Published in final edited form as:

Langmuir. 2017 January 17; 33(2): 600–610. doi:10.1021/acs.langmuir.6b03406.

Curvature-driven migration of colloids on tense lipid bilayers

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Abstract

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? We report the migration of Janus microparticles adhered to giant unilamellar vesicles elongated to present spatially varying curvatures. In our experiments, colloids migrate only when the membranes are tense, suggesting that they migrate to minimize membrane area. By determining the energy dissipated along a trajectory, the energy field is inferred to depend on the local deviatoric curvature, like curvature driven capillary migration on interfaces between immiscible fluids. In this latter system, energy gradients are larger, so colloids move deterministically, whereas the paths traced by colloids on vesicles have significant fluctuations. By addressing the role of Brownian motion, we 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, we report here an exciting and previously unexplored modality to actively direct the migration of colloids to desired locations on lipid bilayers.

TOC graphic

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Keywords

Vesicles; membrane trafficking; Janus particle; micropipette aspiration; directed assembly; self assembly; inclusions; Helfrich energy; capillarity; adhesion

1. Introduction

Curvature generating and sensing proteins are exciting to biologists and materials scientists alike. When such proteins associate with a lipid bilayer membrane, they can be moved about simply by changing the membrane shape¹⁻³. For example, when a tether is pulled from a giant unilamellar vesicle (GUV) whose membrane initially contains sparse concentrations of such peripherally bound proteins, they may concentrate on the tether^{4,5}. This remarkable coupling of chemical potential to geometry is not only a key mechanism in membrane-mediated trafficking of proteins in cells, it is also an inspiring and powerful tool for directing assembly of synthetic particles. Could related interactions occur for materials at the microscale? This would imply that, by simply dictating the geometry of a vesicle, materials like microscale colloids associated with membranes could be spatially relocated. This could enable material organization on lipid bilayers, which is a system of broad significance in biology and biophysics^{6,7}. However, the motivating system of curvature generating proteins is in some ways quite specific; while there are several groups of proteins that have been identified to be curvature generators and sensors, most membrane-associated proteins do not respond in this way^{1,2}.

When colloids adhere to membranes, the associated distortion field can, in principle, depend on the underlying membrane geometry. 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 reported⁸. What role could membrane tension play? We explore this question here. For bilayers with finite bending rigidity κ and membrane tension σ , the natural length scale $\lambda = \sqrt{\kappa/\sigma}$ determines whether minimization of bending energy or of excess area dictate system behavior⁹. For colloids of radius a , tension and excess area minimization would determine system response only if $\lambda \ll a$. By making membranes tense, membrane tension-mediated migration could be assessed.

The adhesion of colloids to membranes can occur through various non-specific mechanisms, including electrostatic and hydrophobic interactions, and may also involve specific ligand-receptor interactions^{8,10,11}. At equilibrium, the degree of wrapping of the particle, and the

associated “contact line” where adhered and un-adhered membrane meet at the particle surface are determined by a balance of the adhesion energies and energies related to membrane bending and tension¹²⁻¹⁵. Alternatively, the membrane can become trapped at pinning sites, with an undulated contour at the contact line (Fig. 1 (A), (B)). Since contact lines may pin on asperities or patches with high adhesion energy, there can be considerable variation from particle to particle in this case. There is a third possibility: membranes can wrap colloids nearly completely so that they are connected to the outer membrane only via a catenoid-shaped neck¹⁵. In this state, particle-sourced distortions might be isolated from the surrounding membrane and effectively decouple from the membrane geometry around the particle. To explore and develop membrane curvature-mediated particle migration, we limit the degree of particle wrapping by using a Janus colloid fabricated via seeded emulsion polymerization which has two faces, one primarily comprising polystyrene (PS), the other polyacrylic acid (PAA) (Fig. 1 (C))¹⁶. These particles are only partially wrapped by the membrane (Fig. 1 (D)); this 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.

To determine whether the colloids indeed interact with membrane geometry, we study particles adhered to GUVs that are deformed to impose a well-defined membrane shape and held at constant aspiration pressure within a micropipette to impose a well-defined membrane tension. We find that, over the course of a typical experiment, particles move only when the tension is higher than roughly 0.05 mN/m. Furthermore, experiment reveals that particles can dissipate more than $100 k_B T$ of energy along a trajectory for the range of tensions over which we study migration, (0.24-0.69 mN/m). This dependence on tension implies that these migrations do not occur to minimize membrane bending energy, but rather occur to minimize membrane area. We use experiment and theory to determine this mechanism, and explore features of this new modality for membrane-mediated colloidal assembly.

