)*dx_lds(i) + z_l(i)*dz_lds(i) );
d2edsdq4 = q3*( x_right*d2x_rdsdB(i) + z_right*d2z_rdsdB(i) +
                   x_left*d2x_ldsdB(i) + z_left*d2z_ldsdB(i) ) +
                   q3^2*( dx_rdB(i)*dx_rds(i) + dz_rdB(i)*dz_rds(i) + dx_ldB(i)*dx_lds(i) +
                   dz_ldB(i)*dz_lds(i) );

dedq1 = x_right - x_left;
\[ \text{dedq2} = z_{\text{right}} + z_{\text{left}}; \]
\[ \text{dedq3} = x_{\text{right}}*x_{r(i)} + z_{\text{right}}*z_{r(i)} + x_{\text{left}}*x_{l(i)} + z_{\text{left}}*z_{l(i)}; \]
\[ \text{dedq4} = q_3*( x_{\text{right}}*dx_{\text{rdB}(i)} + z_{\text{right}}*dz_{\text{rdB}(i)} + x_{\text{left}}*dx_{\text{ldB}(i)} + z_{\text{left}}*dz_{\text{ldB}(i)}); \]

\[ d2edq1dq1 = 2; \]
\[ d2edq1dq2 = 0; \]
\[ d2edq1dq3 = x_{r(i)} - x_{l(i)}; \]
\[ d2edq1dq4 = q_3*( dx_{\text{rdB}(i)} - dx_{\text{ldB}(i)} ); \]
\[ d2edq2dq1 = d2edq1dq2; \]
\[ d2edq2dq2 = 2; \]
\[ d2edq2dq3 = z_{r(i)} + z_{l(i)}; \]
\[ d2edq2dq4 = q_3*( dz_{\text{rdB}(i)} + dz_{\text{ldB}(i)} ); \]
\[ d2edq3dq1 = d2edq1dq3; \]
\[ d2edq3dq2 = d2edq2dq3; \]
\[ d2edq3dq3 = x_{r(i)}^2 + z_{r(i)}^2 + x_{l(i)}^2 + z_{l(i)}^2; \]
\[ d2edq3dq4 = x_{\text{right}}*dx_{\text{rdB}(i)} + z_{\text{right}}*dz_{\text{rdB}(i)} + x_{\text{left}}*dx_{\text{ldB}(i)} + z_{\text{left}}*dz_{\text{ldB}(i)}; \]
\[ d2edq4dq1 = d2edq1dq4; \]
\[ d2edq4dq2 = d2edq2dq4; \]
\[ d2edq4dq3 = d2edq3dq4; \]
\[ d2edq4dq4 = q_3*( x_{\text{right}}*dx_{\text{rdB}(i)} + z_{\text{right}}*dz_{\text{rdB}(i)} + x_{\text{left}}*dx_{\text{ldB}(i)} + z_{\text{left}}*dz_{\text{ldB}(i)}; \]

% objective function
\[ e = e + 0.5*( x_{\text{right}}^2 + z_{\text{right}}^2 + x_{\text{left}}^2 + z_{\text{left}}^2); \]

% gradient
\[ g(1) = g(1) + \text{dedq1}; \]
\[ g(2) = g(2) + \text{dedq2}; \]
\[ g(3) = g(3) + \text{dedq3}; \]
\[ g(4) = g(4) + \text{dedq4}; \]

% hessian
\[ h(1,1) = h(1,1) + d2edq1dq1 - d2edsdq1*d2edsdq1/d2eds2; \]
\[ h(1,2) = h(1,2) + d2edq1dq2 - d2edsdq1*d2edsdq2/d2eds2; \]
\[ h(1,3) = h(1,3) + d2edq1dq3 - d2edsdq1*d2edsdq3/d2eds2; \]
\[ h(1,4) = h(1,4) + d2edq1dq4 - d2edsdq1*d2edsdq4/d2eds2; \]
\[ h(2,1) = h(2,1) + d2edq2dq1 - d2edsdq2*d2edsdq1/d2eds2; \]
\[ h(2,2) = h(2,2) + d2edq2dq2 - d2edsdq2*d2edsdq2/d2eds2; \]
\[ h(2,3) = h(2,3) + d2edq2dq3 - d2edsdq2*d2edsdq3/d2eds2; \]
\[ h(2,4) = h(2,4) + d2edq2dq4 - d2edsdq2*d2edsdq4/d2eds2; \]
\[ h(3,1) = h(3,1) + d2edq3dq1 - d2edsdq3*d2edsdq1/d2eds2; \]
\[ h(3,2) = h(3,2) + d2edq3dq2 - d2edsdq3*d2edsdq2/d2eds2; \]
\[ h(3,3) = h(3,3) + d2edq3dq3 - d2edsdq3*d2edsdq3/d2eds2; \]
\[ h(3,4) = h(3,4) + d2edq3dq4 - d2edsdq3*d2edsdq4/d2eds2; \]
\[ h(4,1) = h(4,1) + d2edq4dq1 - d2edsdq4*d2edsdq1/d2eds2; \]
\[ h(4,2) = h(4,2) + d2edq4dq2 - d2edsdq4*d2edsdq2/d2eds2; \]
\[ h(4,3) = h(4,3) + d2edq4dq3 - d2edsdq4*d2edsdq3/d2eds2; \]
\[ h(4,4) = h(4,4) + d2edq4dq4 - d2edsdq4*d2edsdq4/d2eds2; \]
end

