Supplementary Information

Controlled adhesion, membrane pinning and vesicle transport by Janus particles

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List of abbreviations

AC – alternating current
BSA – bovine serum albumin
COM – centre of mass
DOPC - 1,2-dioleoyl-sn-glycero-3-phosphocholine
DOTAP - 1,2-dioleoyl-3-trimethylammonium-propane (chloride salt)
GUV – giant unilamellar vesicle
ITO – Indium tin oxide
LUV – large unilamellar vesicle
NA – numerical aperture
Rh-DPPE - 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine-N- (lissamine rhodamine B sulfonyl) (Ammonium salt)
ROI – region of interest

S1. Materials and methods

S1.1. Materials.

1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), 1,2-dioleoyl-3-trimethylammonium-propane (chloride salt) (DOTAP) and 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine-N- (lissamine rhodamine B sulfonyl) (Ammonium salt) (Rh-DPPE) were acquired from Avanti Polar Lipids (Alabaster, AL); the lipid structures are given in Fig. S1. 100 nm pore diameter polycarbonate membranes were obtained from Whatman (Maidstone, UK). Indium tin oxide (ITO) coated glasses (ITO film thickness < 100 nm, resistance 50 Ω) were obtained from Präzisions Glas & Optik (Iserlohn, Germany). Glucose, sucrose, sodium chloride and bovine serum albumin (BSA) were all obtained from Sigma Aldrich (Darmstadt, Germany). The polystyrene particles used for direct adhesion to LUVs and GUVs (Polybead® microspheres, 6 µm non-functionalised polystyrene (exposed sulphate groups), 4 µm functionalised and non-functionalised (exposed amine or sulphate surface groups respectively)) were obtained from Polysciences (Germany). The uniform polystyrene particles used for Janus particle preparation were purchased from Bangs Lab Inc. (Indiana, USA). Iron and chromium pellets were purchased from Kurt J. Lesker Co. (Clairton, PA, USA).

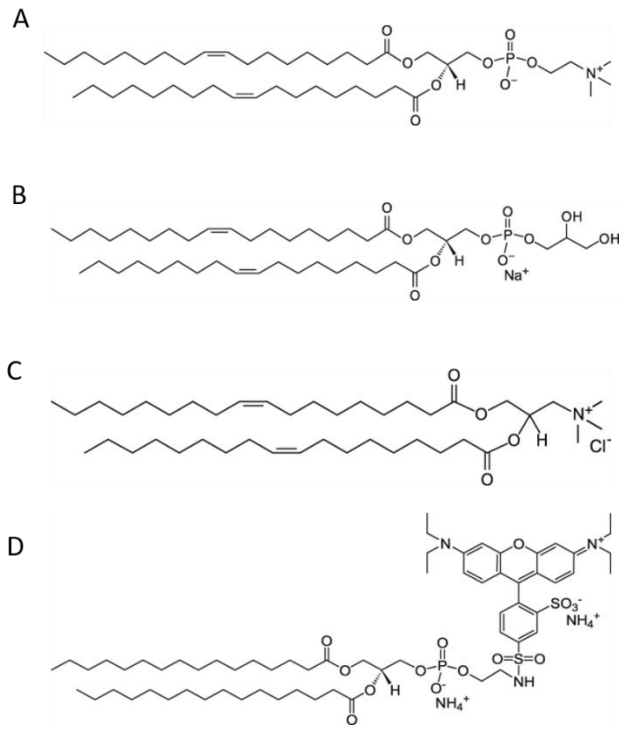

Fig. S1 Structures of lipids used in this study. (A) 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC). (B) 1,2-dioleoyl-sn-glycero-3-phospho-(1'-rac-glycerol) (sodium salt) (DOPG). (C) 1,2-dioleoyl-3-trimethylammonium-propane (chloride salt) (DOTAP). (D) 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine-N-(lissamine rhodamine B sulfonyl) (ammonium salt) (Rh-DPPE). *Images from Avanti Polar Lipids.*

S1.2. Vesicle preparation and mixing with particles.