2. Materials and Methods

Materials

Lipids including 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), 1,2-dioleoyl-3-trimethylammoniumpropane (DOTAP) were purchased from Avanti Polar Lipids (Alabaster, AL). Lipid dyes BODIPY FL DHPE, triethylammonium salt, and Texas Red DHPE, triethylammonium salt were purchased from Invitrogen (Carlsbad, CA). Glass beads with diameter of 10-30 μm and Polybead carboxylate microspheres with a diameter of 1 μm were obtained from Polysciences, Inc. (Warrington, PA). D-sucrose, dextrose (D-glucose) anhydrous, phosphate buffered saline concentrate powder, and chloroform were purchased from Fisher Scientific (Hampton, NH). Poly-L-lysine 1 mM solution and bovine serum albumin were purchased from Sigma-Aldrich.

Janus particles fabrication

Janus particles are fabricated via seeded emulsion polymerization followed by hydrolysis. First, monodispersed linear polystyrene (LPS) seeds with diameter of 700 nm are 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 (*t*BA, 98 %), 1 vol % divinylbenzene (DVB, 55 %), and 0.5 wt % initiator 2,2'-azobis(2,4-dimethylvaleronitrile), are tumbled in an oil bath to complete the seeded emulsion polymerization. Finally, the *t*BA is hydrolyzed by stirring the particles in 80 vol % trifluoroacetic acid (TFAA, 99 %) and 20 vol % formic acid (FA, 95 %). In Tu et al.¹⁶, a more detailed protocol is described.

Upon seeded polymerization, the PS rich hemisphere is 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 is completely covered with carboxylic acids, forming a smooth surface that is negatively charged at pH 7.

GUV formation

GUVs are formed by a standard electroformation method¹⁷. Lipids of desired compositions (40 % DOTAP, 59.5 % DOPC and 0.5 % lipid dye. Two dyes are used in the study, including a green dye, 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), and a red dye, 1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine (Texas Red DHPE)). The lipids and dye are mixed in chloroform at a total concentration of 1 mM, and 40 μ L of the mixture are deposited on each indium tin oxide (ITO) glass slide. After solvent evaporation in a vacuum chamber for two hours, chambers formed by sandwiching a silicon spacer between two ITO slides are hydrated by 430 μ L of 700 mM sucrose solutions and subjected to an alternating current of 0.5 A and 10 Hz for two hours. Prior to transfer, GUVs are diluted 20 fold in 850 mM glucose with 10 mM phosphate-buffered saline (PBS) buffer.

Particle adhesion and determination of degree of wrapping

The behavior of two types of particles that adhere electrostatically to this membrane is compared; these include isotropic, carboxy-functionalized PS microparticles and Janus PS-PAA microparticles, both of radius $a=0.5 \mu\text{m}$. The degree of wrapping is calculated, with 0° being non-wrapped and 180° being completely wrapped, by tracking the positions of both the membrane and the particle. In Fig. 1 (D), Janus particles, infused with Nile red in toluene, are imaged while adsorbed on the membrane labeled with Bodipy FL DHPE (green). This image indicates that the center of mass of the particle and the lipids are collocated, consistent with the Janus particles being only partially wrapped by the membrane. The degree of wrapping for the Janus particles, determined by tracking the relative positions of the membrane and particle, is around 80° , whereas for the homogeneous particles, the average degree of wrapping is 160° (Fig.1 (E), (F)), indicating that they are nearly completely wrapped. A more detailed explanation of the degree of wrapping analysis is available in the SI.

GUV shape and membrane tension analysis

A single vesicle is isolated from the chamber in which the GUVs are suspended in. The vesicle is held via pipette-aspiration under constant pressure (details in SI), protected from dehydration via a transfer capillary¹⁸, and moved from this suspension to a chamber

containing a microparticle suspension with otherwise identical solution conditions, i.e., 800 mM glucose and 10 mM PBS; this allows us to study microparticle migration without interference from other vesicles in the field of view.

Once the GUV is introduced into the chamber, particles begin to adhere to the membrane. Concomitantly, the GUV is deformed using a large, clean, glass bead of radius 10-30 μm glued to a second micropipette. Using micro-manipulators, the bead is tapped gently against the GUV to promote adhesion to the membrane, and gently retracted to elongate the GUV into a lemon-like shape (Fig. 2 (A)). The entire arrangement is then held fixed. The micropipettes are configured so that the elongated vesicle shape is axisymmetric. A GUV aspirated by the pipette, shown in Fig. 2 (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 Fig. 2 (B). We define 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, we observe that the GUV has constant mean curvature H . By minimizing an objective function characterizing the difference between the experimental contour (black line in Fig. 2 (B)) and numerically generated curves, the location for the axis of symmetry (blue line in Fig. 2 (B)), values for H and values the principle curvatures of the membrane along the contour were determined^{19,20}. The fitted profile (yellow dashed line, Fig. 2 (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. (See SI for discussion of fitting error).