% dimensionalize the calculated curve

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R_dim = zeros(length(S),1);
Z_dim = zeros(length(S),1);
V_dim = zeros(length(S),1);
A_dim = zeros(length(S),1);
needle_height = 0;
j = 1;
for i = 1:length(S);
    S(i) = S(i)*q3;
    R_dim(i) = Y(i,2)*q3;
    Z_dim(i) = Y(i,3)*q3;
    V_dim(i) = Y(i,10)*q3^3;
    A_dim(i) = Y(i,11)*q3^2;
    if R_dim(i) < needle_ID/2 && Z_dim(i) > q3,
        needle_height(j) = i;
        j = j + 1;
    end
end
if needle_height(1) == 0,
    needle_height(1) = length(S);
    needle_height(length(needle_height)) = length(S);
end
Volume = V_dim(needle_height(1));
Area = A_dim(needle_height(1));
so_needle = S(needle_height(1));

%center experimental drop with optimized values for position
for i = 1:length(Z)
    Z(i) = Z(i)-q2;
    X_l(i) = X_l(i)-q1;
    X_r(i) = X_r(i)-q1;
end

curve1 = [ compare(:,1) compare(:,3) ];
curve2 = [ compare(:,2) compare(:,4) ];

%shows both the calculated curve with the current parameter
iteration
%and the experimental curve
figure(3)
plot(R_dim(:,1),Z_dim(:,3),'b',-
R_dim(:,2),Z_dim(:,1),'b',X_l,Z,'r',X_r,Z,'r',curve1', curve2','k')
figure(4)
plot(R_dim(1),Z_dim(1),'b',-R_dim(1),Z_dim(1),'b')

end
end
The function “ylp_full_grad_hess”

function [dy] = ylp_full_grad_hess(t,y)
    global bond
    dy = zeros(12,1);
    if y(2) == 0,
        dy(1) = 1;
    else
        dy(1) = 1 - bond*y(3) - sin(y(1))/y(2); % +/- before bond says sessile/pendant
    end
    dy(2) = cos(y(1));
    dy(3) = sin(y(1));
    if y(2) == 0,
        dy(4) = 0.0;
    else
        dy(4) = sin(y(1))*y(5)/y(2)^2 - y(3) - bond*y(6) - cos(y(1))*y(4)/y(2); % +/- before bond says sessile/pendant
    end
    dy(5) = -y(4)*sin(y(1));
    dy(6) = y(4)*cos(y(1));
    if y(2) == 0,
        dy(7) = 0.0;
    else
        dy(7) = -2*sin(y(1))*y(5)^2/y(2)^3 + 2*cos(y(1))*y(5)*y(4)/y(2)^2 + sin(y(1))*y(8)/y(2)^2 - 2*y(6) - bond*y(9) + sin(y(1))*y(4)^2/y(2) - cos(y(1))*y(7)/y(2); % +/- before bond says sessile/pendant
    end
    dy(8) = -y(4)^2*cos(y(1)) - y(7)*sin(y(1));
    dy(9) = pi*y(2)^2*sin(y(1));
    dy(10) = 2*pi*y(2);
    dy(11) = 2*pi*y(2);
    %dy(1)=dpsi/ds, dy(2)=dr/ds, dy(3)=dz/ds
    %dy(4)=d2psi/dsdbond, dy(5)=d2r/dsdbond, dy(6)=d2z/dsdbond
    %dy(7)=d3psi/dsdbond2, dy(8)=dr/dsdbond2, dy(9)=dz/dsdbond2
    %dy(10)=dVds
    %dy(11)=dAds
    %dy(12)=ds/ds
end

The function “ylp_volume”

function [dy] = ylp_volume(t,y)
    dy = zeros(5,1);
    dy(1) = 1 - sin(y(1))/y(2); % +/- before bond says sessile/pendant
    %normalized by mean curvature
    dy(2) = cos(y(1));
    dy(3) = sin(y(1));
    dy(4) = pi*y(2)^2*sin(y(1));
    dy(5) = 2*pi*y(2);
    %y(1)=psi, y(2)=r, y(3)=z, y(4)=V, y(5)=A
end
F. Non-dimensionalization and numerical integration of the Langevin equation

To non-dimensionalize the Langevin equation, we define: \( \tilde{x} = \frac{x}{x^*} \), \( \tilde{t} = \frac{t}{\tau_c} \), \( \tilde{M} = \frac{M}{M_c} \), \( \Delta \tilde{c} = \frac{\Delta c}{\Delta c^*} \), \( \tilde{R}(\tilde{t}) = R(t)\tilde{x}_c^{1/2} \) and \( \tilde{h}_{qp} = \frac{h_{qp}}{h_{qp}^*} \).

