

## Membrane Mediated Attraction and Ordered Aggregation of Colloidal Particles Bound to Giant Phospholipid Vesicles

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We report a light microscopy study of interactions between colloidal particles either chemically bound or physisorbed onto flexible giant vesicle fluid membranes. The particles induce pinched shape deformations of the membrane and are driven to negative curvature regions on nonspherical vesicles. The membrane distortions were found to induce interparticle attraction with a range approximately equal to the particle diameter. Multiple particles decorating fluid membranes aggregate into finite-sized two-dimensional close packed aggregates or, unexpectedly, one-dimensional ringlike aggregates. [S0031-9007(99)08522-1]

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Lipid bilayer membranes provide a unique structural environment for integral and peripheral membrane proteins [1]. Macromolecules or nanoparticles may be embedded or attached to membranes, while they are free to diffuse laterally. The physical forces that act between such membrane inclusions are believed to play an important role in the aggregation behavior of some naturally occurring transmembrane proteins. For example, gap junctions consist of ordered protein channel arrays which allow intercellular molecular transport, and bacteriorhodopsin forms hexagonal crystals within the archaeobacterial membrane.

Aside from the protein-specific short-range interactions, recent theoretical work has suggested that membrane mediated forces should be important. These may be caused by a spontaneous splay deformation of the lipid bilayer [2], by a thickness mismatch of the hydrophobic transmembrane protein part and the lipid bilayer chain length [3], or by variation of the local lipid composition [4]. Recently, it has been pointed out that thermal membrane shape fluctuations can cause long-range attractive interactions between rigid inclusions [5]. However, there are few experimental data available that allow measurements of the membrane mediated interactions between inclusions or absorbed proteins.

Membrane inclusions can be mimicked in model experiments by microscopic colloidal particles bound to lipid membranes. Because of their extreme softness, giant unilamellar lipid vesicles are sensitive transducers for membrane interactions. Recent optical microscopy studies on giant vesicles allowed close comparison of their observed shapes with theoretical predictions derived from elastic continuum models [6], providing a good understanding of the "ground state" vesicles with no inclusions. Here, we directly demonstrate that both chemically attached and electrostatically physisorbed particles can cause elastic deformations of flexible giant vesicles and experience membrane mediated attraction.

Giant unilamellar liposomes were prepared from phospholipids POPC (1-Palmytoyl-2-Oleoyl Phosphocholine)

or DMPC (Dimiristoyl Phosphocholine) (Avanti) and 0.5% biotin-X DHPE (molecular probes) following a standard protocol [7]. The liposomes were observed using differential interference contrast with a Nikon Diaphot 300 inverted microscope (64 $\times$ , 1.4 NA objective). Video images were obtained with a high resolution tube camera (Dage-MTI) in combination with video enhancement and recorded on tape. The vesicle solutions were observed in a flat temperature stabilized chamber with a standard cover slip glass at the bottom. The samples showed a mixture of uni- and multilamellar vesicles with isolated thin-walled vesicles exhibiting known shapes of minimal curvature energy [6]. Latex beads (0.3 and 0.9  $\mu\text{m}$  in diameter) were chemically grafted with streptavidin [8]—a water soluble protein which specifically binds to biotin [Fig. 1(c)]. Streptavidin beads were linked to giant vesicles doped with biotinylated lipids by mixing dilute suspensions of each component.

Observations of the bead-membrane hybrid vesicles by video microscopy revealed that vesicles with a single attached bead showed clearly distorted contours with a pinched angle around the bead. Most notably, beads attached to nonspherical vesicles are found primarily at regions with negative curvature. An example of an oblate shaped vesicle together with a schematic sketch is given in Figs. 1(a) and 1(b). The contour shown is naturally distorted due to the thermal fluctuations of the fluid vesicle membrane. However, the bead remains at the center position of the concave region which exhibits a pinched local deformation. To further demonstrate this fact we captured vesicles using low suction glass pipettes [Fig. 1(d)]. Within seconds upon release from the pipette, vesicles reverted to the minimum bending energy shape with the bead centered at a concave region [Fig. 1(e)].