The pressure drop across the aspiration pipette was controlled by the height of a water reservoir connected 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). We measure the pressure drop between inside and outside of the pipette, $P_{out} - P_{in}$, where P_{in} and P_{out} are the pressures inside of the pipette and in the bulk solution external to the aspiration pipette, respectively. We measure the radius of the aspirated cap, R_p , and the mean curvature of the elongated GUV, H , from image analysis as mentioned. Applying the Young-Laplace equation to the portion of vesicle in the aspirated cap, and to the elongated vesicle yields two equations with two unknowns, the membrane tension σ , and the pressure inside the GUV. We can therefore calculate the membrane tension from the relationship²¹:

$$\sigma = \frac{P_{out} - P_{in}}{2} \frac{R_p}{1 - R_p H} \quad (1)$$

While H is constant along the deformed GUV, the deviatoric curvature c , defined by the difference between the principal curvatures (such that $c > 0$), varies with arc length s . Gradients in c are zero near the middle of the GUV, and are steepest near the bead and

micropipette (Fig. 2 (C)). Below, we show that particle migration is related to this quantity. To probe particle migrations on tense vesicles, tensions are varied over the range $0.24 \text{ mN/m} < \sigma < 0.69 \text{ mN/m}$. To probe for migration at very low tension, we also run the same experiment under a tension of 0.05 mN/m .

Image acquisition and data analysis

Fluorescence images were obtained by a confocal fluorescence microscope (IX81, Olympus, Japan) with a $60\times$ 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). Particle motion was captured by the CCD camera at a frame rate of 30 frames/second. Particle position was tracked by ImageJ and further processed by Matlab.

3. Results

We compare 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 $a=0.5 \mu\text{m}$. On elongated GUVs, over lag times of tens of seconds, the PS microparticles move with linear mean square displacement (MSD) in arc length s versus lag time (Fig. 1 (G)); this indicates that their migration is diffusion-dominated, and that the motion of the wrapped particle is 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, is not sufficient to drive particle migration.

The dynamics differ strongly for the partially wrapped Janus microparticles. Once attached to a spherical GUV, the lateral particle movement on the membrane can be described by a linear MSD, i.e., they move diffusively (Fig. 3 (A)). On elongated GUVs, however, the particles move super-diffusively (Fig. 3 (A), (B)), with motions that can approach deterministic limits, over significant distances to sites of high deviatoric curvature. In some cases, particles traverse distances in excess of 30 particle radii, corresponding to arc length ranging from 15 to $20 \mu\text{m}$ within 10 to 15 seconds. The associated s -directed migration velocity U can be large enough in regions of steepest curvature gradient that particles move with only weak fluctuations, implying that the driving force far exceeds forces owing to Brownian fluctuations (Fig. 3 (C)). This migration is not related to the weak drift velocities in the fluid, as verified by using particles in suspension around the vesicle as tracer particles (see SI text, Fig. S4). Such trajectories are observed only for tense membranes.

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 shape²². 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 we focus on the role of membrane shape, assuming pinned contact lines.

Both bending energy and membrane tension are associated with membrane shape. In section 4, below, we investigate the contributions from both contributions by calculating the energy change during particle migration. In experiment, the particle trajectories can be analyzed to

reveal the dependence of the energy field driving this migration on membrane geometry. The particles move with negligible inertia, i.e., the Reynolds number $Re = \rho_P U a / \mu \ll 1$, where ρ_P is the particle density, and μ 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 play a role. The noisiness is most significant in the regions of a weak deviatoric curvature gradient. We show below that we can 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 (Fig. 3 (C)), the velocity U can be determined. Neglecting Brownian contributions in this region, the energy balance on the particle implies:

$$\Delta E = \frac{k_B T}{D} \int_{s_i}^{s_f} U ds, \quad (2)$$

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 Eq. 2, 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. We initiate this integration at an arc length s_i within 20 particle radii from the boundaries; at distances greater than this value, the motion is diffusive. We also terminate 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.

We 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 $D = 0.12 \pm 0.05 \mu\text{m}^2/\text{s}$. 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, we were 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 $D = 0.07 \mu\text{m}^2/\text{s}$ for one trajectory, and $D = 0.09 \mu\text{m}^2/\text{s}$ for another (See SI text, Fig. S3). For these two cases, we report E in units of $k_B T$ (Fig. 4); $|E| > 100 k_B T$ are found. For all other cases in which we 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 $ED / k_B T$ is reported for those cases (Fig. 4, inset). Assuming $D = 0.12 \mu\text{m}^2/\text{s}$ for these trajectories, the associated energy dissipated along these trajectories ranges from $50 k_B T < |E| < 350 k_B T$. For all cases, E depends linearly on the difference in the deviatoric curvature $C = c(s) - c(s_i)$, the difference in deviatoric curvatures along a particle path from between its instantaneous position s and its initial position, with coefficient of linear regression $R^2 \sim 0.99$. For cases reported in the inset, the slopes vary significantly from trajectory to trajectory and no systematic dependence on tension is observed in the energy dissipated along trajectories (Fig. S5). 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). We hypothesize that these random aspects of the adhesion process obscure any dependence on membrane tension.