Insert above and equation 4.9 into equation 4.62:

\[
\frac{x^*}{\tau_c} \frac{d\tilde{x}}{dt} = M \tilde{M} \frac{\pi}{2} \gamma a^2 \tilde{h}_q \tilde{h} \frac{d\Delta \tilde{c}}{d\tilde{x}} \frac{\Delta c^*}{x^*} + \sqrt{2kTM} \tilde{R}(\tilde{t}) \tau_c^{-1/2} \quad A5
\]

Rearrange:

\[
\frac{d\tilde{x}}{dt} = \frac{\tau_c M}{x_*^2} h_{qp}^* \Delta c^* \gamma a^2 \tilde{h}_q \tilde{h} \frac{d\Delta \tilde{c}}{d\tilde{x}} \frac{\Delta c^*}{x^*} + \sqrt{2kTM} \frac{\tau_c}{x_*^2} \sqrt{M \tilde{R}(\tilde{t})} \quad A6
\]

Define \( x^* = a \) and \( M_c = \frac{D}{kT} \):

\[
\frac{d\tilde{x}}{dt} = \frac{\tau_c}{\gamma h_{qp}^* \Delta c^*} \frac{D}{kT} \frac{h_{qp}^* \Delta c^* \gamma a^2 \tilde{h}_q \tilde{h} \frac{d\Delta \tilde{c}}{d\tilde{x}}}{x^*} + \sqrt{2D\tau_c} \frac{1}{a^2} \sqrt{M \tilde{R}(\tilde{t})} \quad A7
\]

Define \( \tau_c = \frac{kT}{D \gamma h_{qp}^* \Delta c^*} \):

\[
\frac{d\tilde{x}}{dt} = \frac{\tau_c \tilde{M} \tilde{h} \frac{d\Delta \tilde{c}}{d\tilde{x}}}{x_*^2} + \sqrt{2kT} \frac{1}{a^2 \gamma h_{qp}^* \Delta c^*} \sqrt{M \tilde{R}(\tilde{t})} \quad A8
\]

Define the dimensionless number Peclét number:

\[
P_e = \frac{\gamma a^2 h_{qp}^* \Delta c^*}{2kT} \quad A9
\]

Equation A8 becomes:
\[
\frac{d\tilde{x}}{dt} = \frac{\pi}{2} \tilde{M} \tilde{h}_{q_p} \frac{d\Delta \tilde{c}}{d\tilde{x}} + Pe^{-1/2} \sqrt{M} \tilde{R}(\tilde{t}) \quad \text{A10}
\]

\(\tilde{R}(\tilde{t})\) was generated by function randn(1) in Matlab. All other parameters were obtained from experiments.

Discretizing equation A10:

\[
x_{i+1} = x_i + \delta \tilde{t} \frac{\pi}{2} \tilde{M} \tilde{h}_{q_p} \frac{d\Delta \tilde{c}}{d\tilde{x}} + \sqrt{\delta \tilde{t}} Pe^{-1/2} \tilde{R}(\tilde{t}) \quad \text{A11}
\]

To plot the result in real time, the time is made dimensional by:

\[
t = \tilde{t} \underline{\tau} = \tilde{t} \frac{kT}{D \gamma} \frac{1}{\Delta c^*} \quad \text{A12}
\]

Where \(D\) is obtained from experiment.

To initiate the simulation, we need to guess a value for \(h_{q_p}\) as input to equation A11. From experience, we fitted the part of the trajectories where the particles are between 5 and 15 radii away from contact for an initial guess in \(h_{q_p}\).

Matlab code:

```matlab
clear all
%load r
load data1_2015_07_15_result
load data1_2015_07_15_GUV_p
load data1_2015_07_15_p_s
s=sall;
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%characteristics
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
a=0.5*10^(-6); \ %m radius
D= 0.08*10^(-12); \ %m^2/s
kT=1.38*10^(-23)*298;\ %N*m
hqpc=30*10^(-9); \ %m
delcc=0.01*10^6;\ % m^-1

%%%%%change these two values for different experiments
hqp=150*10^(-9)/hqpc; \ %m
```

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\[ \text{tension} = 0.00005; \quad \% N/m \]

\[
\tau = \frac{kT}{(D \times \text{tension} \times hqpc \times delcc)};
\]

\[
Pe = \frac{(a^2 \times \text{tension} \times hqpc \times delcc)}{(2 \times kT)};
\]

\[
y_0 = y;
\]

\[
dpix = 0.11;
\]

\[
\psi = Y(:,1);
\]

\[
r = Y(:,2) \times Ro \times 10^{-6};
\]

\[
z = Y(:,3) \times Ro \times 10^{-6};
\]

\[
curp = \sin(\psi) \times r^{-1};
\]

\[
curm = \text{gradient}(\psi, Y(:,12) \times Ro \times 10^{-6});
\]

\[
% \quad \text{curm} = 1/Ro - \text{curp};
\]

\[
de = -\text{curm} + \text{curp};
\]

\[
z = z(1:1500);
\]

\[
\psi = \psi(1:1500);
\]

\[
\text{scalc} = z;
\]

\[
\text{scalc}(1) = 0;
\]

\[
\text{for} \quad ss = 2 : \text{size}(z,1)
\]

\[
\quad \text{scalc}(ss) = \text{scalc}(ss-1) + (z(ss) - z(ss-1))/\sin(\psi(ss));
\]

\[
\text{end}
\]

\[
n = 7;
\]

\[
pd = \text{polyfit} (\text{scalc}, \text{dev}, n);
\]

\[
\text{devfit} = pd(n+1);
\]

\[
\text{for} \quad ii = 1 : n
\]

\[
\quad \text{devfit} = \text{devfit} + pd(n-ii+1) \times \text{scalc} \times (ii);
\]

\[
\text{end}
\]

\[
d\text{devfit} = pd(n);
\]

\[
\text{for} \quad iii = 2 : n
\]

\[
\quad d\text{devfit} = d\text{devfit} + pd(n-iii+1) \times \text{scalc} \times (iii-1) \times iii; \quad \% 1/m
\]