Large unilamellar vesicles (LUVs) were prepared via extrusion at room temperature.[1] The lipid solution was prepared to 2.2 mL in chloroform at a concentration of 2 mM and composed of either DOPC/DOTAP/Rh-DPPE (59.5/40/0.5 molar ratio), DOPC/DOPG/Rh-DPPE (59.5/40/0.5 molar ratio) or DOPC/DOTAP/Rh-DPPE (94.5/5/0.5 molar ratio) and deposited in a small glass vial. The chloroform was evaporated first under a stream of N_2 and then further dried under vacuum for 2 hours. The lipid film was then rehydrated with either 0.2 M sucrose or 150 mM NaCl to a final lipid concentration of 1.2 mM. The solution was vortexed for 2-5 minutes, obtaining multilamellar vesicles. The vesicle solution was then subjected to 11 cycles of extrusion through a 100 nm pore diameter polycarbonate membrane. For the adhesion studies, LUVs were mixed with the microspheres and incubated for 1 hour before imaging, in a vertical rotating mixer. The particles had a final concentration of 8.4×10^4 particles/mL and the LUVs had a final lipid concentration of 0.83 mg/mL.

Giant unilamellar vesicles (GUVs) were prepared via the established electroformation protocol.[2] Lipid solutions were prepared in chloroform at 4 mM with varying ratios of DOPC and DOTAP, as indicated throughout the text. Unless explicitly stated in the text, lipid solutions also contained 0.5 mol% Rh-DPPE fluorescent dye. A total volume of 16 µL of the lipid solution in chloroform was spread on two conductive ITO-coated glasses and dried under vacuum for 2 to 2.5 hours at room temperature. The ITO glasses, together with a rectangular Teflon spacer, were then assembled to form a chamber of 2 mL volume which was filled with 0.2 M sucrose. The chamber was then connected to a function generator which was used

to apply an AC field (1.2 V, 10 Hz) for 1.5 hours at room temperature (for the lipid compositions containing dyes, the electroformation was performed in the dark). The GUVs were then removed from the growth chamber via pipetting and diluted 1:1 in a 0.21 M glucose solution (unless otherwise stated in the main text) containing dispersed particles. GUV suspensions and glucose solutions were measured and the osmolarity adjusted (glucose only) using an osmometer (Osmomat 030, Gonotec, Germany) such that the particle solution had higher osmolarity by ~10 mOsm. The GUVs were incubated with the particles (final concentration of 8.4×10^3 particles/mL) for 1 hour in a vertical rotating mixer before observation.

S1.3. Janus particle preparation.

Iron-patched Janus microspheres were prepared from the uniform polystyrene particles using a metal vapour deposition technique,[3, 4] see sketch in Fig. S2A. Briefly, the polystyrene particles were concentrated and washed by centrifuging at 1500 ×*g* for 5 min and replacing the supernatant with MQ water; this was repeated 2-3 times. A convective assembly method was used to deposit particle monolayers on pre-cleaned glass slides.[5] The dried particle monolayers were coated with hemispherical patches of chromium followed by a layer of iron (5 nm and 20 nm respectively) in a metal evaporator (Cooke Vacuum Products, model CV302). The thickness of the evaporated metals was monitored using a Maxtek Inc. TM350 thickness monitor equipped with SC-101 sensor crystals. The particles were then gently scraped from the deposition surface and resuspended in Milli-Q water. A SEM image of Janus particles is shown in Fig. S2B. The microparticles were then washed 3 times in Milli-Q water, via repeated centrifugation and removal of supernatant, before use. Note that the iron patch on the surface of microspheres transforms to iron oxide (Fe_2O_3) upon their resuspension in an aqueous environment.[3] In some brightfield images (depending on the particle orientation), it is also possible to distinguish a region of the particle surface that is darker, which corresponds to the iron oxide cap, see Fig. S2C.