Such a linear dependence of energy dissipated on diviatoric curvature has been reported previously for particles migrating along curvature gradients on interfaces between immiscible fluids²³⁻²⁶. 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:

$$\Delta E = -\sigma \pi r_0^2 \frac{h_{qp} \Delta C}{2}. \quad (3)$$

In this expression, for fluid interfaces, σ 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 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, we 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, we 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 Eqs. 2 and 3, 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.

4. Theory

A colloid of radius a is adhered to a curved, tense lipid bilayer membrane. The “contact line”, the contour where adhered membrane, un-adhered membrane and particle meet, is assumed to be a pinned, undulated contour which can be decomposed into Fourier modes with amplitudes small compared to the contact line radius $r=r_0$ where $r_0 \sim a$. In Helfrich’s model, membranes with bending rigidity κ and tension σ define a natural length scale

$\lambda = \sqrt{\kappa/\sigma}$. Typically, for microscale particles, the ratio $\epsilon = \lambda/\ell_0 \ll 1$. For example, in our experiments, for the range of tension studied, ϵ varies from 0.02 to 0.04. To develop an energy expression within the Helfrich model assuming $\epsilon = \lambda/\ell_0 \ll 1$, 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 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 λ . 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. We summarize the main points below with details given in SI.

4.1 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:

$$E = \int_m 2\kappa H^2 + \sigma dA. \quad (4)$$

where m denotes the entire membrane. When a particle adheres to the membrane, the membrane shape changes. The membrane height h above the reference plane tangent to the unperturbed membrane is described in a Monge parametrization in the limit of small slope. For an adhered particle, the Helfrich Hamiltonian becomes:

$$E = E_0 + \frac{1}{2} \iint_{m-p} [\kappa (\nabla^2 h)^2 + \sigma (\nabla h)^2] dS, \quad (5)$$

where p denotes the domain beneath the particle and E_0 is a reference energy. For an arbitrary perturbation in membrane shape δh that decays far from the particle, the perturbation in membrane energy is:

$$\delta E = \iint_{m-p} [\kappa \nabla^2 h \nabla^2 \delta h + \gamma \nabla h \nabla \delta h] dS. \quad (6)$$

Repeated application of Green's theorem allows this expression to be recast:

$$\delta E = \frac{1}{2} \iint_{m-p} [\kappa \nabla^4 h - \sigma \nabla^2 h] \delta h dS + \int_0^{2\pi} \left\{ (\kappa \nabla^2 h) \nabla \delta h + [\nabla h \sigma - \kappa \nabla (\nabla^2 h)] \delta h \right\} \big|_{r=r_0} \cdot \mathbf{e}_r r_0 d\phi$$

where $\mathbf{e}_r$ is the outward pointing 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:

$$E_P = 4\pi a^2 \gamma_1 + \int_0^{2\pi} \int_0^{\theta_c} \left(2 \frac{\kappa}{a^2} + f_{ad} - \gamma_1 \right) a^2 \sin \theta d\theta d\phi, \quad (8)$$

where $f_{ad} (<0)$ is the adhesion energy per unit area between the membrane and the particle, γ_1 is the surface energy of the particle-liquid interface, and where θ_c is the degree of wrapping of the membrane on the spherical cap. The first term under the integral in Eq. 8 is the bending cost to wrap the membrane around a particle with radius a . If the contact line is perturbed by an amount δh , the resulting perturbation in the adhesion energy is:

$$\delta E_P = E_P(h + \delta h) - E_P(h) = \int_0^{2\pi} \left(2 \frac{\kappa}{a^2} + f_{ad} - \gamma_1 \right) a \delta h d\phi, \quad (9)$$

Contributions and constraints from surrounding fluids—The arbitrary perturbation δh also displaces the surrounding fluid. The resulting energy change δE_{bulk} is:

$$\delta E_{bulk} = \int_{V_\alpha} \rho_\alpha \mathbf{g} \cdot \delta \mathbf{u}^\alpha \Delta t dV + \int_{V_\beta} \rho_\beta \mathbf{g} \cdot \delta \mathbf{u}^\beta \Delta t dV, \quad (10)$$