\[
\text{end}
\]

\[
\text{SStot2} = \text{sum}((\text{dev} - \text{mean} (\text{dev})).^2);
\]

\[
\text{SSreg2} = \text{sum}((\text{devfit} - \text{mean} (\text{devfit})).^2);
\]

\[
R22 = \frac{\text{SSreg2}}{\text{SStot2}}
\]

\[
\text{nnmax} = 1;
\]
dt=0.001;
NF=20000;
st=zeros(NF,1);
st(1)=20; % nondim
stc(1)=st(1);

n=7;
for f=2:NF
  devcheck(f)=pd(n+1);
  % finding the gradient of deviatoric curvature
  for ii=1:n
    devcheck(f)=devcheck(f)+pd(n-ii+1)*(stc(f-1)*a)^(ii);
  end
  ddev(f)=pd(n);
  ddevc(f)=pd(n);
  for iii=2:n
    ddev(f)=(ddev(f)+pd(n-iii+1)*(st(f-1)*a)^(iii-1)*iii);
    ddevn(f)=ddev(f)*a/delcc;
    ddevc(f)=(ddevc(f)+pd(n-iii+1)*(stc(f-1)*a)^(iii-1)*iii);
    ddevnc(f)=ddevc(f)*a/delcc;
  end
  % langevin equation
  st(f)=st(f-1)+pi/2*hqp*ddevn(f)*dt+Pe^(-1/2)*randn(1)*sqrt(dt);
  stc(f)=stc(f-1)+pi/2*hqp*ddevnc(f)*dt;
  fc(f)=pi/2*hqp*ddevnc(f)*dt;
end

% t=(0:(NF-1))*tau*dt;
% convert to microns
stc=stc*0.5;
st=st*0.5;

% min(abs(s-s(end))==min(abs(s-s(end))))
% id0=min(find(abs(st-s(ids0))==min(abs(st-s(ids0)))));
id0=NF;
% idc0=min(find(abs(stc-s(ids0))==min(abs(stc-s(ids0)))));
% save('realization_low_tension','st');
% hold on
% plot(t(id0)-t(2:id0),st(2:id0)-s(end));
% hold on
% plot(((ids0-1):-1:0)*0.03,s(1:ids0)-s(end),'o')
% hold on
% plot(t(idc0)-t(1:idc0),stc(1:idc0)-s(end));

% u=(stc(2:end)-stc(1:(end-1)))/(tau*dt);
% Pe=-u*a*10^(-6)/D;
G. Displacement on a sphere and MSD calculation Matlab code

Displacement on a sphere calculated from projected positions:

```matlab
% MSD
% clear all
clear all
name='Data1';
correct3D=1;  
% results saved by the particle tracking code
load position_Data1
load time_Data1
if correct3D==1
load vesicle_Data1
xc=vesicle(2);
yc=vesicle(3);
R=sqrt(vesicle(1)/pi);
else
    xc=0;
yc=0;
end

NNP=2;
n1=1;
n2=2;
dpix=135/(512*1.6);
xx=zeros(size(position,2),NNP)*dpix;
xx(:,:)=position(1,:,:);
yy=zeros(size(position,2),NNP)*dpix;
yy(:,:)=position(2,:,:);
xx(xx==0)=nan;
yy(yy==0)=nan;
% correct for 3D
Pnum=1;

xs=xx(:,Pnum)-xc;
ys=yy(:,Pnum)-yc;
zs=sqrt(R^2-xs.^2-ys.^2);
dpix=135/512/1.6;
position3=[xs,ys,zs]*dpix;
dim=2;

%%%%%%%% on off %%%%%%%%%%
histogram=0;
MSDcalc=0;
DPcalc=1;

NF=size(position3,1);
```
if histogram==1
    dt=6;
    d=zeros(NF-dt,dim);
    for i=1+dt:NF
        d(i-dt,:)=position3(i,:)-position3(i-dt,:);
    end
    [dxyz,H]=hist(11,d,dim);
end

if MSDcalc==1
    MSD3=zeros(NF,1);
    for f=1:NF-1
        D=zeros(NF,1);
        for ff=f+1:NF
            D(ff)=sum((position3(ff,:)-position3(ff-f,:)).^2);
        end
        MSD3(f+1)=sum(D)/(NF-f);
    end
    MSDfit=MSD3(1:50);
    logMSD=log(MSDfit);
    tfit=0.033*(1:50);
    lopt=log(tfit);
    fitMSD= @(p)sum((p(1)*tfit'+p(2)-MSDfit).^2);
    [results,fval]=fminsearch(fitMSD,[1,0]);
end

if DPcalc==1
    xs1=xx(:,n1)-xc;
    ys1=yy(:,n1)-yc;
    xs2=xx(:,n2)-xc;
    ys2=yy(:,n2)-yc;
    if correct3D==1
        zs1=sqrt(R^2-xs1.^2-ys1.^2);
        zs2=sqrt(R^2-xs2.^2-ys2.^2);
    else
        zs1=0;
        zs2=0;
    end