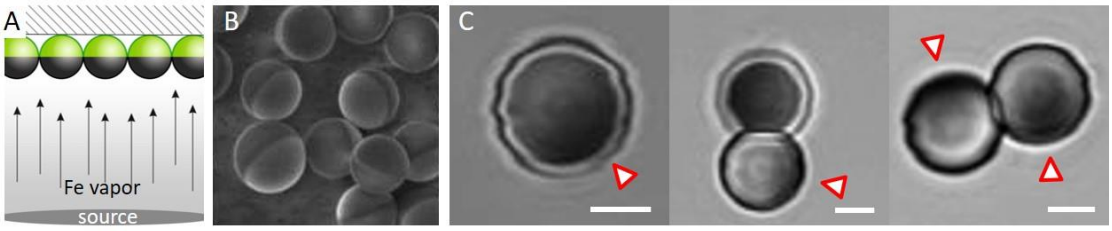

Fig. S2 (A) Schematic of metal vapour deposition on a monolayer of polystyrene colloid spheres dried on a solid substrate, adapted from [4]. (B) SEM image of 4 µm Janus particles adapted from[3], clearly showing the two different surfaces on the same particle. (C) Brightfield images of 4 µm Janus particles in aqueous solution with visible darker patches (arrowheads pointing to them), corresponding to the iron oxide coating. Scale bars: 2 µm. The apparent distorted surface of the particle is an artefact of the slow scanning speed of the confocal microscope with which the images were acquired.

S1.4. Imaging.

Confocal imaging was performed on either a Leica confocal SP8 or SP5 setup (Mannheim, Germany). Rh-DPPE was excited with a 561 nm laser and the emission signal collected between 580-670 nm. The images were acquired with a 63x (1.2 NA) water immersion objective and 1 Airy unit. The subsequent image analysis is described in detail in section S2. Phase contrast imaging was performed on an Axio Observer

D1 (Zeiss, Germany) microscope, equipped with a Ph2 20x (NA 0.5) objective and an ORCA R2 CCD camera (Hamamatsu, Japan).

S1.5. Magnetic manipulation of Janus particles.

A handheld bar magnet was used to generate a magnetic field gradient by placing the magnet close (approximately 2 cm from pole of magnet to glass) to the observation chamber. The magnet is formed from multiple blocks of Neodymium (dimensions 2.0 cm × 2.0 cm × 10.4 cm), with an approximate total magnetic field of 1 mT (at the magnet surface). To change the direction of the magnetic field, the position of the magnet was rotated approximately 180° around the observation chamber. During image acquisition the magnet was held in a fixed position.