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

$$C_{bulk} = \int_{V_\alpha} P^\alpha \Delta t (\nabla \cdot \delta \mathbf{u}^\alpha) dV + \int_{V_\beta} P^\beta \Delta t (\nabla \cdot \delta \mathbf{u}^\beta) dV, \quad (11)$$

where P^α and P^β are Lagrange multipliers. After manipulation, these terms can be written:

$$\begin{aligned}
\delta E_{bulk} + C_{bulk} &= \int_{V_\alpha} (\rho_\alpha \mathbf{g} - \nabla P^\alpha) \cdot \delta \mathbf{u}^\alpha \Delta t dV \\
&\quad + \int_{V_\beta} (\rho_\beta \mathbf{g} - \nabla P^\beta) \cdot \delta \mathbf{u}^\beta \Delta t dV \\
&\quad + \int_{A_\alpha} P^\alpha \delta \mathbf{u}^\alpha \cdot \mathbf{n}^\alpha \Delta t|_S dS + \int_{A_\beta} P^\beta \delta \mathbf{u}^\beta \cdot \mathbf{n}^\beta \Delta t|_S dS,
\end{aligned} \tag{12}$$

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

Total perturbation in energy of the system—Combining all energy contributions and constraints, the perturbation of the total energy owing to the arbitrary displacement field $\delta \mathbf{h}$ is:

$$\begin{aligned}
\delta E_{total} &= \delta E + \delta E_P + \delta E_{bulk} + C_{bulk}, \\
&= \frac{1}{2} \iint_{m-p} \left[\left(\kappa \nabla^4 h - \sigma \nabla^2 h + P^\alpha - P^\beta \right) |_S \right] \delta h dS + \\
&\quad \int_0^{2\pi} \left[\left(\kappa \nabla^2 h \nabla \delta h|_{r=r_0} \right) \cdot \mathbf{e}_r r_0 \right] d\phi + \int_0^{2\pi} \left[\left\{ (\nabla h \sigma - \kappa \nabla (\nabla^2 h)) \cdot \mathbf{e}_r r_0 + \left(2 \frac{\kappa}{a^2} + f_{ad} - \gamma_1 \right) a \right\} \delta h|_{r=r_0} \right] d\phi \\
&\quad + \int_{V_\alpha} [\rho_\alpha \mathbf{g} - \nabla P^\alpha] \cdot \delta \mathbf{u}^\alpha \Delta t dV + \int_{V_\beta} [\rho_\beta \mathbf{g} - \nabla P^\beta] \cdot \delta \mathbf{u}^\beta \Delta t dV,
\end{aligned} \tag{13}$$

To minimize the energy $\delta E_{total} = 0$; this requires each term in square brackets in Eq. 13 to be zero.

Euler Lagrange equation and associated boundary conditions—The first term in Eq. 13 yields the Euler Lagrange equation:

$$-\kappa \nabla^4 h + \sigma \nabla^2 h = \Delta P, \tag{14}$$

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

The two integrals over ϕ in Eq. 13 yield sets of conditions at the contact line:

Contact line boundary condition, set 1:

$$(\kappa \nabla^2 h) \nabla \delta h|_{r=r_0} = 0 \tag{15}$$

Contact line boundary condition, set 2:

$$\left((\nabla h \sigma - \kappa \nabla (\nabla^2 h)) \cdot \mathbf{e}_r \right)_{r=r_0} + \left(2 \frac{\kappa}{a^2} + f_{ad} - \gamma_1 \right) a \delta h|_{r=r_0} = 0 \quad (16)$$

These conditions are given as products in Eq. 15 and 16. 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 $\nabla^2 h|_{r=r_0}$ or $\nabla \delta h|_{r=r_0} = 0$. 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 Eq. 13 give the hydrostatic equations for the bulk fluids.

4.2 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 $\varepsilon = \lambda/r_0$ and analyze Eq. 14 in the high tension limit, i.e. $\varepsilon \ll 1$. We adopt a polar coordinate system (r, ϕ) 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 Eq. 14 by this length scale to find:

$$-\varepsilon^2 \hat{\nabla}^4 \hat{h} + \hat{\nabla}^2 \hat{h} = \Delta \hat{P} \quad (17)$$

where $\hat{\nabla} = r_0 \nabla$, $\hat{h} = h r_0^{-1}$ and $\hat{P} = P r_0 / \sigma$. The highest order derivative in Eq. 17 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 Fig. 5 (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, Eq. 14 will be rescaled with characteristic length scale λ . Outside of this region, tension alone influences the membrane shape according to Eq. 17. 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^{Tin} 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 ε

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. Details are provided in SI.