    DP=sqrt((xs1-xs2).^2+(ys1-ys2).^2+(zs1-zs2).^2)*dpix;
    DPr=2*asin(DP/(R*dpix))*R*dpix;
    DP(DP>6)=nan;
    [dDP,HDP] =hist(20,DP,1);
    plot(t(1:size(DP,1)),DP,'-o');
    save(['DP_' name],'DP');
end
MSD calculation function:

```matlab
function [MSD3]=MSDfun(DP,R)
NF=size(DP,1);
MSD3=zeros(NF,1);
for f=1:NF-1
    D=zeros(NF,1);
    Ds=D;
    theta=D;
    for ff=f+1:NF
        D(ff)=nansum((DP(ff,:)-DP(ff-f,:)).^2);
        theta(ff)=2*asin(0.5*D(ff)/R);
        Ds(ff)=R*theta(ff);
    end
    MSD3(f+1)=nansum(Ds)/(NF-f);
end
end
```

H. Energy integration and fitting code for Janus particle migration

```matlab
clear all
%load r
load data1_2015_07_15_result
load data1_2015_07_15_GUV_p
bead=1;
dpix=0.11;

t=T=1.38*10^(-23)*298;
y0=y;
yn=yn(1:end-0)*dpix-y0(2);
yn=yn;
dt=0.03; % s
zexall=yall;
NFall=size(zexall,2);
tall=(0:(NFall-1))*dt;
% tend=2.69;
% if tall(end)< tend
%     diffstart=(tall-tall(end)).^2;
% else
%     diffstart=(tall-tend).^2;
% end
% start=NFall-find(diffstart==min(diffstart))+1;

diffend=(zexall-zexall(end)-2.5).^2;
cutend=NFall-find(diffend==min(diffend));
diffstart=(zexall-zexall(end)-10).^2;
start=find(diffstart==min(diffstart));
yn=yn(start:end-cutend);
```
t=(cutend:(NFall-start))*dt;
zeug
NF=size(zeug,2);

plotall=0;
plotdevE=1;
plotd=0;
%yn=yn(1:180);
%Y=Y*0.11/0.1;
%Ro=Ro*0.11/0.1;

%converting geometries to microns
psi=Y(:,1);
r=Y(:,2)*Ro;
z=Y(:,3)*Ro;
scalc=z;
scalc(1)=0;
for ss=2:size(z,1)
    scalc(ss)=scalc(ss-1)+(z(ss)-z(ss-1))/sin(psi(ss));
end
%calculating curvatures
curm=gradient(psi,Y(:,12)*Ro);
curp=sin(psi).*r.^(-1);
dev=-curn+curp;
for ii=1:size(z,1)-2
    ddevds(ii)=((dev(ii+2)-dev(ii))/(scalc(ii+2)-scalc(ii)))*10^(12); %m^-2
    ddevdz(ii)=((dev(ii+2)-dev(ii))/(z(ii+2)-z(ii)));
end
Ro;
D=0.11*10^(-12); %Diffusivity
%D=1;
a=500*10^(-9);
kbT=1.38*10^(-23)*298;
tension=0.000536759; %N/m

for i=1:NF;
    diff=0;
    diff=abs(zeug(i)-z);
    id(i)=find(diff==min(diff));
    s(i)=scalc(id(i));
end
for i=1:NFall;
    diff=0;
    diff=abs(zeugall(i)-z);
idall(i)=find(diff==min(diff));
sall(i)=scalc(idall(i));

n=3;
p=polyfit(t,s,n);
sfit=p(n+1);
for ii=1:n
    sfit=sfit+p(n-ii+1)*t.^(ii);
    if ii>1
        ds=ds+p(n-ii+1)*t.^(ii-1)*ii;
    end
end

for i=1:NF
    diff=0;
    diff=abs(sfit(i)-scalc);
    ids(i)=find(diff==min(diff));
    devplot(i)=dev(ids(i));
end
E=zeros(NF,1);

for j=2:NF-1
    for i=1:j
        v(i)=ds(i)*10^(-6);
        F(i)=kbT/D*v(i); %kg*m/s^2=N
        ds1(i)=ds(i)*10^(-6)*dt;
        E(j+1)=-sum(F.*ds1)/kbT;
    end
end

%fitting devplot vs. E with a linear line and R2
fitqua=1;
fitlin=0;
fitquaonly=0;
cut=0;
devfit=devplot(1:end-cut)'*10^(6);%m
Efity=E(1:end-cut);
if fitqua=1
    Sfit=@(p)sum(abs(p(1)*devfit+2.9*10^(-9)*devfit.^2+p(2)-Efity));
    [result,fval1]=fminsearch(Sfit,[0.001,30]);
    %slope=p(2);
    %qua=p(1);
    %ratio=qua/slope
    fit=result(1)*devfit+2.9*10^(-9)*devfit.^2+result(2);
end
if fitlin==1
    p=polyfit(devfit,Efity,1);
slope=p(1)
    fit=p(1)*devfit+p(2);
if fitquaonly==1
    \text{p=polynomial\(fitv\text{.^2,Efit,1}\);}  \\
    \text{fit=p(1)*devfit.^2+p(2);}  \\
end

\text{SStot=sum((Efit-mean(Efit)).^2);}  \\
\text{SSreg=sum((fit-mean(Efit)).^2);}  \\
R2=SSreg/SStot  \\
\text{diff10=abs(2.5+zex(end)-zex);}  \\
\text{id10=find(diff10==min(diff10));}  \\
\text{id10=min(id10);}  \\
\text{if id10==1}  \\
\text{id10=nan;}  \\
end