The equilibrium contours of unilamellar bilayer vesicles are determined by the curvature free energy and geometrical constraints imposed by the fixed membrane area. Our observations can be interpreted as perturbed shapes of minimal curvature energy. We therefore consider the

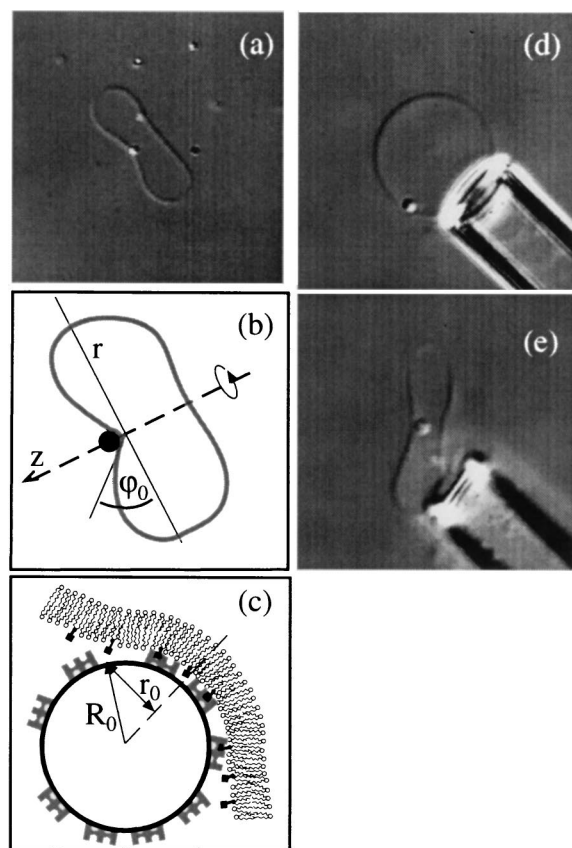


FIG. 1. (a) POPC/DHPE-biotin liposome with a single  $0.9 \mu\text{m}$  bead attached to it. The bead is located in the middle of the concave region of a prolate vesicle. (b) Schematic of a pinched hatlike membrane deformation around the bead. (c) Schematic drawing of the lipid membrane region covalently linked to a bead via biotin-streptavidin bonds. (d) A single bead on a vesicle held in a suction pipette. (e) The same bead and vesicle after releasing the pipette suction.

deformation field around an adhering bead. The bound part of the membrane forms a spherical cap around the bead, which is connected at a boundary gradient angle  $\varphi_0$  to the surrounding membrane. The membrane shape is given by an axisymmetric surface of minimal curvature energy. For an infinite flat membrane with a boundary gradient angle of  $\varphi_0 = \pi/2$  this surface would be the catenoid [9]. In the limit of a partially encapsulated bead the contour  $z(r)$  around the bead for  $r > r_0$  can be approximately described by [10]

$$z - z_0 \approx \pm \frac{r_0^2}{R_0} \ln \frac{2r}{r_0}, \quad (1)$$

where  $r_0$  denotes the radius of the encapsulated spherical cap, and  $R_0 = 0.45 \mu\text{m}$  is the bead radius as shown in Fig. 1(c). Fitting experimentally observed contours of several vesicles using Eq. (1) yielded an estimate of  $r_0 \approx 0.3 \mu\text{m}$ .

In order to explain the preferred localization of the beads in regions of negative curvature, we apply a zeroth order perturbation approach and superimpose the long-ranged deformation of Eq. (1) onto the shapes of finite curvature

vesicles. The biotin-streptavidin interaction favors maximizing the bead-membrane area of contact. The competition of adhesion ( $\gamma$ ) and membrane curvature energies will determine the degree of wrapping of the membrane around the bead [11]: For  $\kappa \gg \gamma$  the membrane will remain flat, while it will completely wrap the bead if  $\kappa \ll \gamma$  ( $\kappa \sim 30k_B T$  is the membrane bending modulus). Since Eq. (1) with  $r_0 < R_0$  describes membrane deformation in our experiment, the bead is only partially wrapped by the membrane, as shown in Figs. 1(b) and 1(c). Thus the adhesion and elastic energies are comparable with  $\gamma \sim \Delta F_e \approx 2\kappa\sigma/R_0^2 \sim 100k_B T$ , where  $\sigma = \pi r_0^2$  is the projected contact area between the bead and the membrane. The bead induced membrane deformation is asymmetric: It effectively introduces a spontaneous curvature  $\sim 1/R_0$  into the membrane around it. Hence, the vesicle-bead system lowers its energy if the membrane curvature  $H_0$  has the same sign as the bead-imposed concave deformation (additional energy penalty would be  $\sim \kappa\sigma H_0/R_0 \sim k_B T$  for a vesicle with undisturbed curvature  $H_0 \sim 1/10 \mu\text{m}$ ). For an isolated single bead, its observed localization on non-spherical vesicles in regions of negative curvature is thus energetically favored.