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 ϵ , e.g. $\hat{h}^{T_{in}} = \hat{h}_{(0)}^{T_{in}} + \epsilon \hat{h}_{(1)}^{T_{in}} + \epsilon^2 \hat{h}_{(2)}^{T_{in}} + \dots$. Since we discuss only the leading order solutions in the main text, we 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 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:

$$\hat{h}_0^{T_{out}} = \frac{\Delta c_0 r_0}{4} \hat{r}^2 \cos 2\phi + \frac{H_0 r_0}{2} \hat{r}^2. \quad (18)$$

In the T_{in} domain, to leading order in ϵ , Eq. 18 reduces to the Young-Laplace equation:

$$\nabla^2 \hat{h}^{T_{in}} = 2Hr_0. \quad (19)$$

The undulated contact line imposes a quadrupolar mode to leading order with magnitude $\hat{h}_{qp}$ ²⁷:

$$\hat{h}^{T_{in}}(\hat{r}=1, \phi) = \hat{h}_{qp} \cos 2\phi + \omega, \quad (20)$$

where ω is a shift in the center of mass of the particle. Far from the particle:

$$\lim_{\hat{r} \rightarrow \infty} \hat{h}^{T_{in}} = \hat{h}_0^{T_{out}}. \quad (21)$$

Using these two boundary conditions, $\hat{h}^{T_{in}}$ is:

$$\hat{h}^{T_{in}} = \frac{\Delta c r_0}{4} \cos 2\phi \left(\hat{r}^2 - \frac{1}{\hat{r}^2} \right) + \frac{\hat{h}_{qp}}{\hat{r}^2} \cos 2\phi + \frac{H r_0}{2} \hat{r}^2, \quad (22)$$

where $\omega = \frac{H r_0}{2}$. This solutions was derived previously for particles at fluid interfaces^{24,26,28}.

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 λ . We define a stretched local coordinate $\tilde{\xi} = (\hat{r} - 1)/\epsilon = (r - r_0)/\lambda$ originating from the particle

surface, shown schematically in Fig. 5 (B). We expand the membrane shape in powers of ϵ , i.e. $\hat{h}^{BT} = \hat{h}_{(0)}^{BT} + \epsilon \hat{h}_{(1)}^{BT} + O(\epsilon^2)$, and find the equation governing the membrane shape, to leading order:

$$-\frac{\partial^4 \hat{h}_{(0)}^{BT}}{\partial \xi^4} + \frac{\partial^2 \hat{h}_{(0)}^{BT}}{\partial \xi^2} = 0, \quad (23)$$

The boundary conditions include the Van Dyke matching condition:

$$\lim_{\xi \rightarrow \infty} \hat{h}^{BT} = \lim_{\substack{\phi \rightarrow 0 \\ \xi \text{ fixed}}} \hat{h}^{T_{in}} = \hat{h}_{qp} \cos 2\phi + \frac{Hr_0}{2}, \quad (24)$$

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:

$$\frac{\partial^2 \hat{h}_{(0)}^{BT}(\tilde{\xi}=0)}{\partial \tilde{\xi}^2} = 0. \quad (25)$$

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 $\theta_c \ll 1$ in the small slope limit this requires:

$$\frac{\partial \hat{h}_{(0)}^{BT}(\tilde{\xi}=0)}{\partial \tilde{\xi}} = 0. \quad (26)$$

The leading order solution for either case is:

$$\hat{h}_{(0)}^{BT} = \hat{h}_{qp} \cos 2\phi + \frac{Hr_0}{2}. \quad (27)$$

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 in SI.

4.3 The energy landscape around an adhered particle

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

$$E_1 = \frac{\kappa}{2} \iint_m (\nabla^2 h_0)^2 dS + \sigma \iint_m \left(1 + \frac{\nabla h_0 \cdot \nabla h_0}{2}\right) dS - \nabla P \iint_m h_0 dS \quad (28)$$

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 p attached to the particle. The particle-sourced distortion field changes the energy over the domain $m - p$ exterior to the contact line.

$$E_2 = \frac{\kappa}{2} \iint_{m-p} (\nabla^2 h)^2 dS + \sigma \iint_{m-p} \left(1 + \frac{\nabla h \cdot \nabla h}{2}\right) dS - \Delta P \iint_m h dS + E_0, \quad (29)$$

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 $E = E_2 - E_1$. In the SI, we evaluate this energy change to leading order and first order corrections. The leading order solution is:

$$E = E_2 - E_1 = \left\{ E_0 + \sigma \pi h_{qp}^2 - \sigma \pi r_0^4 \frac{3H^2}{4} \right\} - \sigma \pi r_0^2 \frac{h_{qp} \Delta c_0}{2}. \quad (30)$$

In Eq. 30, 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 $h_{qp} c_0$ is the curvature capillary energy driving migration; in this expression, the deviatoric curvature 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 E is given by Eq. 3. Thus, gradients in c along a particle path drive colloids to sites of high deviatoric curvature to minimize the excess membrane area.