\% figure  \\
\% subplot(3,1,1);  \\
\% plot(z,r,'-r','LineWidth',2)  \\
\% hold on  \\
\% plot((ynl*0.11-y0(2)),-  
\% xnl*0.11,'ko','MarkerFaceColor','k','MarkerSize',2);  \\
\% xlim([5,35]);  \\
\% set(gca,'fontSize',15,'LineWidth',2)  \\
\% ylabel('r (\mu m)','FontSize',20)  \\
\% xlabel('z (\mu m)','FontSize',24)  \\
\% subplot(2,1,1);  \\
\% plot(scalc,dev,'k','LineWidth',2);  \\
\% xlim([0.3,35]);  \\
\% set(gca,'fontSize',15,'LineWidth',2)  \\
\% ylabel('\Delta c (\mu m^{-1})','FontSize',20)  \\
\% subplot(2,1,2);  \\
\% plot(scalc(2:end-1),ddevds*10^(-12),'k','LineWidth',2);  \\
\% xlim([0.3,35]);  \\
\% set(gca,'fontSize',15,'LineWidth',2)  \\
\% ylabel('d\Delta c/ds (\mu m^{-2})','FontSize',20)  \\
\% xlabel('z (\mu m)','FontSize',24);

% figure  \\
% plot(z(2:end-1),ddevdz,'k','LineWidth',2);  \\
% xlim([0.2,35]);  \\
% set(gca,'fontSize',15,'LineWidth',2)  \\
% ylabel('d\Delta c/dz (\mu m^{-2})','FontSize',20)  \\
% xlabel('z (\mu m)','FontSize',24);

if plotall==1 || plotdevE==1
    if bead==1

if bead==1

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plot((devplot-devplot(1))*10^6,E,'o','MarkerEdgeColor','k','MarkerFaceColor','k','MarkerSize',4);
else
    plot(devplot-devplot(1),E,'o','MarkerEdgeColor','b','MarkerFaceColor','b','MarkerSize',4);
end
hold on;
plot(devfit-devfit(1),fit,'-.','LineWidth',2);

%plot(devplot(id10)-devplot(1),E(id10),'o','MarkerEdgeColor','r','MarkerFaceColor','r','MarkerSize',8);
set(gca,'fontsize',25)
xlabel('{\Delta}c-\Delta c_{0} (m^{-1})','FontSize',25);
ylabel('{\Delta}E (kbT)','FontSize',25);
end

%ids0=min(find(abs(sall-sall(end))==min(abs(sall-sall(end)))))
if plotall==1 || plotd==1
    if plotall==1
        figure
    end
    hold on
    if bead==1
        plot(tall(end:-1:1),sall,'o','MarkerEdgeColor','k','MarkerFaceColor','k','MarkerSize',4);
    else
        plot(tall(end:-1:1),(sall),'o','MarkerEdgeColor','b','MarkerFaceColor','b','MarkerSize',4);
    end
    hold on
    plot(t(end:-1:1),(sfit),'color',[0.5,0.5,0.5],'LineWidth',2);
    set(gca,'fontsize',25)
xlabel('t_{max}-t(s) ','FontSize',25);
ylabel('s-s_{o} ({\mu}m) ','FontSize',25);
end

% [ax,p1,p2] = plotyy(tall(end:-1:1),sall-sall(end),tall(end:-1:1),Fcap*a/kT);
% set(ax(2),'FontSize',20);
% pp=polyfit(scalc(2:1401),ddevds(1:1400)',3);
% z=z(1:1500);
% dev=dev(1:1500);
% scalc=scalc(1:1500);
% n=7;
% pd=polyfit(scalc,dev,n);
% devfit=pd(n+1);
% for ii=1:n
% devfit=devfit+pd(n-ii+1)*scalc.^ii;
% end
% ddevfit=pd(n);
% for iii=2:n
% ddevfit=ddevfit+pd(n-iii+1)*scalc.^(iii-1)*iii; %1/m
% end
% hq=100;
% Fcap=hq*tension*pi*a^2/2*ddevfit*10^(-9)*10^12; %N
% hold on
% box on
% D=0.1*10^(-12);
% p1=plot(scalc,Fcap*sqrt(D)/kT,'--','LineWidth',2);
% hold on
% p2=plot(scalc(2:id(1)),Fcap(1:(id(1)-1))*sqrt(D)/kT,'LineWidth',2);
% legend(p2,'\sigma=0.54')
% set(gca,'FontSize',20,'LineWidth',2);
% ylabel('F_cD^{0.5}/k_BT','FontSize',20)
% xlabel('s({\mu}m)','FontSize',20)
% xlim([0,35])
I. Power spectrum calculation for fluctuating GUV

clear all
close all
clc
k=1.38*1e-23;
T=298;
load data620140426734rames
%%%%%%%%%%inputs that you can change%%%%%%%%%%%%%%%%
gamma=1*10e-8;
kappa=20*k*T;
startmode=1;
exact=0;
frame=50;
dpix=135/512;
track=1;
fit=0;
r=2e-5;
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
if track==0
    rinum=r*1e6;
data=simdom4_GUV(rinum,kappa,gamma,frame,exact);
%data=y;
end
if track==1
    for i=1:frame
    data{i,1}=guv(i).R*10^(-6)*dpix;
data{i,3}=mean(guv(i).R)*10^(-6)*dpix;
ri(i)=mean(guv(i).R)*10^(-6)*dpix;
    end
    r=mean(ri);
end
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%2