When two beads were attached to a quasispherical vesicle, where there is no preferred location for a single bead, we found that over a period of a few minutes the beads approached each other and eventually bound together as shown in Fig. 2. The lipid mobility was a prerequisite for aggregation, since it did not occur on DMPC liposomes at temperatures  $T$  below the lipid chain melting temperature  $T_m = 24^\circ\text{C}$ . In this case the beads adhered to the frozen vesicles but did not aggregate [Fig. 2(a)]. When a large unilamellar vesicle was monitored as the

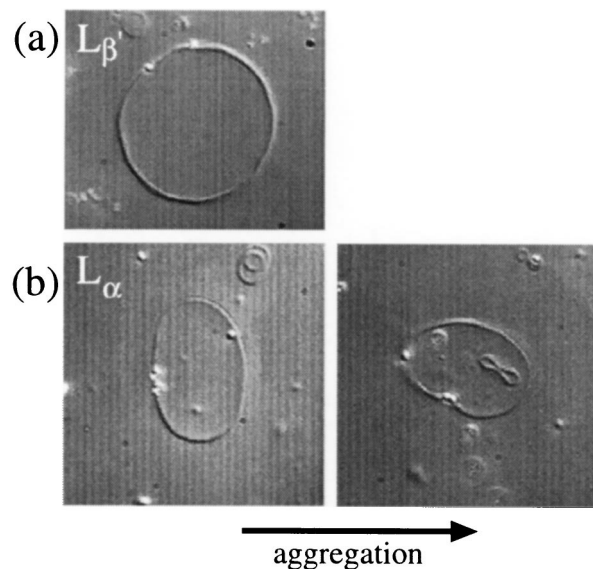


FIG. 2. (a) Giant quasispherical DMPC/DHPE-biotin vesicle in the chain frozen  $L_{\beta'}$  phase with two attached beads. (b) The same liposome as shown in (a) at a temperature above the chain melting transition. The beads aggregate after a period of several minutes.

temperature was raised above  $T_m$ , the two beads that previously were located in close proximity but did not aggregate started to drift towards each other as the temperature exceeded  $T_m$  [Fig. 2(b)]. Importantly, the colloidal interactions alone will not explain bead aggregation, since the beads in the solution did not show any tendency for aggregation over a period of weeks, even in the presence of nonbiotinilated vesicles.

In Fig. 3(a) we present a time sequence of two  $0.9\text{-}\mu\text{m}$ -diameter beads interacting on a surface of a giant quasi-spherical vesicle. The plot of the center-center distance  $R$  of the beads as a function of time [Fig. 3(b)] reveals a Brownian walk motion on the membrane, until the beads get closer than a critical capture radius  $R < 4R_0$  (arrow). The beads remained bound together from then on. This indicates that the range of the membrane mediated attraction in our experiments is of the order of the bead diameter, and the strength of the interaction is of several  $k_B T$ .

Deformation of vesicles can be caused by any adhering object which is sufficiently adhesive and rigid. For example, we investigated cationic giant vesicles decorated with negatively charged DNA-lipid aggregates. As reported previously, DNA and cationic liposomes condense

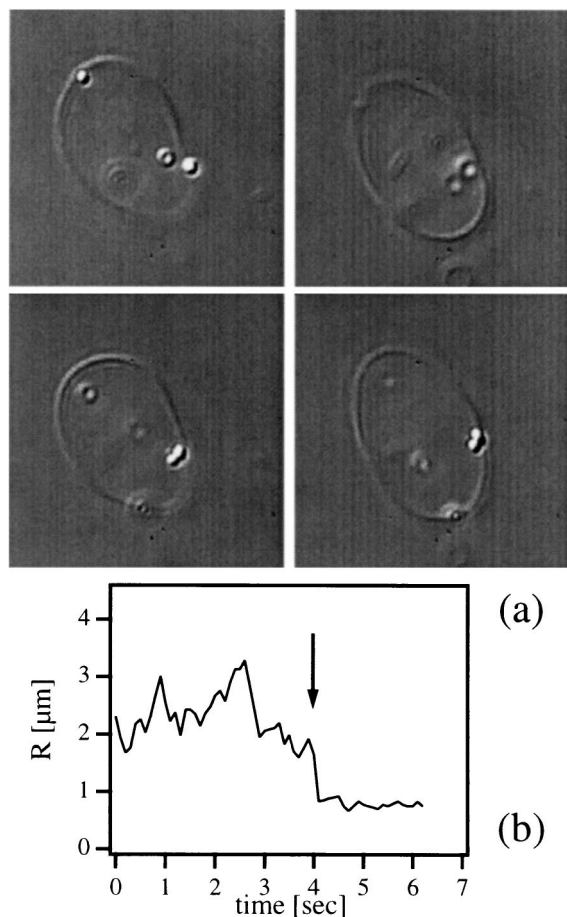


FIG. 3. (a) A time sequence of POPC/DHPE-biotin vesicle decorated with several beads. (b) Center-center distance between the pair of beads in (a) as a function of time just before aggregation.