5. Discussion

Our analysis assumes that the particles have pinned contact lines, and that particle-sourced distortions decay rapidly compared to the vesicle radii of curvature. This latter assumption was relaxed in the analysis of Zeng et al. for particles with equilibrium contact lines on cylindrical isotropic fluid interfaces, for which terms quadratic in the curvature were obtained²⁹. The extension of this analysis to the case of pinned contact lines is a potentially fruitful direction for future work. Similarly, there is a treatment for curvature driven capillary migration for particles with pinned contact lines that predicts terms quadratic in c ³⁰. To probe for evidence of such terms in the present study, the energy dissipation reported in Fig. 4 is fit to a quadratic form $E = a C^2 + b C$; we find that a/b is on the order of 10^{-5} for all cases, indicating that terms quadratic in c do not contribute significantly to the observed migration.

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. A force balance in the s direction (a Langevin equation) includes a capillary force, given by the negative gradient of the energy expression in Eq. 3, and a random force owing to thermal fluctuations:

$$\frac{k_B T}{D} \frac{ds}{dt} = - \frac{d\Delta E}{ds} + \sqrt{\frac{2(k_B T)^2}{D}} R(t), \quad (31)$$

where $R(t)$ is a random number that has the following characteristics: $\langle R(t) \rangle_t = 0$ and $\langle R(t)R(t-\tau) \rangle_t = \delta(\tau)$. We integrate Eq. 31 for a typical value of $h_{qp} = 150$ nm. Because of the Brownian term, each integration is different; 15 simulated trajectories, depicted as grey solid lines in Fig. 3 (C), show distinct zones of behavior. Qualitatively, where curvature gradients are weakest, the simulated trajectories are 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 are steepest, the predicted and experimental trajectories converge, and agree with the polynomial fit to this region. We define a local Péclet number $Pe = U(s)a/D$ along a trajectory, defined in terms of the migration velocity of the particle $U(s)$; domains with $Pe \gg 1$ 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 Fig. 3 (C). This corresponds to the domain of arc lengths over which E was estimated in Fig. 4.

Particles failed to migrate to sites of high curvature for the lowest tension that we 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 Eq. 31 for the vesicle shapes like those in experiments for $\sigma = 0.05$ mN/m indeed predicts 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 (see SI text, Fig. S6, S7). 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. (see SI text, Fig. S7). For tensions an order of magnitude lower, however, $\varepsilon \approx 1$, and our analysis does not apply. Both bending and tension would determine the energy landscape. This remains an open regime for study.

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. We 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 (Fig. S8, Video S2). Are other effects associated with capillary migration observed? On interfaces between isotropic fluids, particles interact and assemble³¹. We report such a pair interaction on a tense membrane in Fig. 6 (A). However, since membrane tension is much less than typical interfacial tensions (e.g., 72 mN/m for the air-water interface), and the colloids here have

relatively small diameters compared to those studied at isotropic interfaces, these interactions occur only when particles are within roughly 10 particle radii. The observed trajectories do not obey a power law consistent with interacting quadrupolar modes (Fig. S9)^{27,28,32}, presumably owing to other, near field details in the deformation fields around the particles. Similar pair interactions were recently reported for isotropic colloids on moderately tense membranes^{33,34}. On curved fluid interfaces with finite deviatoric curvature, anisotropic particles rotate owing to curvature capillary torque to align along the principal axes²³. To explore this phenomenon, we exploit two Janus particles paired to form a dumbbell structure. Once this dumbbell adheres, we elongate the GUV (Fig. 6 (B), Video S3). The dumbbell rotates at roughly fixed position, and then translates, maintaining its alignment along the meridional direction, with rate of translation increasing with the local curvature gradient.

Colloids at interfaces have been assembled in structures resembling a square lattice, consistent with weak, quadrupolar modes on surfaces with weak curvature gradients³⁵, and more complex structures, guided by curvature field gradients^{23,24}. Those results, and the results of this study, suggest that complex colloidal assemblies with directional bonds can be formed guided by bilayer curvature, to provide building blocks for hierarchical structures, or to provide insights into directional-dependent interactions, an important emerging focus colloidal assembly³⁶. This has exciting implications for many particle interactions and interactions between complex particles aggregates on vesicles, which will be the focus of our future studies.

6. Conclusion

While analysis allows us to support the observed colloid migration and colloid interactions

within a Helfrich model in the limit of high tension (i.e. $\varepsilon = \frac{\lambda}{r_0} \ll 1$), the similarity between this system and colloid migration at the interface between isotropic fluids is remarkable. 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.