% $\%$ calculate power spectrum $\%$ $\%$ $\%$
meanpowsum=0;meanpow=[];
for i=1:size(data,1)
    lengths(i)=length(data{i,1}(1,:));
end
maxlength=max(lengths);
powers=zeros(maxlength,size(data,1));
trans=zeros(maxlength,size(data,1));
for l=1:size(data,1)
    trans(1:lengths(l),l)=fft(data{l,1}-data{l,3});
    trans(:,l)=trans(:,l)/(size(data{l,1},2));
powers(:,l)=(abs(trans(:,l))).^2; %4 comes about because Fourier coefficients occur twice % Ningwei: No need to times 4 here
% (plus and minus q) and here are only accounted for once. The square
% makes it a 4
meanpowsum=meanpowsum+powers(:,l);
R0vec(l)=data{l,3};
meanradiusvec(l)=mean(data{l,1}); %mean radius angularly averaged along every trace
%compute radial autocorrelation function

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G. Detail derivations for bending corrections in the energy for Janus particles on lipid bilayers

Define the universal solution described in equation 4.59 into separate terms:
\[ \hat{h}^{UV} = \hat{h}_0 + \hat{h}_p + \hat{\eta}_c + \hat{h}^{BT} \] \hspace{1cm} \text{(A13)}

\( \hat{h}_0 \) is the host interface shape:

\[ \hat{h}_0 = \frac{\Delta c_d r_0}{4} \hat{\rho}^2 \cos 2\phi + \frac{H_d r_0}{2} \hat{\rho}^2 \] \hspace{1cm} \text{(A14)}

\( \hat{h}_p \) is the deformation made by the particle:

\[ \hat{h}_p = \hat{h}_{wp} \cos 2\phi \] \hspace{1cm} \text{(A15)}

\( \hat{\eta}_c \) is the coupling term between the particle's quadrupole and the deviatoric curvature of the interface:

\[ \hat{\eta}_c = -\frac{\Delta c_d r_0}{4} \frac{1}{\hat{\rho}^2} \cos 2\phi \] \hspace{1cm} \text{(A16)}

\( \hat{h}^{BT} \) is the correction from bending:

\[ \hat{h}^{BT} = -\sqrt{2} \varepsilon \exp\left(-\frac{\hat{\rho} - 1}{\varepsilon \sqrt{2}}\right) \left( \hat{h}_{wp} \cos 2\phi + \theta_r \right) \] \hspace{1cm} \text{(A17)}

The energy of the membrane can be calculated from the membrane shape as in equations 4.50 - 4.52. Before and after particle adsorption, the change in energy can be expressed as:

\[ E = E_2 - E_1 = E_o + \sigma \int \mathcal{M-P}_UV \cdot \mathcal{M-P}_UV - \sigma \frac{\nabla h_0 \cdot \nabla h_0}{2} dS - \sigma \int \left( 1 + \frac{\nabla h_0 \cdot \nabla h_0}{2} \right) dS \] \hspace{1cm} \text{(A18)}

\[ -\Delta P \int \mathcal{M-P}_UV - h_d dS + \frac{\kappa}{2} \int (\nabla^2 h^{UV})^2 dS \]

In the tension term (first term) in equation A18, the bending tension domain solution, \( \hat{h}^{BT} \), has contributions described in the following, with \( \hat{h}^{UV} \) broken down into contributions mentioned above:
\[ \sigma \int \nabla \hat{h}_o \nabla \hat{h}_{BT} dS \]
\[ = \sigma \int_\mathcal{M} \frac{\partial \hat{h}_o}{\partial \hat{r}} \frac{\partial \hat{h}_{BT}}{\partial \hat{r}} \hat{r} d\hat{r} d\phi \]
\[ = \sigma \int_\mathcal{M} \left( \frac{\Delta c_r}{2} + H_o f_0 \right) \hat{r}^2 \exp \left( - \frac{\hat{r} - 1}{\sqrt{2} \epsilon} \right) \left( \hat{r}_q \cos 2\phi + \theta_c \right) d\hat{r} d\phi \]
\[ = \sigma \epsilon \left( \frac{\sqrt{2} \Delta c_r}{2} \hat{r}_q + 2 \sqrt{2} H_o f_0 \theta_c \right) \tag{A19} \]

\[ \sigma \int \nabla \hat{h}_p \nabla \hat{h}_{BT} dS \]
\[ = \sigma \int_\mathcal{M} \frac{\partial \hat{h}_p}{\partial \hat{r}} \frac{\partial \hat{h}_{BT}}{\partial \hat{r}} \hat{r} d\hat{r} d\phi \]
\[ = -\sigma \int_\mathcal{M} \frac{\hat{r}_q}{\hat{r}^2} \frac{\partial \hat{r}_q}{\partial \hat{r}} \exp \left( - \frac{\hat{r} - 1}{\sqrt{2} \epsilon} \right) \left( \hat{r}_q \cos 2\phi + \theta_c \right) d\hat{r} d\phi \]
\[ = -\sigma \epsilon 2 \sqrt{2} \hat{r}_q^2 \tag{A20} \]