S2. Particle-membrane affinity with homogeneous and Janus particles

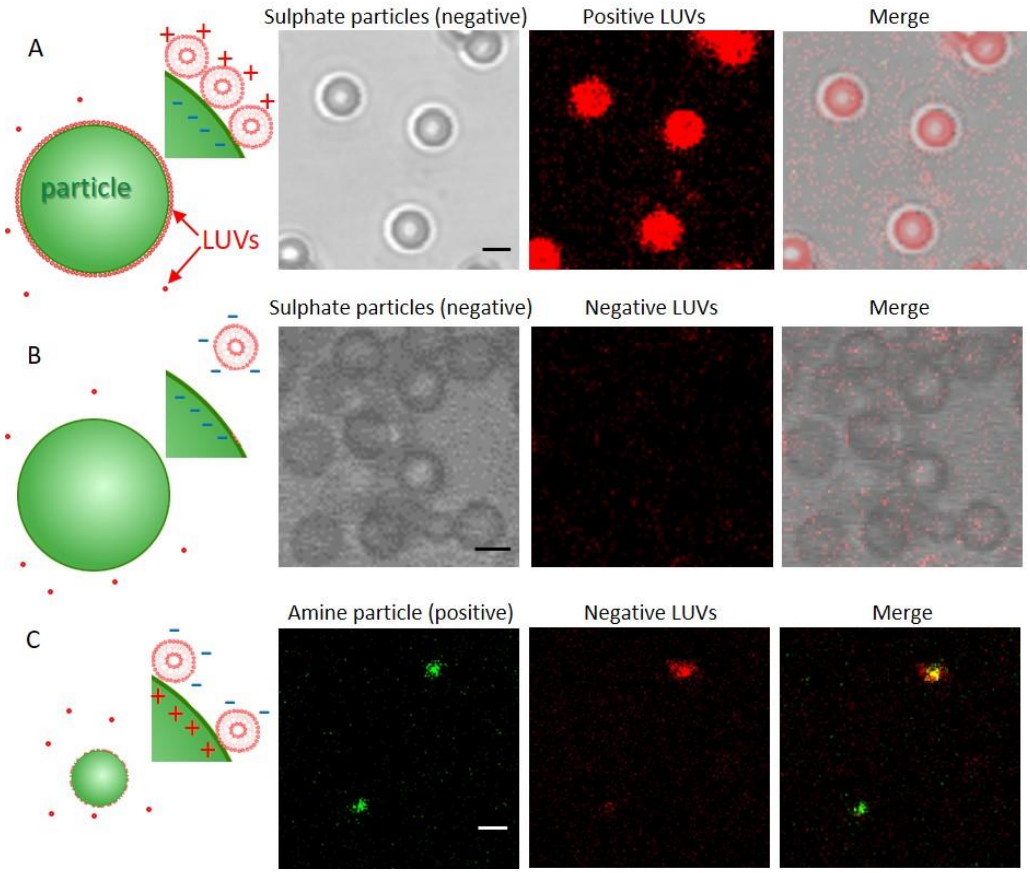

Fig. S3 Adhesion of fluorescently labelled LUVs of varying lipid compositions (and labelled with 0.5 mol% Rh-DPPE, red) to microparticles with different surface charges shown with schematic representations of the different degrees of interactions and bright-field and confocal images. The sketches roughly represent the relatively large size of the microparticles (6 μm or 1 μm) relative to that of the LUVs (100 nm). (A) 6 μm polystyrene particles with a negative surface charge (sulphate groups) and positively charged LUVs (DOPC/DOTAP 60/40 mol%). The LUVs (as observed from the fluorescence signal from the red dye Rh-DPPE in the membrane) completely cover the surface of all of the particles in the sample. (B) The same 6 μm polystyrene particles and negatively charged LUVs (DOPC/DOPG 60/40 mol%). The LUVs do not adhere

to the particles' surface, which we conclude from the lack of fluorescence signal in the particles' location in the merged image. (C) 1 μm polystyrene particles with amine functional groups (positively charged, labelled with green fluorescent dye) incubated with negatively charged LUVs (DOPC/DOPG 60/40 mol%, red) showed heterogeneous adhesion of LUVs to the particles' surfaces, as can be seen from the difference in fluorescence signals from the red LUVs on the two particles (middle image). All scale bars correspond to 5 μm. The merged images on the right show overlay of the signal detected in the channels showing the particles and the LUVs individually.

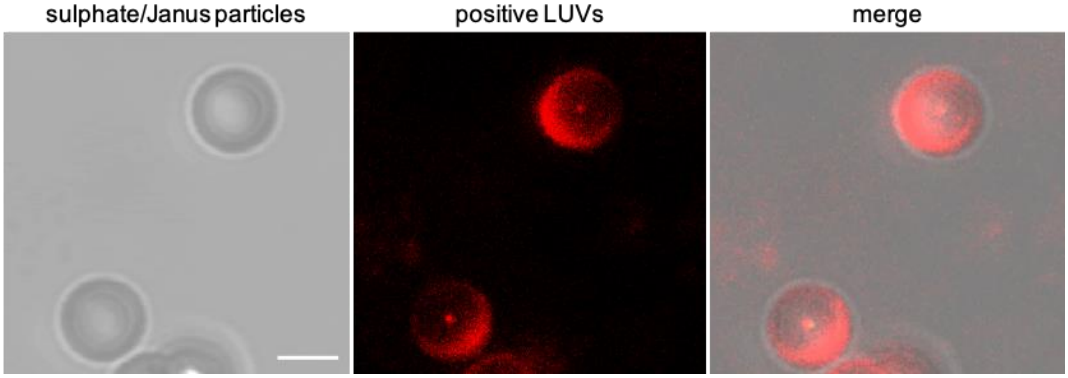

Fig. S4 Preferential adhesion of LUVs to one region of Janus particles. DOTAP (positively charged) doped LUVs (labelled with Rh-DPPE visualized in red) only adhere to portions of Janus particle surfaces, indicating a stronger adhesion for one region compared to another. Scale bar: 3 μm. The non-adhesive region, exhibiting less fluorescence from the LUVs (middle image), corresponds to the metal patch, which appears darker in the brightfield image (left). The merged image shows an overlay of both.