Nanoparticles^{3,37-39} and proteins^{2,40,41} interact with lipid bilayers, and can, through collective interactions, drive pronounced changes in vesicle morphologies including pearling, and tubule formation. Since a particle-sourced disturbance has magnitude comparable to the particle radius and decays over similar distances, nanoscopic particles with $a \ll \lambda$ are not anticipated to respond to the curvature field via the capillary curvature mechanism explored here. Nor are curvature generating and sensing proteins, which are also thought to generate disturbances with magnitudes and decay lengths comparable to molecular dimensions. However, we hypothesize that larger entities, which generate finite disturbances over distances from their contact lines larger than λ , should migrate. This would have strong implications for collective interactions of entities adhered to fluid vesicles; the exploration of the behavior of particles or protein aggregates with sizes from

tens of nanometers to microns on curved, tense vesicles is an important focal point for future research.

Finally, it is interesting to consider whether the interactions studied here are potentially important in biology. For cells, the apparent membrane tension is determined by contributions from the cortical tension and the lipid bilayer; the latter contribution can range from 10^{-3} mN/m up to 0.1 mN/m⁴²⁻⁴⁴. Bilayer membrane tension regulates important cellular functions, including the extension of actin filaments⁴⁵, the formation of lamellipodia and cell spreading⁴³, and the determination of cell shape⁴⁶. Cell membranes also have reservoirs of membrane area, e.g., in microvilli and caveolae^{44,47}. Cell membranes exchange membrane area with these reservoirs to regulate tension when they are in hypertonic solutions or if they are osmotically stressed⁴⁴. Is it possible for tension-mediated migration to occur for tensions like those observed in cell membranes? The simulated trajectories (SI text, Fig. S6) for $\sigma=0.05$ mN/m indicate that, given sufficient time, such migrations could indeed occur. These results suggest that the tension in lipid bilayers in cell membranes could play an additional role, i.e., that colloids and other membrane-bound microscale structures might migrate on lipid bilayers in cell membranes to reduce excess membrane area via tension-mediated mechanisms. To assess this possibility, the roles of cortical tension and barriers owing to the cytoskeleton would have to be addressed. This is an open area for fruitful research.

To conclude, we have shown that microscale colloids adhere to lipid bilayers and migrate to sites of high deviatoric curvature. We hypothesize that the particles “generate” a distortion via their adhesion state, and “sense” membrane geometry. We find that, on tense membranes, particles move with nearly deterministic trajectories. The dependence on membrane tension (migration is not observed at low tension) suggests that this interaction is related to the elimination of excess area of the membrane, not to minimization of curvature energy. We confirm that this migration is a form of curvature capillary migration, with membrane tension playing the role of interfacial tension, and demonstrate the versatility of this previously unexploited modality for colloidal interaction on lipid membranes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors acknowledge support from NSF CBET Grant 1133267, NSF MRSEC Grant DMR11-20901 and NIH GM Grant 097552.

N.L., T.B., and K.J.S. conceived of this study. N.L., T.B., and K.J.S. designed the experiments. N.L., N.S-M, R. R. and K.J.S. developed the theory. F.T. and D.L. synthesized the Janus colloids. N.L. performed the experiments. N.L. and T.B. analyzed the experiments. All authors reviewed the manuscript.

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Figure 1. Homogeneous and Janus colloids adhering via electrostatic interactions to a GUV
(A) Schematic of a particle partially wrapped by a lipid bilayer with undulated contact line.
(B) Heat map of the leading order mode in the particle-sourced membrane deformation field, a decaying quadrupolar mode, top view. Scale bar: 2.5 μm . **(C)** Scanning electron microscope image of Janus particles with PAA side (smooth) and PS side with PAA domains (rough). Scale bar: 1 μm . **(D) & (E)** Confocal fluorescence microscopy image of a Janus particle (in panel D) and a homogenous particle (in panel E) attached to a lipid bilayer. 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. **(F)** Degree of wrapping for homogeneous and Janus particles on vesicle. $p < 0.01$, error bars are standard deviations. Inset: schematic of degree of wrapping.

(G) Trajectory of the homogeneous particle shown in (E) on an elongated vesicle. Inset: mean square displacement calculated from this trajectory.

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Figure 2. 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 .

Figure 3. 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 μm . (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 $h_{qp}=150$ nm; solid gray lines: migration trajectories predicted by integration of Langevin

equation for $D=0.09 \mu\text{m}^2/\text{s}$ and $T=298 \text{ K}$. Inset: Peclet number of the migrating particle plotted against the arc length.

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Figure 4. 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.

Figure 5. Domain layer schematics

(A) Top view of the domain layers; 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 m includes all the domains described. (B) A zoomed-in schematic of the BT domain. A stretched local coordinate $\tilde{\xi}$ that originates from the particle is defined.

Figure 6. Pair interaction (Top) and Evidence of curvature alignment of anisotropic colloid (Bottom)

(A) 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 μm . (B) 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 .