into small liquid crystalline aggregates (complexes) due to their strong electrostatic attraction [12]. The DNA-lipid complexes can stick to giant cationic liposomes without rupturing the vesicles. Figure 4(a) depicts such a situation with one aggregate attached to a giant liposome and viewed by the lipid fluorescence microscopy. The liposome exhibits a deformed pearlike shape with the complex attached at the neck region. Figure 4(b) shows three complexes adhering to a spherical liposome, and in Fig. 4(c) a sequence of images captures their aggregation by tracking the fluorescently labeled DNA molecules. The distance between the complexes is plotted in Fig. 4(d) as a function of time. Clearly an attractive force acts over a distance of several diameters of the inclusions.

In order to estimate the order of magnitude of the attractive force it is useful to determine the lateral mobility  $\mu$  of the beads. As shown in Fig. 1(d), a glass pipette allows one to hold onto a vesicle and to suppress membrane thermal fluctuations. In this case the lateral diffusion of an attached bead can be measured by particle tracking. We found that  $D = \mu/kT \approx 0.4 \mu\text{m}^2/\text{sec}$  for  $0.9\text{-}\mu\text{m}$ -diameter beads [13]. The approach between two beads due to an attractive force  $f(r)$  can now be described by a ballistic equation  $f(r) = \mu v(r)$ . This ansatz can be applied to estimate the effect of entropic membrane fluctuation induced attraction proposed by Goulian *et al.* [5]. We derive extremely long aggregation times of about 10 h

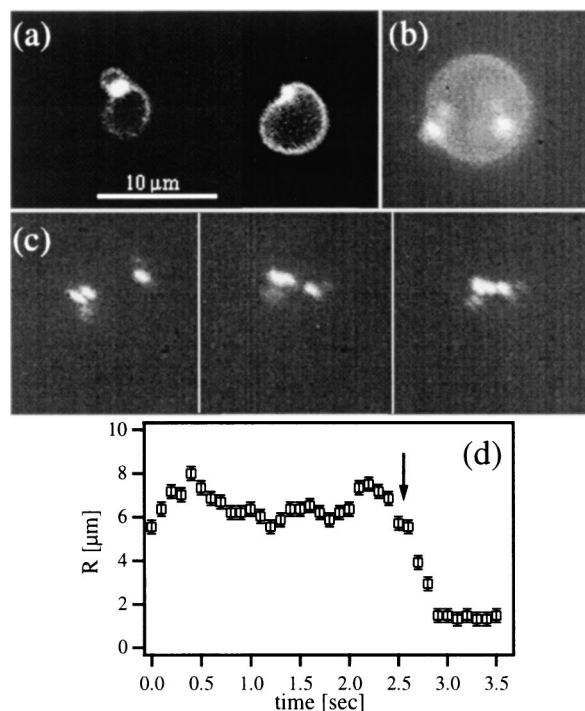


FIG. 4. (a) Lipid fluorescence micrograph of a cationic liposome with an electrostatically bound DNA-lipid complex. The shape of the vesicle is determined by the fluctuating position of the complex. (b),(c) Three complexes aggregating on a cationic liposome imaged in lipid (b) and DNA (c) fluorescence modes. (d) Plot of the relative distance of the single complex from the duplex.

for beads that have been initially  $3 \mu\text{m}$  apart. In fact, for large separation distances Brownian motion would dominate the particle trajectory. This can be seen in Fig. 3(b), where the measured distance between the beads varied randomly until they came close enough that the interaction was able to capture the beads in a directed motion. Furthermore, the fluctuation induced attraction is long range, decaying as  $1/R^4$  as opposed to the observed short-range force. Therefore, the possible entropic attraction is overwhelmed by Brownian motion and is not the cause of bead aggregation in our experiments.

We propose that on spherical vesicles the beads interact via the local elastic deformation of the membrane, similar to the mechanism suggested by Dan *et al.* [3]. The beads deform the membrane locally, stretching or bending it near the bead boundary. This leads to an oscillatory perturbation of the bilayer curvature which decays exponentially with the distance and results in an attractive interaction with a range of, roughly, the inclusion (bead) diameter. Comparing the images of Fig. 4 with those of Figs. 1–3, one notices a larger perturbation of vesicle shapes and longer-range attraction in the case of physisorbed DNA-lipid complexes than covalently bound colloids. This is because the latter interacted with the membranes via a low density coverage covalent bond, while the former interacted due to an electrostatic attraction. This led to a greater contact area between the membrane and inclusion and a larger membrane deformation.