S3. Calculating particle penetration depth

The particle penetration depth into the GUVs was calculated from a stack of confocal z-slices of each vesicle-particle pair. First, the (x,y) centre of mass (COM_V in Fig. S5) of the vesicle was determined by fitting a circle to the vesicle contour in the z-stack with the largest diameter (the error on this value is the standard deviation of three such measurements on the same vesicle). The z-position of the COM(s) were calculated as the image number in the stack (e.g., z = 14) multiplied by the z step height (the error on this value was determined to be the z step height of the confocal stack, and is introduced when selecting the correct contour to measure). The centre of mass of the particle (COM_P) was determined in the same way from the brightfield channel. The distance between the vesicle COM and particle COM was calculated using Equation 1:

$$D = \left|\overrightarrow{COM_V COM_P}\right| = \sqrt{x^2 + y^2 + z^2} \quad (1)$$

in which $COM_V = [x_V, y_V, z_V]$, $COM_P = [x_P, y_P, z_P]$ and subsequently $x = x_V - x_P$ (and similarly for y and z). The depth of particle penetration into the vesicle (d) is defined as the distance between the vesicle membrane on the particle surface (solid line in contact with particle) and where it is projected to be (dashed red line) if the particle were not present (by assuming the vesicle is spherical). This distance is therefore calculated using the following equation:

$$d = R_P + R_V - D \quad (2)$$

where R_P and R_V are the particle and vesicle radii respectively, as indicated in the diagram below.

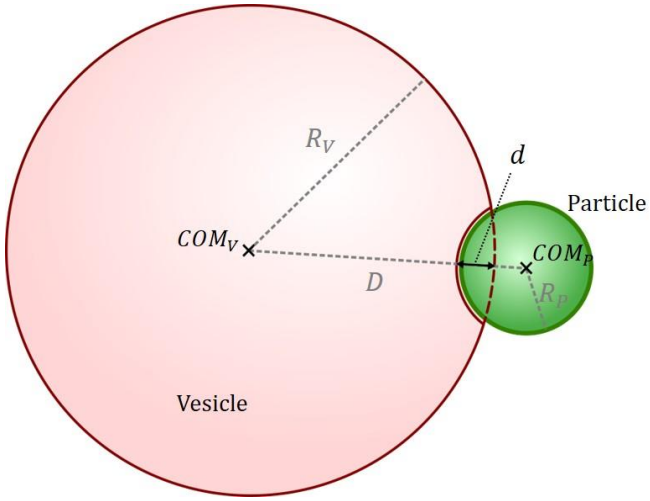

Fig. S5 Schematic presentation of the vesicle and particle with indicated relevant dimensions of the system.

S4. Imaging and evaluating LUV fluorescence on particle surface

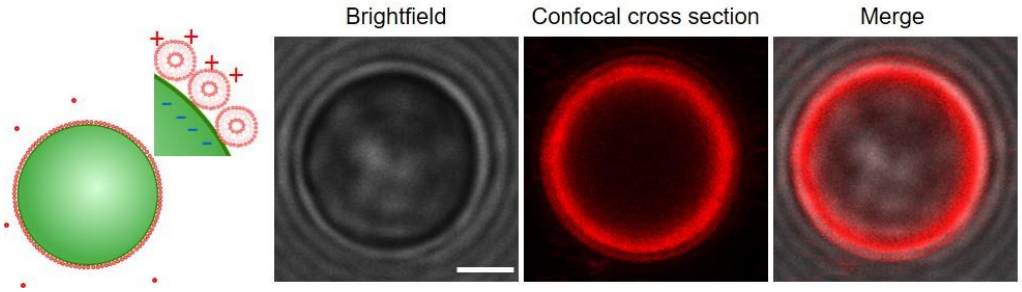

Fig. S6 Brightfield and confocal cross-section with LUVs (red) adsorbed to the surface of a 6 µm polystyrene (sulphate surface groups, negative charge) particle (visible in brightfield image and overlay of the brightfield and red fluorescent channels) in the absence of salt. Scale bar 2 µm.