\[ \sigma \int \nabla \hat{h}_c \nabla \hat{h}_{BT} dS \]
\[ = \sigma \int_\mathcal{M} \frac{\partial \hat{h}_c}{\partial \hat{r}} \frac{\partial \hat{h}_{BT}}{\partial \hat{r}} \hat{r} d\hat{r} d\phi \]
\[ = \sigma \int_\mathcal{M} \frac{\Delta c_u f_0 \cos 2\phi}{2 \hat{r}^2} \exp \left( - \frac{\hat{r} - 1}{\sqrt{2} \epsilon} \right) \left( \hat{r}_q \cos 2\phi + \theta_c \right) d\hat{r} d\phi \]
\[ = \sigma \epsilon \left( \frac{\sqrt{2}}{2} \Delta c_u \hat{r}_q \right) \tag{A21} \]

\[ \frac{\sigma}{2} \int \nabla \hat{h}_{BT} \nabla \hat{h}_{BT} dS \]
\[ = \frac{\sigma}{2} \int_\mathcal{M} \frac{\partial \hat{h}_{BT}}{\partial \hat{r}} \frac{\partial \hat{h}_{BT}}{\partial \hat{r}} \hat{r} d\hat{r} d\phi \]
\[ = \frac{\sigma}{2} \int_\mathcal{M} \exp \left( - \sqrt{2} \frac{\hat{r} - 1}{\epsilon} \right) \left( \hat{r}_q \cos 2\phi + \theta_c^2 \right) d\hat{r} d\phi \]
\[ = \sigma \epsilon \left( \frac{\sqrt{2}}{4} \hat{r}_q^2 + \frac{\sqrt{2}}{2} \theta_c^2 \right) \tag{A22} \]

Combining equations A19-A22, the correction on the order of \( \epsilon \) for the tension terms is:
\[ \Delta E^{(1)}_\sigma = \sigma \pi e \left( \sqrt{2} \Delta c_{\theta_0} \hat{h}_\psi + 2 \sqrt{2} H_0 r_0 \theta_c - \frac{5 \sqrt{2}}{4} \hat{h}_\psi^2 + \frac{\sqrt{2}}{2} \theta_c^2 \right) \]  

A23

The pressure term can be decomposed into two domains:

\[ -\Delta P \int_{r} h^{UV}_0 - h_0 dS = -\Delta P \int_{r} h_\rho + \eta_c + h_{\theta r} dS - \Delta P \int_{r} h^{UV}_0 (r = r_0) - h_0 dS \]  

A24

The first term is on the order of \( \varepsilon^2 \). To evaluate the 2nd term, let's use the relation \( \Delta P = \sigma \nabla^2 h_0 = 2 \sigma H_0 \). In dimensionless form, the 2nd term in equation A24 becomes:

\[ -2 \sigma H_0 \int \left[ \frac{H_0}{2} - \sqrt{2} \varepsilon (\hat{h}_\psi \cos 2\phi + \theta_c) - \frac{H_0 r_0 \rho^2}{2} - \frac{\Delta c_{\theta_0} \rho^2}{4} \cos 2\phi \right] r \, d\phi \, d\theta \]  

\[ = -\sigma \frac{H_0 \rho^2 \pi}{2} + \sigma \pi e 2 \sqrt{2} H_0 \theta_c \]  

A25

The last term from equation A18 is the bending energy term. Normalizing it by \( \sigma \):

\[ \sigma \frac{\varepsilon^2}{2} \int \left( \tilde{\nabla}^2 \hat{h}^{UV} \right)^2 d\tilde{S} \]  

\[ = \sigma \frac{\varepsilon^2}{2} \int \left[ \tilde{\nabla}^2 (\hat{h}_0 + \hat{h}_{\theta r}) \right]^2 d\tilde{S} \]  

\[ = \sigma \frac{\varepsilon^2}{2} \int \left( 2 H + \tilde{\nabla}^2 \hat{h}_{\theta r} \right) \hat{h} \hat{r} d\phi d\theta \]  

\[ = \sigma \frac{\varepsilon^2}{2} \int \exp \left( -\sqrt{2} \frac{r - 1}{\delta} \right) \left( \hat{h}_\psi \cos 2\phi + \frac{\theta_c^2}{2 \delta^2} \right) \hat{h} \hat{r} d\phi d\theta \]  

\[ = \sigma \pi e \left( \frac{\hat{h}_\psi^2}{2} + \sqrt{2} \theta_c^2 \right) \]  

A26

In summary, the bending energy has no correction up to the leading order to the energy. Combining equations A23, A25 and A26, the first order correction from bending is as described in equation 4.61:

\[ \varepsilon E^{(1)} = \sigma \pi e \left( \sqrt{2} \Delta c_{\theta_0} \hat{h}_\psi + 4 \sqrt{2} H_0 r_0 \theta_c - \frac{3 \sqrt{2}}{4} \hat{h}_\psi^2 + \frac{3 \sqrt{2}}{2} \theta_c^2 \right) \]  

A27
REFERENCES


(47) Anselmo, A. C.; Kumar, S.; Gupta, V.; Pearce, A. M.; Ragusa, A.; Muzykantov, V.; Mitragotri, S. Exploiting Shape, Cellular-Hitchhiking and Antibodies to Target Nanoparticles to Lung Endothelium: Synergy between Physical, Chemical and Biological Approaches. *Biomaterials* 2015, 68, 1–8.


(64) Shi, Z. Mechanisms of Membrane Remodeling by Peripheral Proteins and Divalent Cations, University of Pennsylvania, 2015.


(84) Schmid, S. L. Clathrin-Coated Vesicle Formation and Protein Sorting: An Integrated