Collective behavior can be observed when many beads are adsorbed on a giant liposome. We found segregated condensed phases of beads located in concave regions of the membrane. Figure 5(a) shows a close packed hexagonal cluster of colloids aggregated on the concave side of a stomatocyte shaped vesicle, while Fig. 5(b) shows a ring shaped string of beads aggregated around the waist of a dumbbell shaped vesicle. The beads exhibited constant mean center-center separation distances  $R \sim 2R_0$  in the hexagonal cluster. In contrast, in the ring shaped cluster the beads were firmly pinned to the waist along the meridional direction but distributed freely with varying separation distances along the azimuthal direction. The observed clusters provide an example for curvature in-

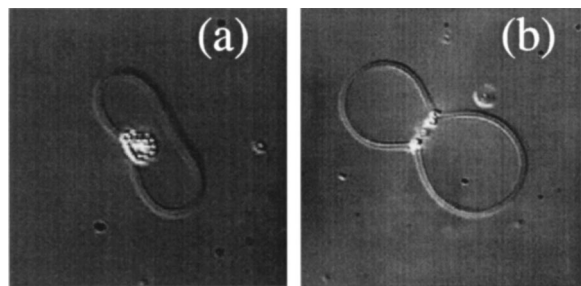


FIG. 5. (a) Phase separation of many beads into a condensed hexagonal packed cluster. The cluster is located at the concave dip of a stomatocyte shaped liposome. (b) Beads clustered in a ring form around the waist of a dumbbell shaped multilamellar vesicle.

duced lateral phase segregation of a two-dimensional colloidal system. The aggregated state should be naturally preferred for the beads on nonspherical vesicles, since it minimizes the membrane deformation energy. The ring shaped cluster has a smaller average curvature than the stomatocyte one, indicating that the beads will adhere together more strongly in the latter, as observed experimentally. This also shows that aggregation of many beads on a vesicle surface can drive transformations of vesicles into dumbbell shapes, which are a precursor to the budding and fission of membranes [6].

In conclusion, we have observed different deformation states of giant liposomes with both chemically attached and physisorbed particles. Single beads cause bilayer deformations that lead to a curvature induced localization of the bead. The same mechanism holds for many beads and leads to their aggregation in concave regions of nonspherical vesicles and to possible vesicle shape transformations. Importantly, we have directly observed an attractive short-range membrane mediated force between particles attached to spherical membranes, which has not been previously experimentally demonstrated and which might be of importance in protein-protein interactions.

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- [1] R. Lipowsky and E. Sackmann, in *Handbook of Biological Physics* (Elsevier, New York, 1995).
- [2] H. Gruler, *Z. Naturforsch. C* **30**, 608 (1975).
- [3] N. Dan *et al.*, *Langmuir* **9**, 2768 (1993); N. Dan *et al.*, *Biophys. J.* **71**, 1267 (1996).
- [4] S. Marcelja, *Biochim. Biophys. Acta* **455**, 1 (1976); J. C. Owicki, M. W. Springgate, and H. M. McConnell, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 1616 (1978).
- [5] M. Goulian *et al.*, *Europhys. Lett.* **22**, 145 (1993); **23**, 155 (1993).
- [6] J. Käs and E. Sackmann, *Biophys. J.* **60**, 825 (1991).
- [7] E. Evans and W. Rawicz, *Phys. Rev. Lett.* **64**, 2094 (1990).
- [8] E. Evans, K. Ritchie, and R. Merkel, *Biophys. J.* **68**, 2580 (1995).
- [9] W. Harbich, R. M. Servuss, and W. Helfrich, *Z. Naturforsch.* **33A**, 1013 (1973).
- [10] W. Helfrich and M. M. Kozlov, *J. Phys. II (France)* **4**, 1427 (1994).
- [11] U. Seifert and R. Lipowsky, *Phys. Rev. A* **42**, 4768 (1990); C. Dietrich *et al.*, *J. Phys. II (France)* **7**, 1651 (1997).
- [12] J. O. Rädler, I. Koltover, T. Salditt, and C. R. Safinya, *Science* **275**, 810 (1997).
- [13] This is similar to the value of  $D$  measured for large membrane-associated proteins. D. W. Tank, E.-S. Wu, P. Meers, and W. W. Webb, *Biophys. J.* **40**, 129 (1981).