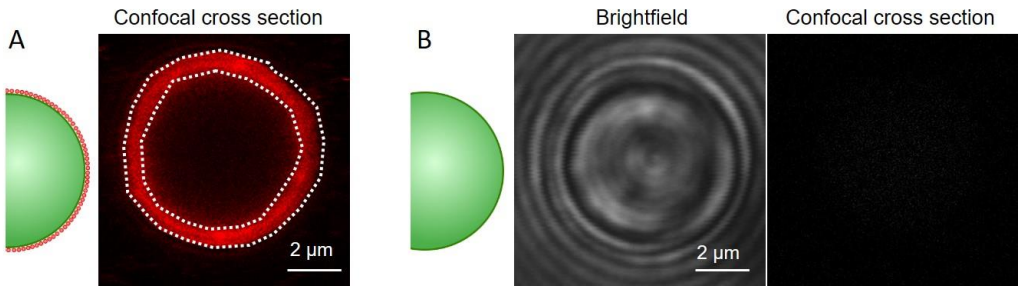

Fig. S7 (A) Evaluating the fluorescently labelled LUV coverage on 6 µm polystyrene particles: a confocal cross section of one particle with dashed line indicating the ROI (region of interest) that is selected for fluorescence intensity analysis of LUV coverage. The analysis is carried out in LAS X, a confocal imaging

and analysis software from Leica. As the particles are roughly of the same size, the equatorial cross-section is imaged at the same height from the glass for all particles. When the ROI is selected, regions with visible lipid/vesicle aggregation (and thus higher intensity) are excluded from the selection. Example demonstrated here is for 200 mM sucrose (no salt). (B) Images taken at the same imaging settings and image brightness/contrast for comparison of particles in the absence of LUVs demonstrate that none of the fluorescence signal results from particle reflection or autofluorescence (there is no red signal in the confocal panel).

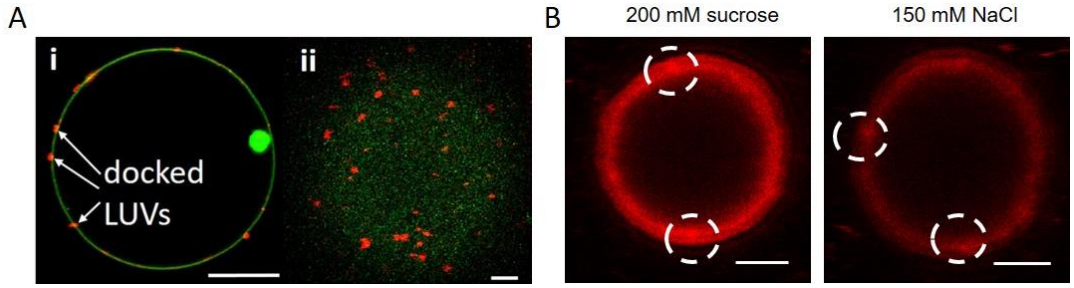

Fig. S8 Comparison of LUVs fluorescence observed on GUV surface and on particle surface. (A) Confocal images of LUVs docked on the surface of a GUV; (i) mid-plane confocal cross section of GUV (green) with docked LUVs (red) indicated and (ii) upper GUV surface. Images adapted from [6]. Scale bars (i) 10 µm, (ii) 2 µm. (B) Confocal image of LUVs present on the surface of a polystyrene particle in the conditions of Fig. 3 in the main text. Scale bars 2 µm. The fluorescence from individual docked LUVs (A) appear to produce stronger signal compared to the more homogeneous fluorescence that we observe over the particles (B), but certain spots of bright fluorescence (encircled in B) appear to result from LUV docking.

Movie S1 Time series showing Janus particle adhered to a GUV moving through observation chamber in the presence of a magnetic field gradient; for details see Fig. 4 in the main text.

References:

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